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Oxamyl

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ANNEX B.6
Toxicology and metabolism data and assessment of risks for humans

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May 2016	First RAR of Italy	
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B.6 TOXICOLOGICAL AND METABOLISM DATA

Introduction

This document contains summaries of toxicity tests completed for oxamyl and overviews by groups of tests. Test summaries cover the individual study types and a discussion of the primary toxicological effects.

Test substance specification can be determined from the test substance code which is a research and development code number given to a specific batch of produced material (either technical or formulated). The approximate composition of the materials used in the various tests is given in Table 1.

Table 1 Test substance specification

Test substance code	Type	Composition
Annex I inclusion EU approval review		
Not given	[^{14}C]oxamyl	Not given
E52467-29	[1-^{14}C]oxamyl	Radiochemical purity >96%
DPX-D1410-196	Oxamyl PAI (Pure Active Ingredient)	97.1%, 97.2%, 96.9%, 98.3%, 97.6%, 98.21%
DPX-D1410-196A	Oxamyl PAI	98.09%
DPX-D1410-196B	Oxamyl PAI	98.21%
Not given	Oxamyl PAI	96.9%, 100%, 98%, 97.1%, Not given
DPX-D1410-304	Oxamyl technical 42	42%
DPX-D1410-7	Oxamyl PAI	Not given
DPX-D1410-15	Oxamyl PAI	95%
Not given	IN-A2213 technical metabolite	Not given
IN-A2213-1	IN-A2213 technical metabolite	Not given
Not given	IN-L2953 technical metabolite	Not given
IN-N0079-1	IN-N0079 technical metabolite	Not given
IN-N0079-2	IN-N0079 technical metabolite	Not given
IN-N0079-3	IN-N0079 technical metabolite	100%
Not given	IN-N0079 technical metabolite	100%
IN-D2708-3	IN-D2708 technical metabolite	Not given
2015 renewal submission		
DPX-D1410-535	Oxamyl PAI	97.5%
1841000	[1-^{14}C]oxamyl PAI	Radiochemical purity: 99.7%
DPX-D1410-196	Oxamyl PAI	98.0%, 98%
DPX-D1410-532	Oxamyl PAI	99.1%

The detailed composition and impurity profiles of the test substances used in the toxicological studies are provided in the Oxamyl RAR Vol. 4. The toxicological profile of oxamyl was assessed using isolated oxamyl pure active ingredient (PAI). However, it is the only 42% technical that is manufactured and used to prepare the formulations; therefore, representative toxicology studies for the 42% which includes cyclohexanone as solvent for stabilization were conducted in order to describe the hazards for transportation and formulation processes. The detailed composition of oxamyl technical 42 can be found in the Oxamyl RAR Vol. 4 . Oxamyl is being manufactured using an integrated process resulting in a solution containing 42% or 10% oxamyl. Oxamyl is never isolated during the manufacturing process. The detailed description of the manufacturing process is contained in the Oxamyl RAR Vol. 4.

Unless specifically indicated, all reports in this section are submitted to address mandatory data requirements for the approval of active substance.

Unless specifically indicated, all tests submitted in this section, which involve vertebrate animals, address mandatory data requirements which could not be met with alternative methods. Studies were conducted according to prescribed guidelines.

Unless specifically indicated, this section does not contain reports of studies duplicating previous tests on vertebrate animals.

B.6.1 Absorption, distribution, metabolism and excretion in mammals

B.6.1.1 Absorption, distribution, metabolism and excretion by oral route

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.1.1/01

Reference: --	**Report:**	Han, J.C.-Y, Harvey, J (1977); Metabolism of oxamyl and selected metabolites in the rat **J. Agric. Food Chem. 26:902-910 1978** **DuPont Report No.:** O/ME 33 **Guidelines: Not given** (No OECD Guideline was available at the time the test was performed) **GLP: NO** (GLP system was not in place at the time the test was performed)

1. Test material: [^{14}C]oxamyl
   Lot/Batch #: Not given
   Purity: Not given

Materials and Methods:

The metabolism of Oxamyl and its principal plant metabolites, methyl N',N'-dimethyl-N-[1- glucosyl)oxyl]-1-thiooxamimidate (metabolite A - the glucose conjugate of the oximino metabolite, IN-A2213) and N',N'-dimethyl-1-cyanoformamide (DMCF or IN-N0079) was investigated by incubation with rat liver microsomes and by oral administration to 2 preconditioned male rats (Charles River CD).

Incubation with liver microsomes:

Liver microsomes were prepared from Charles River-CD rats. A typical reaction mixture in a 50 mL test tube contained:- 8 mL of liver microsome fraction, 8 mL of 0.2 M tris buffer (pH 7.3), 8 mL of NADPH (2 mg/mL) in buffer solution, and 2 mL of an aqueous solution of the radiolabelled test substance. Three dose levels of the 3 ^{14}C-labelled compounds were investigated:- Oxamyl (0.3, 1, and 2 mg), IN-A2213 (0.3, 1, and 5 mg), and IN-N0079 (0.3, 1, and 5 mg) and IN-A2213-glucoside (0.3, 1, and 5 mg). A separate control incubation lacking in liver microsomes and containing 1 mg of each ^{14}C-labeled compound was included in each test. All test solutions were incubated for 2 hours at 37°C.

Treatment of rats with ^{14}C-oxamyl:

Two male rats (rat A and rat B) were preconditioned on a diet containing unlabelled Oxamyl. One rat received 50 ppm for 32 days and the second rat received 150 ppm for 18 days, delivered in both cases in ground Laboratory Chow containing 1% corn oil. At the end of the preconditioning period, the body weights of rat A and rat B were 393 g and 225 g, respectively. They were dosed with 2.54 and 4.58 mg/kg bw ^{14}C-Oxamyl in 2 mL of peanut oil, respectively, by intragastric intubation. Dose solutions contained enough radiolabel to deliver approximately 3.7 µCi (1.4 MBq) and 5.6 µCi (2.1 MBq) for rats A and B, respectively. Faeces, urine, and volatile trap solutions were collected at 24-hour intervals for analysis of radioactive residues. Seventy-two hours following administration of ^{14}C-Oxamyl, blood samples were taken, the rats were killed and tissues and organs were collected.

Oxidation of volatile organic compounds from the rat chamber:

After rat A had been dosed with 2.54 mg/kg bw ^{14}C-Oxamyl, it was immediately placed in a metabolism chamber through which 500 mL/minute CO_2-free air was drawn. The effluent air from the chamber was first scrubbed with 4 N aqueous NaOH to remove respiratory ^{14}C-carbon dioxide. Subsequently, organic volatiles

were oxidized over cupric oxide heated at ca. 700°C by an electric furnace. Any ^{14}C-carbon dioxide generated due to oxidation was collected in a second NaOH trap.

Treatment of rats with IN-A2213-glucoside and IN-N0079:

Two additional male rats were dosed separately with ^{14}C-IN-A2213-glucoside and ^{14}C -IN- N0079. One rat was preconditioned for 8 days with water containing 1540 ppm unlabeled IN- A2213 glucoside and dosed by intragastric intubation with 4 mL of an aqueous solution of ^{14}C -labelled IN-A2213-glucoside equivalent to 2.42 mg/kg bw (3.77 µCi; 1.39 MBq). The weight of the rat at the time of dosing was 277 g. The rat was transferred immediately to a metabolism chamber. Similarly, the second rat was preconditioned with 450 ppm of unlabeled IN-N0079 administered in ground Purina Laboratory Chow for seven days. Then the rat (283 g) was given a single dose with 4 mL of aqueous solution containing ^{14}C-IN-N0079 equivalent of 3.9 mg/kg bw (10.7 µCi; 0.40 MBq) and transferred immediately to a metabolism chamber. Faeces, urine and the trap solutions were collected at 24 hour intervals. The rats were sacrificed and dissected 72 hours after dosing and tissues and organs collected.

Urine analyses of ^{14}C *-Oxamyl dosed rats:*

Urinary metabolites were purified using two gel permeation columns (Sephadex G-15 and Sephadex LH-20). Urine samples were either extracted with ethyl acetate prior to, or after, treatment with β-glucuronidase-aryl sulphatase to determine the extractable ^{14}C-residues. Freeze-dried urine samples were also subjected to acid hydrolysis using anhydrous methanolic HCl reagent. The reagent was evaporated and the residues re-dissolved in methanol and analysed on silica gel TLC plates. The hydrolysate was also analysed by gas chromatography using a glass column (6 ft x 0.25 inch; 30% OV-101 on 80-100 mesh Chromosorb W). The column temperature was programmed from 100-250°C with a ramp of 15°C/min.

The bands on the TLC plate containing polar metabolites were scraped off and eluted with 50% methanol. This was evaporated and the residues incubated for 24 hours at 37°C in BCl3/2-chloroethanol reagent. Following the evaporation of the reagent, the ^{14}C -residues were dissolved in ethyl acetate. The organic solution was extracted with water to remove inorganic contaminants. The organic phase was evaporated to 2 mL and the concentrated extract analysed by silica gel TLC together with reference standards.

Urine analyses of ^{14}C-*IN-A2213-glucoside dosed rat:*

Urine samples were combined, lyophilised and the solids extracted sequentially with ethyl acetate, methanol, and water. The ethyl acetate and water extracts were not investigated further as these fractions contained low levels (< 2%) of radioactivity. The concentrated methanol extract was analysed using a Sephadex LH-20 column. The eluate fractions from the Sephadex LH-20 column were further analysed using Aminex A-6 (BioRad Laboratories) and Porasil A chromatography columns. The column fractions were individually subjected to methanolic HCl and/or Lewis acid hydrolysis. The major fractions were converted to trimethylsilyl derivatives and analysed by GC/MS.

Urine analyses of ^{14}C-*IN-N0079 dosed rat:*

The freeze-dried urine samples were extracted as described previously. The methanol extract was hydrolysed with BCl_3/chloroethanol and the hydrolysate analysed by TLC.

Faeces analyses:

All faecal samples were extracted with distilled water using ultrasonic vibration. The extract was partitioned with ethyl acetate and lyophilised. Aliquot samples from the faeces extracts were hydrolysed separately by methanolic HCl and by BCl3/chloroethanol. The hydrolysates were analysed using TLC as described for the urine.

Hydrolysis of proteins in the blood and skin:

After extraction, the skin was treated with Pronase (CalBiochem) to hydrolyse protein. The radioactivity solubilised by Pronase treatment was characterised by Bio-gel P-2. The amino acids were isolated as a fraction by absorption on 50-100 mesh Dowex-X8 (H+ form) cation exchange resin.

Rat blood was centrifuged to precipitate the blood cells which were washed with water and the washings added to the original serum supernatant. The aqueous phase was extracted with ethyl acetate and heated to precipitate coagulated protein. The original precipitate and heat- precipitated protein were treated with Pronase and the resulting solutions characterised.

Findings:

In vitro studies:

Identification of all metabolites was confirmed by mass spectral analysis after isolation by TLC and HPLC as appropriate.

Incubation of Oxamyl with liver microsomes for 2 hours resulted in the 6 compounds listed in Table 2. The control incubation produced IN-A2213 (Metabolite I) in approximately the same amount as the microsomal incubation, suggesting that the formation of this compound was due to a non-enzymatic reaction. IN-N0079 (Metabolite V) and IN-D2708 (Metabolite III) were identified as enzymatic reaction products. Small amounts of IN-D1409 (Metabolite VI) and IN-L2953 (Metabolite II) were detected, indicating that N-demethylation was occurring at slower rates. Unchanged Oxamyl was present in all incubation experiments. IN-D2708 was the only product of ^{14}C-IN-N0079 incubation with liver microsomes.

Liver microsomes had almost no effect on either IN-A2213 or IN-A2213-glucoside with approximately 99% and 90%, respectively, being recovered unchanged. A trace level of polar material was tentatively identified as the glucuronide conjugate of IN-A2213, an oxidation product of IN-A2213-glucoside.

Oxamyl degraded in the presence of liver microsomes by two major and distinctly different pathways. The first route consisted of hydrolysis of Oxamyl to the corresponding oximino compound (IN-A2213), which is itself resistant to further degradation by liver microsomes. The second pathway was the enzymatic metabolism of Oxamyl to IN-N0079 which was in turn metabolised to dimethyloxamic acid (IN-D2708). Superimposed on both of these pathways is another slower enzymatic process which results in partial demethylation of the dimethyl carbamoyl group.

Table 2: Liver microsomal metabolites of ^{14}C-Oxamyl and ^{14}C-IN-N0079

Compound incubated	mg tested	Metabolites identified as % of incubated ^{14}C- compound					
		Oxamyl	IN-A2213 (I)	IN-N0079 (V)	IN-D1409 (VI)	IN-L2953 (II)	IN-D2708 (III)
^{14}C-Oxamyl (-mic)	1.0	85.7	12.9	ND	ND	ND	ND
^{14}C-Oxamyl	0.3	37.6	15.5	31.6	3.8	1.1	8.8
^{14}C-Oxamyl	1.0	59.0	12.7	17.1	2.5	0.7	7.3
^{14}C-Oxamyl	2.0	60.0	10.4	19.6	2.1	0.5	6.8
^{14}C-IN-N0079 (-mic)	1.0	NA	NA	99.9	NA	NA	NA
^{14}C-IN-N0079	0.3	NA	NA	79.6	NA	NA	20.4
^{14}C-IN-N0079	1.0	NA	NA	87.9	NA	NA	12.1
^{14}C-IN-N0079	5.0	NA	NA	93.9	NA	NA	6.1

ND = not detected
NA = not applicable as IN-N0079 cannot metabolise to its precursor compounds.

In Vivo studies:
Disposition and Mass Balance:

The results of disposition and mass balance following dosing of Oxamyl are summarised in Table 3. Each rat displayed a remarkably similar pattern of elimination of radioactivity regardless of which ^{14}C-compound was administered. Most of the dosed radioactivity (68-72%) was eliminated within 72 hours of dosing with the major amount in urine (48-64%). No major radioactivity (<0.03%) appeared in the expired air. Lower levels of radioactivity were distributed throughout the body at the conclusion of the experiment with a similar pattern in tissues and organs, regardless of the compound administered.

Table 3: Mean disposition and material balance of ^{14}C-Oxamyl after oral dosing to male rats

Fraction	Distribution of radioactivity as % administered dose of ^{14}C-test substances			
	Oxamyl (Rat A)	**Oxamyl (Rat B)**	**IN-A2213-glucoside**	**IN-N0079**
Pre-furnace gas trap	ND	0.24	0.10	0.28
Post-furnace gas trap	ND	0.06	0.00	0.00
Sub-total	ND	0.030	0.1	0.28
Total urine	61.2	48.4	63.5	64.3
Total faeces	6.42	23.00	5.26	4.84
Total eliminated	**67.6**	**71.7**	**68.9**	**69.4**
Body Fractions				
Hide (skin and hair)	6.98	12.55	4.16	4.88
Carcass	6.34	4.18	4.18	5.07
Gastro intestinal	4.76	1.32	1.67	1.83
Liver	1.58	0.020	1.43	2.69
Blood	1.55	2.13	1.57	1.65
Kidneys	0.19	0.27	0.25	0.41
Testes	0.13	0.09	0.11	0.08
Lungs	0.11	0.16	0.22	0.37
Heart	0.11	0.14	0.08	0.17
Muscle	0.11	NA	0.11	0.15
Spleen	0.05	0.06	0.06	0.12
Brain	0.03	0.36	0.02	0.05
Fat	0.03	0.08	0.01	0.02
Sub-total	22.0	21.5	13.9	17.5
Total Recovery	**89.6**	**93.2**	**82.8**	**86.9**

ND = not detected; the detection limits were not specified in the published paper

Urinary and Faecal metabolites from the ^{14}C-Oxamyl treated rats:

TLC analysis of the fresh urine from rats treated with ^{14}C-Oxamyl showed that no radioactivity moved from the origin. The polar nature of the ^{14}C-residues was confirmed by the absence of radioactivity in the ethyl acetate extract of urine. Gel permeation chromatography eluted compounds earlier than Oxamyl, indicating the presence of higher molecular weight compounds, such as conjugates of Oxamyl, IN-A2213, and IN-D2708. Anion exchange chromatography indicated that the ^{14}C -residues were acidic in nature, presumably glucuronides and/or sulphates. These conjugated metabolites were acid hydrolysed to identify the aglycones.

Approximately 79% and 72% of the original radioactivity in urine and faeces, respectively, were accounted for as conjugates of IN-A2213, IN-L2953, IN-D2708, and IN-KP532 (Metabolite IV). Conjugates of Oxamyl and IN-A2213 were cleaved by methanolic HCl back to Oxamyl and IN-A2213, which were identified by TLC and GC. These conjugates were not cleaved by the Lewis acid (BCl3/chloroethanol) catalyst. On the other hand, the Lewis acid cleaved conjugates of N,N'-dimethyloxamic acid (IN-D2708) and N-methyl oxamic acid (IN-KP532) to the corresponding β-chloroethyl esters which were identified by TLC. These conjugates do not appear to be attacked by β-glucuronidase-aryl sulphatase enzyme treatment just as the glucosides of compounds I and II formed in plants are not attacked by β- glucosidase. Acid hydrolysis of these conjugates did not yield quantitative results. The results are shown in Table 4.

Table 4: Oxamyl metabolites in urine and faeces after dosing with ^{14}C-Oxamyl

Conjugates of metabolites	% Total radioactivity	
	Urine	Faeces
IN-A2213 (I)	14.5	12.6
IN-L2953 (II)	23.9	22.2
IN-D2708 (III)	18.0	16.7
IN-KP532 (IV)	22.6	20.7
Total	**79.0**	**72.0**

Urinary and faecal metabolites from the ^{14}C-IN-A2213-glucoside dosed rat:

The radioactivity eliminated in urine and faeces from the rat dosed with ^{14}C-IN-A2213- glucoside is shown in Table 3. Unmetabolised IN-A2213-glucoside (45% of the urine radioactivity; 30% of the original dose) was identified by GC/MS. Other minor radioactive residues were isolated and hydrolysed separately using methanolic HCl and Lewis acid. IN- A2213, IN-L2953, IN-D2708, and IN-KP532 were characterised from the hydrolysates. Liberation of these compounds by acid hydrolysis suggested that IN-A2213, IN-L2953, IN-D2708, and IN-KP532 were in the form of conjugates, presumably glucuronides and/or sulphates.

Urinary and faecal metabolites from the ^{14}C -IN-N0079 dosed rat:

The radioactivity eliminated in urine and faeces from the rat dosed with ^{14}C-IN-N0079 is shown in Table 3. Urine from rats treated with ^{14}C -IN-N0079 was also found to be free of organosoluble ^{14}C-metabolites. A portion of the methanol extract on treatment with Lewis acid gave 15% of the total radioactivity as conjugates of IN-D2708 and 7% as conjugates of IN-KP532. Furthermore, when a portion of the methanol extract of the freeze dried urine was fractionated on a Dowex cation exchanger, 27% of the radioactivity was retained and was eluted with ammonium hydroxide similar to amino acids. This observation suggests that much of the remainder of the radioactivity had been incorporated into natural products, particularly amino acids.

Tissue Residues:

Ethyl acetate extraction of tissues containing the highest ^{14}C-residues from the ^{14}C-Oxamyl treated rats failed to remove any radioactivity (< 1%), indicating the absence of organosoluble metabolites in the tissues. Ethyl acetate extraction after acid hydrolysis again failed to remove any significant (< 3%) radioactivity, indicating the absence of conjugated metabolites.

About 51% and 43% of the original radioactivity in the hide (skin and hair) and blood, respectively, were found to be incorporated into amino acids (Table 5). The mass spectra of the standard n-butyl-trifluoroacetate-derivatised amino acids were in excellent agreement with those obtained from hydrolysis of rat protein. Following solvent extraction, pronase treatment of liver, carcass and blood from the ^{14}C-IN-A2213-glucoside treated rat liberated nearly all of the remaining radioactive residues. When the Pronase-solubilised radioactivity was characterised by gel filtration chromatography most of the radioactivity was recovered in the molecular weight fraction characteristic of amino acids. The amino acid fraction, isolated on a cation-exchange resin, accounted for about 64% of the Pronase- solubilised radioactivity in liver, 64% of that in the carcass, and 65% of that in the blood.

Table 5: Distribution of radioactivity in amino acids from skin and hair or blood from ^{14}C-Oxamyl-treated rats

Tissue	% the Total radioactivity in the sample
Skin and hair	
Alanine and Valine	5.1
Glycine	9.1
Leucine	5.4
Proline, Methionine	3.2
Phenylalanine, Aspartic acid	10.2
Glutamic acid, Lysine, Arginine	10.2

Tryptophan	8.0
Total	**51.2**
Blood	
Alanine, Valine, Glycine	7.3
Leucine	9.4
Proline, Methionine, Aspartic acid	5.1
Glutamic acid, Lysine	11.8
Arginine, Tryptophan	9.3
Total	**42.9**

Ethyl acetate extracts of liver, carcass, hide and blood from the ^{14}C-IN-N0079-dosed rat showed very low amounts of organosoluble radioactivity. When the insoluble residue in the liver was treated with Pronase all remaining radioactivity was solubilised. When this soluble ^{14}C -residue was analysed by cation-exchange, 45% of the injected radioactivity was retained and subsequently eluted with 1 N ammonium hydroxide. This is characteristic for amino acids. Similar results were obtained after Pronase treatment of carcass and hide/hair with recoveries of 89% and 60% for the ^{14}C residues, respectively. Of the solubilised radioactivity, about 40% and 23% were retained on a cation-exchange column from the carcass and hide, respectively, as amino acids.

Blood serum contained 46% and 36% of the high-molecular weight and amino acid fractions, respectively. When the high-molecular weight fraction was treated with Pronase and re-characterised on Bio-gel, 74% of the radioactivity was converted into the amino acid fraction. Although the remainder of the radioactivity in tissues has not been identified, the fact that it has not been found as known metabolites of Oxamyl together with the finding of extensive labeling of normal amino acids suggests that many other natural products in tissues have also become radiolabelled as a result of complete breakdown of the oxamyl molecule.

CONCLUSIONS:

The absorption, distribution, metabolism, and excretion by oral route study O/ME 33 J. Agric. Food Chem. 26:902-910 1978, originally submitted under EU Rev8 Point IIA 5.1.1 and conducted with test material [^{14}C]oxamyl, was conducted under an internal protocol; no guideline was given, no GLP was used. A review of this study indicates that although it was conducted prior to development of metabolism testing guideline, used methodologies are sound and the study partially meets current guidelines B.36 and OECD 417 being consistent with supplementary data requirements of OECD 417 (2010). However, relevant limitations are noted, starting from the poor characterization of the test item (no batch or putity reported. In addition:

1) In vitro study: only microsomes are tested, accounting for phase I metabolism and not further phase II biotransformation. A further control incubation containing inactivated microsomes is missing.

2) In vivo studies: the number of tested animals is definitely limited, being reduced to 1 animal per experimental condition (rat A and B were pre-treated with different doses of cold Oxamyl for different period of time, and received two different radiolabelled Oxanyl doses). Doses were quite high when compared to LD_{50} values (the higher dose is expected to give rise to some toxic effects, based on the acute toxicity of oxamyl, therefore value obtained at that dose are not fully reliable). Only repeated administration of Oxamyl was studied; only male rats were used.

Nevertheless some useful information can be obtained regarding Oxamyl metabolism: The primary metabolism of Oxamyl and its metabolites was consistent in both *in vitro* and *in vivo* experiments. Oxamyl was hydrolysed to IN-A2213 which was found as the only major degradation product in the control *in vitro* experiments, suggesting that this reaction was not enzymatic. The pattern of *in vivo* metabolism was confirmed by the fact that the same conjugates appeared in the urine regardless of which compound was administered to the rats.

No Oxamyl or other organo-soluble metabolites were detected in urine, faeces and tissues. More than 50% of the tissue radioactivity was incorporated into the natural pool of amino acids. IN-A2213-glucoside was somewhat resistant to degradation as approximately 30% of the dose (45% of urine radioactivity) was eliminated unchanged and the remainder was converted to conjugates. IN-N0079 was degraded and eliminated mainly as conjugates of IN-D2708 and IN-KP532.

RMS comments and conclusion for renewal
Despite the above limitations, the paper provides useful information and can be considered acceptable as a supporting study.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.1.1/02

Reference:	**Report:**	[redacted] (1990); Biokinetics and metabolism of ^{14}C-oxamyl in rats **DuPont Report No.:** AMR 1226-88 **Guidelines: U.S. EPA 85-1 (1982)** **GLP: YES**
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1. Test material: [1-^{14}C]oxamyl
   Lot/Batch #: E52467-29
   Purity: Radiochemical purity - >96%

Deviations: Based on OECD test guideline 417 the following deviations were identified in the study protocol:-

- ± 20% weight variation on test day 0 was not determined
- a single dose level was investigated instead of two doses as recommended by the guideline

Materials and methods:

The study has been conducted using [1-^{14}C] Oxamyl ((methyl 2-(dimethylamino)-N-[(methylamino)carbonyloxy]-2-oxoethanimidothioate) and [1-^{14}C] Oxime (methyl 2- (dimethylamino)-N-hydroxy-2-oxo-[1-^{14}C]ethanimidothioate). Due to the acute toxicity of Oxamyl, only one low dose (1 mg/kg bw) was used in these studies. In order to obtain sufficient amounts of metabolites for identification, additional rats were dosed with the ^{14}C-oxime (IN-A2213: the hydrolysis product and principal metabolite of oxamyl) at a higher dose level of 100 mg/kg bw. The oxime has been shown to be considerably less toxic with a lethal dose of about 1100 mg/kg bw.

Test substance: Oxamyl (DPX-D1410); Oxime (IN-A2213)
[1-^{14}C] Oxamyl: Pilot study – Batch E52467-24; purity 98 – 99%
Main study – Batch E52467-29; purity 96 – 98%
[1-^{14}C] Oxime: Batch E62727-8-A; purity 97 – 100%
Oxamyl: Batch D1410-222; purity 99.2%
Oxime: Batch A2213-10; purity 100%

Main study:

Five male and five female [redacted] CD rats were administered (dosing method unspecified) a single oral dose of ^{14}C -Oxamyl (specific activity 85 µCi/mg, 3.14 MBq/mg, >96% radiochemical purity) at 1.0 mg/kg bw. Individual urine samples were collected from each animal at 0-6 and 6-24 hours and 24-hour intervals thereafter for 168 hours. Faeces were collected at 24-hour intervals up to 168 hours after dosing. Expired air was not collected because in the pilot study, only 0.5% of the administered radioactivity was detected in the expired air. Animals were sacrificed at 168 hours post dosing and selected tissues/organs (heart, lungs, liver, kidneys, spleen, gastrointestinal tract, brain, ovaries/testes, total skin, samples of muscle, fat, and bone) were analysed for total retained radioactivity. Blood samples were taken at sacrifice. The carcass was also retained for analysis.

Qualitative Tissue Distribution:

In addition, one male and one female rat were administered ^{14}C -Oxamyl at 1 mg/kg bw dose level, sacrificed 168 hours after dosing and subjected to whole-body autoradiography.

Additional Study:

In order to provide sufficient quantities of metabolites for identification, single oral doses of ^{14}C-IN-A2213 (specific activity 116 µCi/mg, 4.29 MBq/mg, >97% radiochemical purity) were administered to 10 male rats at 100 mg/kg bw and to 2 male rats at 1 mg/kg bw. Urine was collected from paired animals at 0-8, 8-24 and 24-48 hours after dosing. Faeces were collected at 0-24 and 24-48 hours after dosing. Animals were sacrificed 48 hours after dosing.

Measurement of Radioactivity:

Aqueous samples and solvent extracts were mixed with scintillation cocktail and analysed by liquid scintillation counting (LSC). Tissue and faeces samples were mixed with dry cellulose powder and analysed by combustion and LSC analyses. Skin samples and carcasses were solubilised with 23 mL and 1000 mL of 2M sodium hydroxide:methanol: Triton X405 (6:3:1 v/v/v), respectively and then analysed by LSC.

Metabolite Analysis:

Metabolite characterisation/identification was done by co-chromatography of available reference standards (A2213, L2953, N0079, and D2708) using high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and gas chromatography/mass spectroscopy (GC/MS).

Urine Analysis:

Urine samples (0-24 and 24-48 hours) from ^{14}C -Oxamyl dosed male and female rats were separately pooled to provide representative samples. Samples were lyophilised, resuspended in methanol, centrifuged and the supernatant removed. The residues were extracted twice with methanol and the extracts were combined.

Enzymatic Hydrolysis:

Samples of the 0-24 hour pooled urine were mixed with 0.1 M sodium acetate (pH 5) and incubated overnight at 37°C with β-glucosidase/sulphatase. The incubated samples were analysed by TLC.

Metabolite Isolation:

Approximately 25% of the 0-24 hour urine of the male rats dosed with ^{14}C -IN-A2213 was pooled and lyophilised overnight. The residue was resuspended in methanol, sonicated and centrifuged 4 times. The supernatants were removed after each step, combined and concentrated under nitrogen at 37°C to approximately 600 µl in volume. An additional 2 mL of methanol was added followed by mixing and centrifugation. The supernatant was transferred to a centrifuge tube and an aliquot counted. The residue was resuspended in methanol, mixed and centrifuged. The methanol wash and a final methanol/water wash of the residues were combined with the pooled supernatant.

The concentrated urine extract was analysed by TLC using Kieselgel plates with butanol: 35% ammonia:95% ethanol (2:1:1) as the developing solvent. Plates were placed against Amersham β-film overnight and the film developed the next day. The metabolite bands were scraped from the plate and the silica gel containing each band was extracted 4 times with methanol. The extracts were concentrated to dryness under a stream of nitrogen at 37°C, reconstituted in methanol and subsequently analysed by HPLC and TLC.

Findings:
Pilot Study:
Excretion:

After oral administration of 1 mg/kg bw ^{14}C-Oxamyl, approximately 93% and 95% of the dose was excreted in male and female rat urine, respectively over 0-168 hours. Most of the radioactivity was excreted in the urine (84%) over 0-24 hours. The 0-168 hour faeces contained approximately 2% of the dose and ≤0.5% was found in the expired air traps. The carcass contained about 4% of the dose.

Main Study:
Excretion:

A total of 91% of the orally administered doses of 1 mg/kg bw of Oxamyl was excreted in the urine of males and females within 0-168 hours after dosing. Approximately 80% was eliminated within 0-24 hours (Table 6).

Faecal excretion was 3% and 2% for males and females, respectively, and approximately 1% of the dose was retained in the carcass (after removal of the tissues).

Table 6: Excretion (% of dose) of radioactivity by rats after single oral doses of 1 mg/kg bw ^{14}C-Oxamyl

Sample	Time (hours)	Mean Male (n = 5)	Mean Female (n = 5)
Urine	0 - 24	80.20	79.50
	24 - 168	10.33	11.54
Cagewash	0 - 168	0.74	0.79
Total urine and cagewash		91.27	91.83
Total Faeces		2.59	2.41
Carcass*	0 - 168	1.10	1.34
Total		94.94	95.58

*After removal of tissues/organs

Radioactivity concentrations at 168 hours after dosing were highest in whole blood (approximately 0.1 µg equivalents/g) and in the heart, liver, kidneys, lungs, spleen and gastro-intestinal tract (approximately 0.04 to 0.09 µg equivalents/g). Concentrations in all other tissues were approximately 0.01 to 0.03 µg equivalents/g. There were no significant differences in tissue concentrations between males and females (Table 7).

Whole body autoradiography showed a wide distribution of radioactivity in tissues with the highest concentrations in stomach mucosa and as a urinary contaminant on fur. Lower concentrations of radioactivity were present in the stomach contents, kidney, ureter, blood, hair follicles, liver, and the lungs. The distribution pattern of administered radioactivity was similar in males and females.

After single oral doses of ^{14}C -IN-A2213 at 1 and 100 mg/kg bw, the urinary excretion pattern was similar to that of ^{14}C -Oxamyl with approximately 102 and 96% of the dose eliminated within 48 hours after dosing, respectively. Approximately 1-2% of the dose was eliminated in the 0-48 hour faeces after either dose.

Table 7: Mean proportion (% of dose) of radioactivity in tissues of rats at 168 hours after single oral doses of 1 mg/kg bw ^{14}C-Oxamyl

Tissue	Mean Male (n = 5)	Mean Female (n = 5)
Bone	0.01	0.01
Brain	0.01	0.01
Fat*	0.04	0.05
G. I. TRACT	0.56	0.36
Heart	0.03	0.03
Kidneys	0.05	0.05
Liver	0.33	0.29
Lungs	0.02	0.03
Muscle*	0.84	1.18
Spleen	0.01	0.01
Testes/Ovaries	0.01	<0.01
Whole blood	0.55	0.70
Plasma*	0.08	0.11
Carcass	1.10	1.34
Skin	1.56	1.69

*Data calculated assuming the following proportions of bodyweight:- plasma (4%), whole blood (7%), fat (7%) and muscle (45%)

Urine Analysis:

TLC analysis was used to separate and quantify radioactive urine components. The major (0- 48 hour) urinary components after dosing with ^{14}C-Oxamyl was the glucuronide conjugate (metabolite A) of IN-A2213 (31-38% of the dose) and IN-A2213 (13-18% of the dose). Unmetabolised Oxamyl represented 7-11% of the dose within 24 hours. Three additional components each accounted for 7%, 10% and 2% of the dose. No radioactivity was associated with IN-L2953, IN-D2708, or IN-N0079. Metabolite A was partially hydrolysed (declining from 36 to 24% of the dose) after treatment with glucuronidase/sulphatase and the concentration of IN-A2213 was increased (18% to 34% of the dose). The concentration of two of the three unknown metabolites also decreased. Urine metabolite profiles were similar for both Oxamyl- and IN-A2213-dosed rats except for the absence of Oxamyl in the profile from the ^{14}C-IN-A2213-dosed animals. In these animals, the concentration of metabolite A decreased from 53 to 37% of the dose and the concentration of IN-A2213 increased from 18 to 51% of the dose when treated with β-glucuronidase/sulphatase.

Metabolite Identification:

Mass spectral analysis (fast ion bombardment) of urine from IN-A2213-dosed rats (100 mg/kg bw) confirmed the structure of metabolite A as the major urinary component in Oxamyl- and IN-A2213-dosed rats. IN-A2213 was also confirmed as a component present in the Oxamyl- dosed rats. A component consistent with IN-N0079 was found by mass spectral analysis. However, its chromatographic behaviour was not the same as IN-N0079. Minor unidentified metabolites were considered to be conjugates of IN-L2953 and IN-D2708 (Table 8). The major route of Oxamyl biotransformation was hydrolysis to IN-A2213 (oxime), followed by conjugation.

Table 8: Proportions (% of dose) of radioactive components in urine of rats after single oral doses of 1 mg/kg bw ^{14}C-Oxamyl

Components	Male		Female	
	0 –24 hours	24 – 48 hours	0 –24 hours	24 – 48 hours
IN-A2213 glucuronide (Metabolite A)	37.5	0.6	30.8	0.9
Unknown 1	7.1	1.0	6.9	0.8
Unknown 2	2.2	0.4	2.3	0.4
Unknown 3*	8.0	1.6	6.4	1.2
IN-A2213 (Metabolite E)	12.3	0.7	17.0	1.2
Oxamyl	7.1	0.1	11.1	0.3
Other	3.8	0.2	4.3	0.3
Origin	2.2	-	0.8	-

* Degrades in mass spectrometry to IN-N0079. Proportions measured by TLC

The absorption, distribution, metabolism, and excretion by oral route study AMR 1226-88, originally submitted under EU Rev8 Point IIA 5.1.1 and conducted with test material [1-^{14}C]oxamyl, was conducted under guideline U.S. EPA 85-1 (1982). A review of this study indicates that this is a GLP study that partially meets current OECD 417 guidelines. It was performed according to the original 1982 U.S. EPA testing guideline (85-1) as a tier 1 material balance and tissue distribution experiment using male and female rats (5/sex) treated with a single oral gavage dose of 1 mg/kg bw. This dose level was approximately 30–40% of the known LD_{50} in rats and was considered adequate given the acutely toxic nature of oxamyl (LD_{50} = 2.5–3.1 mg/kg bw).

RMS comments and conclusion for the renewal

The study is considered acceptable as a key study to define Oxamyl kinetics after oral treatment and metabolites identification.

B.6.1.2 Absorption, distribution, metabolism and excretion by other routes

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the article reviewed can be found in Appendix 1.

Study submitted to the EU for the first time in this submission.

B.6.1.2/01

Reference: CA 5.1.2/01	**Report:**	Mingoia, R.T. (2014a); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in human skin **DuPont Report No.:** DuPont-39017 **Guidelines:** OECD 428 (2004), OECD 28 (2004), OECD 156 (2011), EFSA Journal 2012;10(4):2665 **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-39017 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

An *in vitro* absorption study using human skin mounted in a static diffusion cell apparatus was conducted to determine the rate of penetration and distribution of [^{14}C]oxamyl. Data were generated for oxamyl at one concentration, 250 g oxamyl/L in deionized water.

The 250 g oxamyl/L aqueous formulation was applied to the 0.64 cm^2 exposed skin surface in the diffusion cell at a rate of 10 $\mu L/cm^2$. The amount of oxamyl applied per area of skin was approximately 2500 $\mu g/cm^2$. The applied formulation remained in contact with the skin surface for 6 hours. During this period, aliquots of the receptor fluid were removed and analysed for [^{14}C]oxamyl that had penetrated the skin sample. At the end of this exposure phase, all skin surfaces were washed. The skins were left in the diffusion cells for an additional 18 hours, and the monitoring of the receptor fluid continued. The skin samples were terminated at the end of the study, 24 hours post-dose. At termination, the application site skin was tape-stripped to remove the *stratum corneum*, and the total distribution of the applied dose was determined.

The mean in vitro penetration rates during the 6-hour exposure period (1–6 hours) and post exposure period (6–24 hours) were 1.74 and 0.222 μg equiv/cm^2/h, respectively. The absorbed dose at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.597 ±0.379 %) and stripped skin, (0.456 ± 0.282), was 1.7%, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-535
	Purity:	97.5%
	Description:	Solid, powder
	CAS #:	23135-22-0
	Stability of test compound:	The formulation prepared was stable under the conditions used in this study.
2.	Radiolabel test material:	[^{14}C]oxamyl technical
	Lot/Batch #:	1841000
	Radiochemical purity:	99.7%
	Specific activity:	75.6 μCi/mg
	Description:	Solid
	Stability of test compound:	The formulation prepared was stable under the conditions used in this study.
4.	Vehicle:	Test substance was dissolved in deionised water
5.	Human skin:	Samples of human cadaver skin (derived from the abdomen) from the National Disease Research Interchange (NDRI, Philadelphia, Pennsylvania, U.S.A.) were stored frozen at approximately -20°C until prepared for use.
6.	Test substance concentrations:	See Table 9

Table 9 Summary of the formulation, target concentration, and skin dose

Formulation	Target concentration	Target skin dose
Oxamyl aqueous formulation	250 g a.s./L	2500 μg a.s./cm^2

B. STUDY DESIGN AND METHODS

Study start and completion dates

04-June-2014 to 24-July-2014

Dermal penetration and absorption assay

The dermal penetration and absorption of oxamyl was measured *in vitro* through human skin. Frozen samples of human skin (n=8) were thawed, and full thickness skin was dermatomed to approximately 350 μm. Each skin membrane was mounted over the receptor chamber of a glass *in vitro* diffusion cell up to approximately 20 hours before dose application. The receptor chamber, containing deionised water as the receptor fluid, was maintained at 32°C. During the exposure phase, continuous magnetic stirring of the receptor chamber was maintained to facilitate diffusion of the test substance into the receptor fluid such that the rate of diffusion into the receptor fluid did not become a rate-limiting step.

The integrity of each membrane was assessed by measurement of electrical impedance prior to application of test substance. The test concentration was applied *via* the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

The test substance, oxamyl, was dissolved in deionized water and applied as a 250 g a.s./L aqueous formulation. Penetration and absorption were followed using [^{14}C]oxamyl, which was uniformly blended into the formulation prior to application.

The formulated product was applied at a rate of 10 μL/cm^2 to each skin sample. The amount of oxamyl applied per area of skin was approximately 2500 μg/cm^2 for the 250 g/L aqueous formulation. The applied formulation remained in contact with the skins for 6 hours (unoccluded). Serial receptor fluid samples (200 μL) were collected at 1, 2, 4, 6, 12, and 24 hours and put immediately into a glass vial. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume.

At 6 hours, the skin surfaces were washed using a 3x1mL of a 2% Ivory soap solution followed by 1x1mL rinse with deionised water and deied with a lint free wipe. The wash was collected into a glass vial. Exposed skin was maintained until 18 hours post-exposure and then terminated. At termination, the application skin site was removed and tape-stripped up to 10 times using Stripping Discs (CuDerm Corporation, Dallas, USA) ans a D-Squame pressure device to remove the *stratum corneum*. Each of the tape strips was placed into an individual vial and extracted with 1 mL acetonitrile. The remaining skin piece was placed into a separate vial for digestion with Solvable^{TM} .
Then the total distribution of the applied material determined by using liquid scintillation for total radioactivity measurment.

II. RESULTS AND DISCUSSION

A. OXAMYL, 250 G OXAMYL/L AQUEOUS FORMULATION (TABLE 10 TO TABLE 12)

The mean penetration of [^{14}C]oxamyl into the receptor fluid over the 24-hour study duration was 14.9 µg equiv/cm^2, representing 0.597% of the applied dose. The mean penetration rate during the 6-hour exposure period (1-6 h) and post exposure period (6-24 h) was 1.74 and 0.222 µg equiv/cm^2/h, respectively. Approximately 85% of the absorption of [^{14}C]oxamyl in the receptor fluid over 24 hours occurred within the first half of the study duration (12 hours).

The percent of applied dose detected in the receptor fluid was 0.597 ±0.379 %; the one detected in the stripped skin, was 0.456 ± 0.282 %. .

Tape-stripping of the skin to remove the *stratum corneum* accounted for 0.411 (± 0.342) % of the applied dose. The first two tape strips accounted for approximately 0.265 (±1.185) % of the applied dose.

The mean unabsorbed dose, defined as the percent of applied dose detected in the skin wash, tape strips, and donor chamber rinse was 101 (± 2) %.

The mean recovery of [^{14}C]oxamyl in the dermal penetration and absorption assay with human skin was 102 (± 2)%.

The absorbed dose, defined as the percent of applied dose detected in the receptor fluid and stripped skin, **was 1.7%**, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guideline for dermal absorption calculation.

Table 10 Penetration kinetics of [^{14}C]oxamyl from a 250 g oxamyl/L aqueous formulation, 0–24 hours (18-hour post-exposure)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Human	
	Mean	SD
1	1.88	0.69
2	5.37	2.35
4	9.08	5.23
6 (end exposure)	10.8	6.4
12	12.7	7.6
24 (18 hours post-exposure)	14.9	9.5
Penetration rate, 1–6 hours[a]	1.74	1.18
Penetration rate, 6–24 hours[b] (µg equiv./cm^2/h)	0.222	0.179

[a] Slope of mean data, 1–6 hours
[b] Slope of mean data, 6–24 hours

Table 11 Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 250 g oxamyl/L aqueous formulation (18-hour post-exposure)

	Data expressed as a percent of applied dose	
	Human	
	Mean	SD
Absorbed dose		
Receptor fluid	0.597	0.379
Skin (after stripping)	0.456	0.282
Total absorbed	1.05	0.57
Unabsorbed dose		
Skin wash	100	2
Donor chamber	0.00708	0.00755
Tape strips	0.411	0.342
Total unabsorbed	101	2
Total recovered	102	2

Table 12 Percent distribution in tape strips (*stratum corneum*) at 24 hours following a 6-hour topical exposure to a 250 g oxamyl/L aqueous formulation (18-hour post-exposure)

	Data expressed as a percent of applied dose	
	Human	
	Mean	SD
Tape strip 1	0.165	0.108
Tape strip 2	0.100	0.077
Tape strip 3	0.101	0.0742
Tape strip 4	0.0587	0.0341
Tape strip 5	0.0390	NA
Tape strip 6	0.0530	NA
Tape strip 7	0.0466	NA
Tape strip 8	0.0403	NA
Tape strip 9	0.0349	NA
Tape strip 10	0.0220	NA

III. CONCLUSIONS

The results obtained in this study, using an in vitro dermal static diffusion cell model, demonstrate the penetration rate and percent absorption of oxamyl from a 250 g/L aqueous formulation in human skin. The absorbed dose at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.597 ±0.379 %) and stripped skin, (0.456 ± 0.282), was 1.7%, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).

The mean penetration rates during the 6-hour exposure period (1-6 hours) and post exposure period (6-24 hours) were 1.74 and 0.222 µg equiv/cm^2/h, respectively, for the 250 g/L aqueous formulation in human skin.

RMS comments and conclusion for this renewal

The study is acceptable as a key study, although it is noted that only one concentration of Oxamyl in deionised water was tested.

Study submitted to the EU for the first time in this submission.

B.6.1.2/02

Reference: CA 5.1.2/02	**Report:**	Mingoia, R.T. (2014b); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in rabbit skin **DuPont Report No.:** DuPont-39524 **Guidelines:** OECD 428 (2004), OECD 28 (2004), OECD 156 (2011), EFSA Journal 2012;10(4):2665 **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-39524 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

An *in vitro* absorption study using rabbit skin mounted in a static diffusion cell apparatus was conducted to determine the rate of penetration and distribution of [^{14}C]oxamyl. Data were generated for oxamyl at one concentration, 250 g oxamyl/L in deionized water.

The 250 g oxamyl/L aqueous formulation was applied to the 0.64 cm^2 exposed skin surface in the diffusion cell at a rate of 10 µL/cm^2. The amount of oxamyl applied per area of skin was approximately 2500 µg/cm^2. The applied formulation remained in contact with the skin surface for 6 hours. During this period, aliquots of the receptor fluid were removed and analysed for [^{14}C]oxamyl that had penetrated the skin sample. At the end of this exposure phase, all skin surfaces were washed. The skins were left in the diffusion cells for an additional 18 hours and the monitoring of the receptor fluid continued. The skin samples were terminated at the end of the study, 24 hours post-dose. At termination, the application site skin was tape-stripped to remove the *stratum corneum*, and total distribution of the applied dose was determined.

The mean *in vitro* penetration rates during the linear part of the 6-hour exposure period (4-6 hours) and post exposure period (6-24 hours) were 0.786 and 0.501 µg equiv./cm^2/h, respectively. The absorbed dose at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.491 ±0.209 %) and stripped skin, (2.08 ± 0.39), was 2.7%, calculated by summing up the SD to the mean value (when >25% f the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-535
	Purity:	97.5%
	Description:	Solid, powder
	CAS #:	23135-22-0
	Stability of test compound:	The formulation prepared was stable under the conditions used in this study.
2.	Radiolabel test material:	[^{14}C]oxamyl technical
	Lot/Batch #:	1841000
	Radiochemical purity:	99.7%
	Specific activity:	75.6 μCi/mg
	Description:	Solid
	Stability of test compound:	The formulation prepared was stable under the conditions used in this study.
4.	Vehicle:	Test substance was dissolved in deionized water.
5.	Rabbit skin:	Rabbit skin was procured from Product Safety Labs from the dorsal area of male rabbits of approximately 5 months of age. Skins were stored frozen at approximately -20°C until prepared for use.
6.	Test substance concentrations:	See Table 13

Table 13 Summary of the formulation, target concentration, and skin dose

Formulation	Target concentration	Target skin dose
Oxamyl aqueous formulation	250 g a.s./L	2500 μg a.s./cm^2

B. STUDY DESIGN AND METHODS

Study start and completion dates

04-June-2014 to 24-July-2014

Dermal penetration and absorption assay

The dermal penetration and absorption of oxamyl was measured *in vitro* through rabbit skin. The rabbit was considered as the species of choice as this species was used for the dermal toxicity studies, for the evaluation of ixamyl effects through the dermal route of exposure.

Frozen samples of full thickness rabbit skin were thawed and were not processed further prior to use. Each skin membrane was mounted over the receptor chamber of a glass *in vitro* diffusion cell approximately 20 hours before dose application. The receptor chamber, containing deionised water as the receptor fluid, was maintained at 32°C. During the exposure phase, continuous magnetic stirring of the receptor chamber was maintained to facilitate diffusion of the test substance into the receptor fluid such that the rate of diffusion into the receptor fluid did not become a rate-limiting step. Serial receptor fluid samples (200 μL) were collected at 1, 2, 4, 6, 12, and 24 hours. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume. The integrity of each membrane was assessed by measurement of electrical impedance prior to application of test substance. The test concentration was applied *via* the donor chamber as a single application distributed evenly over the exposure area (0.64 cm^2).

The test substance, oxamyl, was dissolved in deionized water and applied as a 250 g a.s./L aqueous formulation. Penetration and absorption were followed using [^{14}C]oxamyl, which was uniformly blended into the formulation prior to application. The formulated product was applied at a rate of 10 μL/cm^2 to one group of eight skins. The amount of oxamyl applied per area of skin was approximately 2500 μg/cm^2 for the 250 g/L aqueous formulation. The applied formulation remained in contact with the skins for 6 hours (unoccluded). Serial receptor fluid samples (200 μL) were collected at 1, 2, 4, 6, 12, and 24 hours and put immediately into a glass

vial. The volume of receptor fluid was maintained by the replacement with a volume of fresh receptor fluid, equal to the total aliquot volume.
At 6 hours, the skin surfaces were washed using a 3x1mL of a 2% Ivory soap solution followed by 1x1mL rinse with deionised water and deied with a lint free wipe. The wash was collected into a glass vial. Exposed skin was maintained until 18 hours post-exposure and then terminated. At termination, the application skin site was removed and tape-stripped up to 10 times using Stripping Discs (CuDerm Corporation, Dallas, USA) ans a D-Squame pressure device to remove the *stratum corneum.* Each of the tape strips was placed into an individual vial and extracted with 1 mL acetonitrile. The remaining skin piece was placed into a separate vial for digestion with Solvable^{TM}.
Then the total distribution of the applied material determined by using liquid scintillation for total radioactivity measurement.

II. RESULTS AND DISCUSSION

A. OXAMYL, 250 G OXAMYL/L AQUEOUS FORMULATION (TABLE 14 TO TABLE 16)

The mean penetration of [^{14}C]oxamyl in the 250 g/L aqueous formulation into the receptor fluid over the 24-hour study duration was 12.3 µg equivalents/cm^2, representing 0.491% of the applied dose. The mean penetration rate during the linear part of the 6-hour exposure period (4-6 hours) and post exposure period (6-24 hours) was 0.786 and 0.501 µg equivalents/cm^2/hour, respectively.

The absorbed dose at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.491 ±0.209 %) and stripped skin, (2.08 ± 0.39), was 2.7%, calculated by summing up the SD to the mean value (when >25% f the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).
Approximately 60% of the absorption of [^{14}C]oxamyl in the receptor fluid over 24 hours occurred within the first half of the study duration (12 hours).

Tape-stripping of the skin to remove the *stratum corneum* accounted for 0.0744 (±0.0275) % of the applied dose

The mean unabsorbed dose, defined as the percent of applied dose detected in the skin wash, tape strips, and donor chamber rinse was 98.7 (±2.5) %.

The first two tape strips accounted for approximately 0.0096% of the applied dose.

Table 14 Penetration kinetics of [^{14}C]oxamyl from a 250 g oxamyl/L aqueous formulation, 0–24 hours (18-hour post-exposure)

Time (hour)	Data expressed in cumulative µg equiv./cm^2	
	Rabbit	
	Mean	SD
1	0.00	0.00
2	0.00	0.00
4	1.42	0.71
6 (end exposure)	2.99	1.55
12	7.31	3.26
24 (18 hours post-exposure)	12.3	5.2
Penetration rate, 4–6 hours[a]	0.786	0.428
Penetration rate, 6–24 hours[b] (µg equiv./cm^2/h)	0.501	0.203

[a] Slope of mean data, 4–6 hours
[b] Slope of mean data, 6–24 hours

Table 15 Recovery of total radioactivity at 24 hours following a 6-hour topical exposure to a 250 g oxamyl/L aqueous formulation (18-hour post-exposure)

	Data expressed as a percent of applied dose	
	Rabbit	
	Mean	SD
Absorbed dose		
Receptor fluid	0.491	0.209
Skin (after stripping)	2.08	0.39
Total absorbed	2.57	0.43
Unabsorbed dose		
Skin wash	98.6	2.5
Donor chamber rinse	0.00766	0.01331
Tape strips	0.0744	0.0275
Total unabsorbed	98.7	2.5
Total recovered	101	2

Table 16 Percent distribution in tape strips (*stratum corneum*) at 24 hours following a 6-hour topical exposure to a 250 g oxamyl/L aqueous formulation (18-hour post-exposure)

	Data expressed as a percent of applied dose	
	Rabbit	
	Mean	SD
Tape strip 1	0.0047	0.0044
Tape strip 2	0.0049	0.0063
Tape strip 3	0.0082	0.0031
Tape strip 4	0.0044	0.0041
Tape strip 5	0.0106	0.0058
Tape strip 6	0.0077	0.0068
Tape strip 7	0.0069	0.0025
Tape strip 8	0.0129	0.0093
Tape strip 9	0.0064	0.0048
Tape strip 10	0.0079	0.0035

III. CONCLUSIONS

The results obtained in this study, using an *in vitro* dermal static diffusion cell model, demonstrate the penetration rate and percent absorption of oxamyl from a 250 g/L aqueous formulation in rabbit skin. The absorbed dose at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.491 ±0.209 %) and stripped skin, (2.08 ± 0.39), was 2.7%, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).. The penetration rates measured over the linear part of the 6-hour exposure period (4–6 hours) and the post-exposure period (6–24 hours) were 0.786 and 0.501 μg equiv./cm^2/h, respectively, for the 250 g/L aqueous formulation in rabbit skin.

RMS comments and conclusion for this renewal

The study is acceptable as a key study, although it is noted that only one concentration of Oxamyl in deionised water was tested.

B.6.1.3 Summary of metabolism studies

Two rat metabolism studies are available to address the absorption, distribution, excretion, and metabolism of ^{14}C -oxamyl in rats. The most recent study (summarised in B.6.1.1/02 and accepted as a key study), evaluated oxamyl metabolism after administration of a single oral dose (1 mg/kg bw) in five male and five female rats. The single oral dose was approximately 30–40% of the LD_{50} in male and female rats (3.1 and 2.5 mg/kg bw,

respectively, see below B6.2.1.). Oxamyl is acutely toxic by the oral route, and the studied dose is considered the highest oral dose that can be administered without causing significant toxicity or death. The majority (>90%) of the dose was excreted in the urine of both genders within 168 hours of dosing; approximately 80% was eliminated within 0-24 hours indicating a rapid and nearly quantitative oral absorption of the test substance. Faecal excretion was 3% and 2% for males and females, respectively; approximately 1% of the dose was retained in the carcass (after removal of the tissues) expired air was a very minor elimination route at ≤0.5%, and tissue residues were low (0.03 to 0.09 mg/kg). There were no significant differences in tissue concentrations between males and females. The highest concentration of radioactivity was found in whole blood (approximately 0.1 □g equivalents/g) and in heart, liver, kidney, lungs, spleen, and the gastro-intestinal tract (approximately 0.04 to 0.09 µg equivalents/g). Bile cannulated rats were not used in the study, but the low % of faecal excretion indicates that the information is not crucial to determine the oral absortion value. Therefore the oral absorption value is >90% (summing up the radioactivity found in the urine, tissues and carcass).

Although a single dose was tested, it is reasonable that a lower dose would be similarly absorbed and eliminated. The metabolism of rats dosed with ^{14}C-IN-A2213 (a less toxic hydrolysis product and principal oxamyl metabolite) was also studied The excretion patterns after single oral doses of 14C-IN-A2213 at 1 mg/kg bw and 100 mg/kg bw were similar to that recorded after Oxamyl administration at 1 mg/kg bw. In addition sufficient metabolites for identification were obtained, thus establishing the metabolic pathway for oxamyl in rats (Fig. 1). The major urinary metabolite in Oxamyl and IN-A2213-dosed rats was the beta-glucuronide of the oxime (IN-A2213). Minor unidentified metabolites were considered to be conjugates of demethylated compounds (e.g., IN-L2953 or IN-D2708). The major route of Oxamyl biotransformation was hydrolysis to IN-A2213 followed by conjugation

The other available study (summarised in B.6.1.1/01**),** is dated and was conducted prior to the adoption of any metabolism test guidelines was adopted. It has some limitations, therefore was accepted as supporting study. It evaluated the metabolism of oxamyl in two male rats (one for each treatment regimen) after oral administration (2.5–4.4 mg/kg bw) of ^{14}C-oxamyl following preconditioning with repeated doses of oxamyl (50 and 150 mg/kg diet corresponding approximately to 2.5–7.5 mg/kg bw daily. The higher dose is expected to give rise to some toxic effects, based on the acute toxicity of oxamyl, therefore value obtained at that dose are not fully reliable. The patterns of absorption, distribution, excretion, and metabolism were consistent with the single dose study. The majority (around 70%) of the dose was eliminated within 72 hours of dosing (most in the urine), and low levels of radioactivity were distributed throughout the body. ^{14}C-IN-N0079 and ^{14}C-IN-A2213 glucoside were also studied in *in vivo* (oral administration to preconditioned rats) experiments. In addition, the metabolism of ^{14}C-oxamyl, ^{14}C-IN-N0079, and ^{14}C-IN-A2213 glucoside (main oxamyl plant metabolites) were examined in *in vitro* (rat liver microsomes) experiments. The results of the *in vitro* experiments were consistent with the whole animal studies and further substantiated the metabolic pathway in rats.

The combination of these studies adequately addresses the regulatory requirements for oxamyl metabolism in rats. Additional studies conducted with higher and/or lower single oral doses of oxamyl would provide no additional information on the absorption, distribution, excretion, and metabolism of ^{14}C -oxamyl in rats.

Further, according to regulation (EU) No 283/2013 comparative *in vitro* metabolism studies are required. For oxamyl, metabolic differences among rats and mice have been assessed: (O/ME 33 for rats and Chang and Knowles, 1979 [Arch. Environm. Contam. Toxicol. 8: 499–508]) for mice. In the Chang and Knowles study (1979), *in vitro* and *in vivo* assessments were reported. In the *in vivo* portion of the study, twenty-nine mice were injected intraperitoneally with a saline solution of [^{14}C]-oxamyl corresponding to a dose of 1.16 mg/kg bw. Mice were sacrificed 96 hours post dosing. Approximately 89% of the dose was eliminated in the urine in 96 hours. Unconjugated urine metabolites included IN-A2213, IN-D2708, IN-N0079, IN-L2953, IN-KP532, and IN-D1409. Minor amounts of conjugated (glucuronide or sulphate) IN-A2213, IN-L2953, IN-D2708, and IN-KP532 were also present. In an *in vitro* experiment, incubation of oxamyl with mouse liver subcellular fractions gave IN-N0079, IN-D2708, IN-KP532, IN-A2213, and IN-D1409. Therefore no major differences were reported.

Furthermore, the metabolic pathway of oxamyl in rats and mice is consistent with the pathways seen in plants and livestock. Oxamyl is hydrolysed rapidly to IN-A2213, an oxime, then a glucuronide is formed either early or late in the pathway. There are no significant differences in the rate of the initial conversion of oxamyl to the oxime metabolite, which is the key event in the detoxification process. Moreover, based on a scientifically sound and ethically conducted volunteer study (HLO−1998-01505, summarised in Point CA 5.7.1), it can be concluded that the metabolism of oxamyl by humans is also rapid.

The most sensitive endpoint in all species is acute neurotoxicity observed due to inhibition of acetylcholinesterase. This effect is rapidly reversible and oxamyl is rapidly hydrolysed. It is well known that species differences are not an issue with OP metabolism. However, in order to address the possible quantitative difference for human metabolism compared to animal species and to account for pathways catalysed in

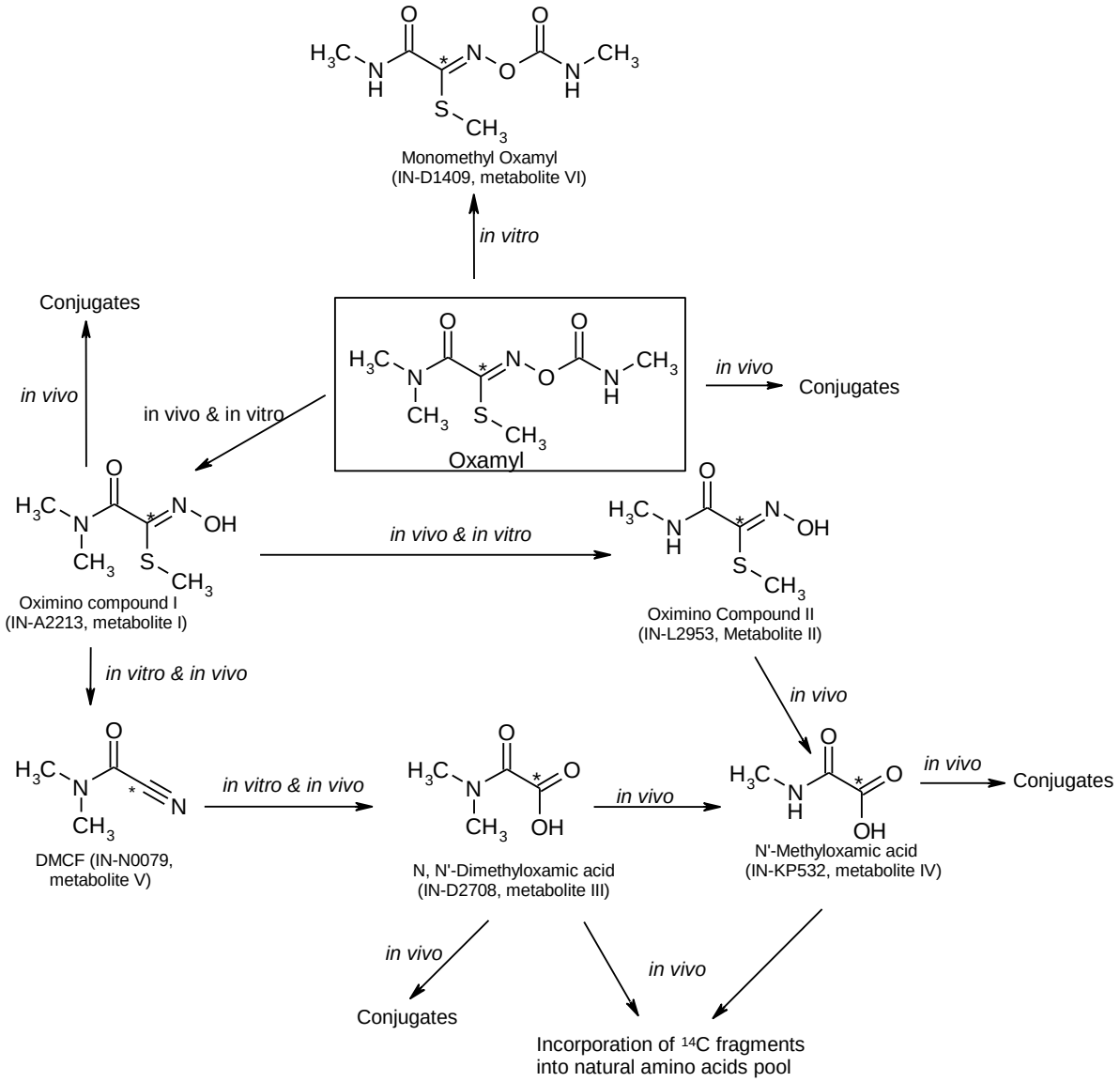
subcellular fractions other than microsomes, a comparative in vitro metabolism study using human and rats cells should be performed.

RMS comments and conclusion for this renewal

Although it is recognized that species differences are not a major issue as far as the OP metabolism is concerned, a comparative in vitro metabolism study using human and at least rats cells should be performed, in order to address also metabolism other than the one catalysed by microsomal enzymes.

A data gap is identified regarding in vitro comparative metabolism

Figure 1 Proposed metabolic pathway of oxamyl and its metabolites *in vitro* and *in vivo* in rats



The dermal penetration of oxamyl (250 g oxamyl/L aqueous formulation) has been measured *in vitro* through human and rabbit skin mounted in a static diffusion cell model, in two recent GLP studies carried out according to OECD TG n°428. Dermal absorption of pure oxamyl (PAI) at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.597 ±0.379 %) and stripped skin, (0.456 ± 0.282), was 1.7%, and 2.7% in human and rabbit skin, respectively, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).

Table 17 Summary of kinetics studies in rats

Oral Absoprtion	>90% after single doses of [^{14}C]oxamyl (1 mg/kg bw) to rats (based on urine excretion and residues in tissues and carcass)
Elimination	After single doses of [^{14}C]oxamyl (1 mg/kg bw) to rats, the majority of the dose was eliminated in the urine. Faeces and expired air were minor elimination routes. No major differences between genders. No major differences after repeated exposure of 2.5 mg/kg bw (supporting study).
Clearance	The highest concentration of radioactivity was found in whole blood (approximately 0.1 µg equivalent/g) and the following tissues (approximately 0.04 to 0.09 µg equivalent/g): heart, liver, kidney, lungs, spleen, and the gastro-intestinal tract. Concentrations in all other tissues were approximately 0.01 to 0.03 µg equivalent/g. There were no significant differences in tissue concentrations between males and females.
Metabolite profile	The major urinary metabolite in oxamyl and IN-A2213 dosed rats was the IN-A2213 glucuronide. Metabolism was characterized by hydrolysis of parent oxamyl to IN−A2213 or enzymatic conversion *via* the N,N-dimethyl- carbonocyanidic amide (IN−N0079) to N,N'-dimethyloxamic acid (IN-D2708) and N-methyloxamic acid (IN−KP523). Minor unidentified metabolites were considered to be conjugates of demethylated compounds (*e.g.*, IN-L2953) or IN-D2708. The major route of oxamyl biotransformation was hydrolysis to IN-A2213, then conjugation. No major species differences observed.
Dermal absorption	1.7% of pure oxamyl (250 g oxamyl/L aqueous formulation) at 18 hours post-exposure in vitro in human skin 2.7% of pure oxamyl (250 g oxamyl/L aqueous formulation) at 18 hours post-exposure in vitro in rabbit skin

B.6.2 Acute toxicity

B.6.2.1 Oral

In the DAR used in the evaluation for Annex 1 inclusion**,** a non GLP study was evaluated ([redacted] 1980). The studyprovided was only a summary report and did not include raw data or details of animal housing and feeding conditions. However, the study at that time was considered acceptable. The acute oral median lethal dose of Oxamyl was estimated to be 3.1 and 2.5 mg/kg bw for male and female rats, respectively, indicating that Oxamyl classifies as highly toxic by the oral route.

A new study was provided for the renewal and is summurised in the following. Results of the new study are consistent with the previous ones.

Study submitted to the EU for the first time in this submission.

B.6.2.1/01

Reference: CA 5.2.1/01	**Report:**	[redacted] (2009); Oxamyl (DPX-D1410) technical (98% w/w): Acute oral toxicity study in rats - up-and-down procedure **DuPont Report No.:** DuPont-26931 **Guidelines:** OPPTS 870.1100 (2002), OECD 425 (2006**)** **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** DuPont-26931 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

A single oral dose of pure oxamyl (PAI; 98% w/w), suspended in deionized water, was administered by oral gavage to two fasted female rats at a dose of 1 mg/kg, to three fasted female rats at a dose of 2.5 mg/kg, and to two fasted female rats at a dose of 6.3 mg/kg body weight. The animals were dosed one at a time at a minimum of 48-hour intervals. Animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing. All animals were examined to detect grossly observable evidence of organ or tissue damage. A software package (AOT425StatPgm) was used to determine the dose progression and to estimate the LD_{50}.

Oral LD_{50} Females = 2.5 mg/kg bw

Death occurred in one of the three rats dosed at 2.5 mg/kg and in both rats dosed at 6.3 mg/kg (Table 18). Clinical signs of toxicity were observed in all rats and included ataxia, clear oral discharge, red nasal discharge, high or low posture, tremors, clear ocular discharge, mydriasis, salivation, stained skin/fur, wet fur, hyperreactivity, absent faeces, or prostrate posture. With the exception of stained skin/fur, no clinical signs were observed after test Day 5. No body weight losses occurred in surviving rats. No gross lesions were present in the rats at necropsy.

Under the conditions of this study, the oral LD_{50} for oxamyl was 2.5 mg/kg bw for female rats.

Under conditions of this study, oxamyl is classified in Category 1 according to the provisions set forth in Regulation EC 1272/2008.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:	Pure oxamyl (PAI)
Lot/Batch #:	D1410-196
Purity:	98%
Description:	White solid
CAS #:	23135-22-0
Stability of test compound:	Not determined.
2. Vehicle and/or positive control:	Deionized water
3. Test animals	
Species:	Rat
Strain:	Crl:CD(SD)
Age at dosing:	Approximately 9–11 weeks old (females)
Weight at dosing:	219.0–272.3 g
Source:	[redacted]
Acclimation period:	At least 6 days
Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*, except when fasted
Water:	Tap water, *ad libitum*
Housing:	Animals were housed singly in stainless steel, wire-mesh cages. Enrichment (*e.g.*, nestlet or nylabone) was placed in each cage.
4. Environmental conditions	
Temperature:	18–26°C
Humidity:	30–70%
Air changes:	Not recorded
Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

   20-November-2008 to 23-December-2008

2. Animal assignment and treatment

   A single oral dose of oxamyl, suspended in deionized water, was administered by oral gavage to two fasted female rats at a dose of 1 mg/kg, to three fasted female rats at a dose of 2.5 mg/kg, and to two fasted female rats at a dose of 6.3 mg/kg. The animals were dosed one at a time at a minimum of 48-hour intervals. The animals were observed for clinical signs just before dosing, once during the first 30 minutes after dosing, two more times on the day of dosing, and once each day thereafter. Animals were weighed on test Days –1, 0, 1, 7, and 14. All animals were euthanised and necropsied to detect grossly observable evidence of organ or tissue damage.

3. Statistics

   A software package (AOT425StatPgm) was used to determine the dose progression and to estimate the LD_{50}.

II. RESULTS AND DISCUSSION

A. MORTALITY

Death occurred on the day of dosing in 1 of the 3 rats dosed at 2.5 mg/kg and in both rats dosed at 6.3 mg/kg. The dose progression and mortality are detailed in Table 18 and Table 19 below.

Table 18 Acute oral toxicity of oxamyl: Dose progression and mortality

Test sequence	Animal ID	Dose (mg/kg bw)	Response within 48 hrs	Response after 14 days
1	2319	1.0	O	O
2	2324	2.5	O	O
3	2475	6.3	X	X
4	2479	2.5	X	X
5	2488	1.0	O	O
6	2537	2.5	O	O
7	2538	6.3	X	X

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.
Stopping criteria met: Likelihood ratio criterion.

Table 19 Acute oral toxicity of oxamyl : Summary of results (after 14 days observation)

Dose (mg/kg)	O	X	Total
1	2	0	2
2.5	2	1	3
6.3	0	2	2
All doses	4	3	7

(X = Died, O = Survived)

Under the conditions of this study, the estimated oral LD_{50} for oxamyl was 2.5 mg/kg for female rats.

B. CLINICAL OBSERVATIONS

Clinical signs of toxicity were observed in all rats and included ataxia, clear oral discharge, red nasal discharge, high or low posture, tremors, clear ocular discharge, mydriasis, salivation, stained skin/fur, wet fur, hyperreactivity, absent faeces, or prostrate posture. With the exception of stained skin/fur, no clinical signs were observed after test day 5.

C. BODY WEIGHT

There were no body weight effects noted in surviving rats.

D. NECROPSY AND GROSS PATHOLOGY

No gross lesions were present in the rats at necropsy.

III. CONCLUSION

Under the conditions of this study, the oral LD_{50} for oxamyl was 2.5 mg/kg bw for female rats.

Under conditions of this study, oxamyl is classified in Category 1 according to the provisions set forth in Regulation EC 1272/2008 and labelled as Acute Tox 2 H300)

RMS comments and conclusion for the renewal

The study is acceptable as a key study

B.6.2.2 Dermal

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.2/01

Reference: --	**Report:**	[redacted] (1988); Acute dermal toxicity study of IN D1410-196 in rabbits **DuPont Report No.:** HLR 114-88 **Guidelines:** U.S. EPA 81-2 (1984) **GLP: YES**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Deviations: Based on OECD test guideline 402 the following deviations were identified in the study protocol:-

- The ± 20% weight variation on test day 0 was not determined.

Materials and Methods:

A single dose of Oxamyl (batch IN D1410-196; purity 97.1%), moistened to a paste with a small amount of distilled water, was applied to the clipped intact skin of 5 male and 5 female New Zealand White rabbits at doses of 2000, 3500 and 5000 mg/kg bw. The test material was spread evenly over 190 cm^2 of exposed skin on the dorso-lumbar area of each animal and held in place under an occlusive dressing for 24 hours. The rabbits were fitted with plastic collars.

Observations for clinical signs of toxicity were made 24 hours after dosing and daily thereafter for 14 days (excluding weekends). Bodyweights were recorded on days 1, 7 and 14 following treatment. Only rabbits that died during the study were subjected to a macroscopic examination post mortem.

Findings:

Deaths occurred in males at the mid and high dose levels and in females at the low and mid dose levels (Table 20). All deaths occurred within 2 days after dosing.

Table 20: Mortality rates in male and female rabbits following dermal administration of Oxamyl

Dose (mg/kg bw)	Mortality Rate	
	Male	**Female**
2000	0/5	1/5
3500	2/5	1/5
5000	2/5	0/5

Erythema and oedema ranging from absent to mild were observed in the rabbits 24 hrs following treatment. These symptoms had resolved by day 5 in all rabbits except for one animal which exhibited slight erythema with epidermal scaling through to day 8. The epidermal scaling resolved in this rabbit by day 12. No dermal irritation was observed in any of the rabbits at study termination.

No clinical signs of toxicity were observed in any of the treated rabbits. Body weight losses of up to 6% of initial body weight were observed at 24 hours in most rabbits but all animals recovered from this initial loss of body weight by day 7.

Gross pathological observations noted in animals that died during the study were consistent with cholinesterase inhibition including a clear, wet discharge of the oral cavity, nasal cavity and/or skin and mild autolysis.

Conclusions:

The dermal study HLR 114-88, originally submitted under EU Rev8 Point IIA 5.2.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 81-2 (1984). A review of this study indicates that it meets the current EEC Method B.3 with the following exceptions: ±20% weight variation on test Day 0 was not determined, but this is considered not to be a major deviation. The dermal absorption in vitro study with rabbit skin indicates a dermal absorption of 2.7%, higher than the one measured with human skin.

The acute dermal median lethal dose of Oxamyl was calculated to be 5027 mg/kg bw in male rabbits. The LD_{50} for females could not be calculated from the data due to the negative slope of the dose response curve, but was considered to be greater than 5000 mg/kg bw. Therefore, **Oxamyl does not classify in terms of acute dermal toxicity.**

RMS comments and conclusion for the renewal
The study is considered acceptable as a key study.

B.6.2.3 Inhalation

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.3/01

Reference: --	**Report:**	[redacted] (2001); Oxamyl (DPX-D1410) Technical (98% w/w): inhalation median lethal concentration (LC_{50}) study in rats **DuPont Report No.:** DuPont-6331 **Guidelines:** 59 NohSan No. 4200 (1985), EEC Method B.2. (1992), OECD 403 (1981), OPPTS 870.1300 (1996) **GLP:YES**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196A
   Purity: 98.09%

Deviations: Based on OECD test guideline 403 the following deviations were identified in the study protocol:-

- The mean weight variation was not calculated prior to exposure.

Materials and Methods:

Oxamyl (batch DPX-D1410-196A; purity 98.09%) was supplied as a white solid which was milled by the sponsor to a fine powder (median particle size 3 – 10 µm).

Groups of 5 male and 5 female Crl:CD®(SD)IGS BR rats were exposed nose-only to mean chamber concentrations of 0.050, 0.054, 0.065 and 0.120 mg/L of Oxamyl dust for a single 4- hour exposure period. Test atmospheres were generated by suspension of Oxamyl particulates in air. The atmospheric concentration of the test substance, the particle size distribution of each test atmosphere, the chamber airflow and the chamber temperature were determined at approximately 30-minute intervals during each exposure. The relative humidity and chamber oxygen concentrations were recorded 3 or 4 times during each exposure period.

Following exposure, surviving animals were observed for 14 days. Clinical observations were made daily. Bodyweights were also recorded throughout the post-exposure period. At the end of the recovery period, all rats were sacrificed and subjected to a gross pathological examination.

Findings:

The achieved test atmosphere characteristics are summarised in Table 21.

Table 21: Chamber atmosphere analysis

Chamber Atmospheric Oxamyl Concentration (mg/L)		MMAD (µm)	GSD	% Particles by Mass		
Mean	**SD**			**< 1 µm**	**< 3 µm**	**< 10 µm**
0.050	0.019	3.5*	1.5*	0.3*	35*	99*
0.054	0.018	3.5 3.2	1.6 1.9	0.3 4.5	35 45	99 96
0.065	0.023	3.8 3.6	1.7 1.7	0.4 1.0	30 40	98 97
0.120	0.018	4.2 3.8	1.6 1.7	0.1 0.5	26 35	97 98

SD: Standard Deviation; MMAD: Mass Median Aerodynamic Diameter - 2 Separate Measurements; GSD: Geometric Standard Deviation - 2 Separate Measurements
*only 1 successful particle size sample was obtained due to clogged impactor jets.

Deaths occurred at all four exposure concentrations. At 0.050 mg/L, 2 of 5 females died. At 0.120 mg/L all rats died (Table 22). All deaths occurred either during exposure or within 2 days of exposure termination.

Table 22: Mortality rates in male and female rats following atmospheric exposure to Oxamyl

Chamber Atmospheric Oxamyl Concentration (mg/L)		Mortality Ratio	
Mean	**SD**	**Male**	**Female**
0.050	0.019	0/5	2/5
0.054	0.018	1/5	5/5
0.065	0.023	3/5	4/5
0.120	0.018	5/5	5/5

During the exposure, red nasal discharge, gasping and salivation were observed and there was a diminished response to an alerting stimulus. Immediately after exposure and during the recovery period, notable clinical signs of toxicity included lethargy, decreased muscle tone, tremors, spasms, fasciculations, abnormal posture, abnormal gait, abnormal hindlimb gait, high carriage and ataxia. Rats showed severe weight loss (> 20 g) in the days after exposure but appeared to return to a normal rate of weight gain within 7 days of exposure. All clinical signs of toxicity returned to normal in the second week after exposure.

Macroscopic observations post mortem were non-specific and not indicative of target organ toxicity.

Conclusions:

The inhalation study DuPont-6331, originally submitted under EU Rev8 Point IIA 5.2.3 and conducted with test material pure oxamyl (PAI), was conducted under guideline 59 NohSan No. 4200 (1985), EEC Method B.2. (1992), OECD 403 (1981), OPPTS 870.1300 (1996). A review of this study indicates that it meets the current EEC Method B.2 with the following exceptions: ± 20% weight variation on test Day 0 was not determined, but this is considered not to be a major deviation.

The median lethal concentration of Oxamyl in male and female rats was 0.056 mg/L/4hr indicating that the substance classifies as **highly toxic by inhalation (Acute tox 2 H330)**

RMS comments and conclusion for the renewal

The study is considered acceptable as a key study

B.6.2.4 Skin irritation

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.4/01

Reference: --	**Report**	[redacted] (2001); Oxamyl (DPX-D1410) technical (98% w/w): primary dermal irritation study in rabbits **DuPont Report No.:** DuPont-7060 **Guidelines:** EEC Method B.4. (1992), OECD 404 (1987), 59 NohSan No. 4200 (1985), U.S. EPA 870.2500 (1996) **GLP: YES**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196A
   Purity: 98.09%

Materials and Methods:

A group of 6 male HM:(NZW)fBR New Zealand White albino rabbits received a single 4- hour application of 0.5 g of Oxamyl (DPX-D1410; batch DPX-D1410-196A; purity 98.09%), moistened with 0.5 mL of deionised water, to a closely-shaven test site of approximately 6 cm^2 on the back of each animal. The test substance was held in place with a semi-occlusive dressing.

Approximately 1 hour after removal of the test patches, the test sites were evaluated for erythema, oedema and other evidence of dermal effects and were scored according to the Draize Scale. Additional evaluations were made 24, 48 and 72 hours after removal of the patches (Table 23). Adjacent areas of untreated skin were used for comparison.

The rabbits were examined for clinical signs of toxicity at each dermal evaluation. Body weights were recorded on the day of treatment and at the last dermal evaluation.

Findings:

Erythema (score of 1) was observed in one rabbit at 1 and 24 hours after test substance removal but all signs of dermal irritation had disappeared by 48 hours (Table 23). The remaining 5 rabbits exhibited no dermal irritation during the study. No clinical signs of toxicity were observed and no body weight loss occurred. The overall mean values for erythema and oedema formation were 0.06 and 0, respectively.

Table 23: Individual and mean skin irritation scores (Draize scale) in the rabbit following dermal application of Oxamyl

Rabbit Number	Hours After Test Substance Removal							
	Erythema				Oedema			
	1	24	48	72	1	24	48	72
35080	0	0	0	0	0	0	0	0
35082	0	0	0	0	0	0	0	0
35089	0	0	0	0	0	0	0	0
35079	1	1	0	0	0	0	0	0
35081	0	0	0	0	0	0	0	0
35083	0	0	0	0	0	0	0	0
Mean		0.17	0	0		0	0	0
Overall Mean: (24hr + 48hr + 72hr)/3		**0.06**				**0**		

Conclusions:

The skin irritation study DuPont-7060, originally submitted under EU Rev8 Point IIA 5.2.4 and conducted with test material pure oxamyl (PAI), was conducted under guideline EEC Method B.4. (1992), OECD 404 (1987), 59 NohSan No. 4200 (1985), U.S. EPA 870.2500 (1996). A review of this study indicates that it fully meets the current EEC Method B.4.

Based on the degree of skin reaction observed at 24, 48 and 72 hours following a single 4- hour application to rabbit skin, **Oxamyl does not classify as a skin irritant.**

RMS comments and conclusion for the renewal
The study is considered acceptable as a key study

B.6.2.5 Eye irritation

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.5/01

Reference: --	**Report:**	[redacted] (2001); Oxamyl (DPX-D1410) technical (98% w/w): primary eye irritation study in rabbits **DuPont Report No.:** DuPont-7059 **Guidelines:** 59 NohSan No. 4200 (1985), EEC Method B.5. (1992), OECD 405 (1987), U.S. EPA 870.2400 (1996) **GLP:YES**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196B
   Purity: 98.21%

Materials and Methods:

Approximately 24 mg of Oxamyl (DPX-D1410; batch DPX-D1410-196B; purity 98.21%) were applied to the lower conjunctival sac of the right eye of 6 male New Zealand White rabbits and the animals were observed for 30 – 60 seconds. The dose of 24 mg was selected based on mortality in a previous eye irritation study which used 42% Oxamyl (HLR 710-87). The left eye was used as the untreated control. The eyes were not rinsed after the introduction of the test substance.

All animals were examined for evidence of eye irritation and for clinical signs of toxicity at approximately 1, 24, 48 and 72 hours after administration of the test substance. At each of these observation periods, eyes were examined using illumination and magnification and scored for ocular reactions according to the Draize scale. Fluorescein stain examinations were conducted at the 24-, 48- and 72-hour evaluations. Rabbits were weighed on the day of treatment and at the 72-hour evaluation.

Findings:

All rabbits pawed the treated eye following instillation. The test substance produced pupillary constriction on the day of instillation in the treated eyes of all rabbits. In all cases the pupils did not react to light. The rabbits exhibited shivering, salivation, effects on balance, rapid or irregular respiration or lung noise on the day of treatment. No clinical signs of toxicity were evident on the day after treatment. No body weight loss occurred.

The test substance did not appear to induce corneal opacity but did produce iritis (score of 1) in 5 rabbits and conjunctival redness (score of 1 or 2) in 4 rabbits (Table 24). Conjunctival chemosis was not observed in any of the test animals but a discharge was produced in the treated eyes of 5 rabbits (score of 1, 2, or 3). All signs of irritation disappeared within 48 hours. Fluorescein stain examinations were negative for corneal injury in the

treated eye. The overall average scores for corneal opacity, iritis, conjunctival redness and conjunctival chemosis were 0, 0, 0.06 and 0, respectively.

Table 234: Individual and mean eye irritation scores (Draize scale) in the rabbit following dermal application of Oxamyl

Rabbit No.	Hours After Test Substance Removal															
	Corneal Opacity				Iritis				Conjunctival Redness				Conjunctival Chemosis			
	1	24	48	72	1	24	48	72	1	24	48	72	1	24	48	72
35088	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
35090	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
35092	0	0	0	0	1	0	0	0	2	1	0	0	0	0	0	0
35087	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
35084	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
35091	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
Mean		0	0	0		0	0	0		0.17	0	0		0	0	0
Overall Mean: (24HR+48HR+72HR)/3		0				0				0.06				0		

Conclusions:

The eye irritation study DuPont-7059, originally submitted under EU Rev8 Point IIA 5.2.5 and conducted with test material pure oxamyl (PAI), was conducted under guideline 59 NohSan No. 4200 (1985), EEC Method B.5. (1992), OECD 405 (1987), U.S. EPA 870.2400 (1996). A review of this study indicates that it meets the current EEC Method B.5 with the following exception; due to toxicity, a dose of 24 mg was used. However reconduct is unlikely to yield a significantly different result as the potential for eye irritation should be evaluated in the absence of systemic toxicity, and this study adequately demonstrates that oxamyl is not an eye irritant.

It is therefore concluded that under the conditions of this study, Oxamyl is not irritating to the rabbit eye and therefore **does not classify as an eye-irritant**

RMS comments and conclusion for renewal

The study is considered acceptable as a key study

B.6.2.6 Skin sensitisation

A study to evaluate the skin sensitisation potential of purified oxamyl (96.9%) was initiated by both the Magnusson-Kligman Maximisation and Buehler methods. The M-K study was discontinued due to animal welfare concerns. When oxamyl was administered according to the recommend test procedures, all animals died following intradermal injection. Following topical treatment, animals either died or showed significant clinical signs of cholinesterase inhibition. Only when applied at one-half the maximum dose rate (0.5 mL of a 50% dilution of the test material in water) according to the Buehler method did the animals survive, although clinical signs of cholinesterase inhibition were observed. The results confirmed that the recommended guideline doses for the dermal sensitisation study are similar to the known dermal LD_{50} for oxamyl and that the intradermal dose is ~20-fold greater than the oral LD_{50}, thereby prohibiting the conduct of a study with the purified material.

Therefore, a study that was performed according to the Buehler method using a 42% solution of oxamyl (oxamyl technical 42) (HLR 179-88) is suggested as the alternative to fill this data point. In this study, all animals survived, and no clinical signs of toxicity were observed with treatment of the undiluted test material (0.4 mL). Therefore, treatment with undiluted 42% oxamyl appears to be near the threshold dose at which neurotoxicity is observed with 50% purified oxamyl, and is nearly the maximum concentration of oxamyl that can be evaluated for skin sensitisation. Therefore, it is proposed that this study adequately fulfils the data requirement.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.6/01

Reference: --	**Report:**	[redacted] (1999); Oxamyl Technical: Evaluation of the potential dermal sensitization in the guinea pig (Magnusson-Kligman maximization and Buehler tests) **DuPont Report No.:** DuPont-3021 **Guidelines:** 59 NohSan No. 4200 (1985), U.S. EPA 81-6 (1984), OECD 406 (1992), EEC Method B.6. (1992) **GLP: YES**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: Not given
   Purity: 96.9%

Materials and Methods:

A dose range-finder study to evaluate the skin sensitisation potential of purified Oxamyl technical (batch unspecified; purity 96.9%) in guinea pigs was conducted using the Magnusson-Kligman Maximisation method. This was subsequently changed to the Buehler method. Both studies were discontinued due to animal welfare concerns.

Maximisation Dose Range-Finder Study:

The dose range-finder Maximisation study consisted of an intradermal, topical induction and topical challenge phase, each with separate groups of 3 animals. In the intradermal phase, each of 3 animals received two 0.1 mL injections of 5% test substance (approximately 10 mg each). In the topical induction phase, each of 3 animals received 0.5 g of the test substance moistened with 0.5 mL normal saline on one test site and 0.5 mL of a 50% dilution of this solution on a second test site. The sites were occluded for 48 hours. The test substance was then removed from the skin by wiping with gauze soaked in normal saline. In the topical challenge phase, each animal received 0.5 g of the test substance moistened with 0.5 mL normal saline on one test site and 0.5 mL of a 50% dilution of this solution on a second test site. The sites were then occluded for 24 hours.

Buehler Dose Range-Finder Study:

In the dose range-finder Buehler study, one group of 4 guinea pigs each received 0.5 g of the test substance moistened with 0.5 mL normal saline and another group of 4 animals each received 0.5 mL of a 50% dilution of this solution. The sites were occluded for 6 hours after which the test substance was removed by wiping the area with gauze soaked in normal saline.

Findings:
Maximisation Dose Range Finder Study

In the intradermal range-finding phase, all 3 animals exhibited convulsions and vomiting within minutes and died within 18-24 minutes. In the topical induction phase, 1 of 3 animals died approximately 3 hours after removal of the test substance. No toxicity was reported in the remaining 2 animals. In the topical challenge phase, 1 of 3 animals showed signs of cholinesterase inhibition following test substance removal and was euthanised 4.5 hours later. No toxicity was reported for the remaining 2 animals (Table 24).

Table 245: Oxamyl: summary of the MK Maximisation dose range-finder study

Phase	Dose	Mortality [a]	Animal weights (g) [b]	Time of death [c]
Intradermal	2 x 0.1 mL injection of 5% solution (2 x 10 mg Oxamyl)	3/3	319-341	18-24 minutes
Topical induction	1 x 0.5 g/0.5 mL and 1 x 50% dilution of this	1/3	317	3 hours
Topical challenge	1 x 0.5 g/0.5 mL and 1 x 50% dilution of this	1/3	328	4.5 hours[d]

[a] number of animals which died/number of animals in dose group
[b] body weight of animals that died
[c] time after application that death occurred
[d] animal sacrificed *in extremis*

Buehler Dose Range Finder Study

One of the 4 animals dosed with 0.5 g of the test substance / 0.5 mL of saline solution was found dead the following morning. One of the 4 animals dosed with 0.5 mL of the 50% test substance dilution showed signs of cholinesterase inhibition approximately 3.5 hours after the initial application of the test substance. These clinical signs had cleared by the following morning and no toxicity was reported for the remaining animals in this dose group. All animals were reported to have severe tape burns away from the test article site (Table 25).

Table 256: Oxamyl: summary of Buehler dose range-finder

Dose	Clinical signs	Mortality [a]	Animal weights (g) [b]	Time of death [c]
0.5 g/0.5 mL	1/4	1/4	361	24 hours
50% dilution of above preparation	1/4	0/4	343	-

[a] number of animals which died/number of animals in dose group
[b] body weight of animals that died
[c] time after application that death occurred

The skin sensitisation study DuPont-3021, originally submitted under EU Rev8 Point IIA 5.2.6 and conducted with test material pure oxamyl (PAI), was conducted under guideline 59 NohSan No. 4200 (1985), U.S. EPA 81-6 (1984), OECD 406 (1992), EEC Method B.6. (1992). A review of this study indicates that it was discontinued due to toxicity observed including clinical signs of carbamate toxicity. However, together with the results of the study below (HLR 179-88), an adequate assessment of the skin sensitisation potential of the relevant technical that is transported and formulated has been completed.

RMS comments and conclusion for the renewal

The study carried out with both **M&K maximization test and the Buehler test were correctly discontinued due to the high toxicity of the a.s.**. No data on skin sensitization can be derived, but the RMS considered it acceptable as supporting study, since it provides the justification for the dose tested in the study described below, which is lower than the one prescribed by the test guideline.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.2.6/02

Reference: --	**Report:**	[redacted] (1988); Closed-patch repeated insult dermal sensitization (Buehler method) with IN D1410-304 in guinea pigs **DuPont Report No.:** HLR 179-88

		Guidelines: U.S. EPA 81-6 (1984) GLP: YES

1. Test material: Oxamyl technical 42
   Lot/Batch #: D1410-304
   Purity: 42%

Materials and Methods:

To fulfil the requirement for a skin sensitisation study, DuPont provided a study which was performed according to the Buehler method using the formulation Vydate® Concentrate 42 which contained 42% Oxamyl technical (batch IN D1410-304; purity 42%). The remaining 58% ingredients comprising the technical test substance, were not specified in the study but were simply described as "inert". In the summaries and assessment documents provided by the notifier (Tier II-Document M-II) the test substance is described as 42% active substance in cyclohexanone and water, but the actual percentage concentrations of cyclohexanone/water are not specified.

Induction phase:

Neat test material was administered to the shaved backs of 10 male and 10 female Dunkin Hartley guinea pigs using a 25 mm Hill Top Chamber at a rate 0.4 mL test substance/animal for 6-hours, once a week for 3 consecutive weeks. Vehicle controls (5 males and 5 females) were administered 0.4 mL of distilled water. Positive controls (3 males and 2 females) were administered 0.4 mL of DNCB (0.2% in 80% aqueous ethanol). In all cases, the treated area was covered with plastic and wrapped with an adhesive bandage. Following each 6-hour exposure, bandages and patches were removed and the test sites were gently washed with warm water to remove excess test material. Irritation responses were scored 24 and 48 hours after treatment.

Challenge phase:

Two weeks after the last induction treatment, 0.4 mL of neat test material was administered (using the same procedures as in the induction phase) to an unexposed test site on the 20 test animals for a 6-hour exposure period. The 10 vehicle control animals received 0.4 mL of distilled water and the 3 male and 2 female positive control animals received 0.4 mL of a 0.1% suspension of DNCB in 80% aqueous ethanol. In addition, two further groups of ten animals (5 males and 5 females) were treated with either 0.4 mL of the neat test material or with 0.1% suspension of DNCB in 80% aqueous ethanol, both groups serving as negative controls.

Application sites (test and controls) were depilated 22 hours after dosing. Irritation was scored 2 hours after depilation (24 hours post dosing) and 48 hours post dosing.

Findings:

Induction phase:

No dermal irritation was observed in the vehicle control or test animals. Slight to severe erythema with necrosis, superficial necrosis or oedema was observed in the positive control animals.

Challenge phase:

All animals survived and the formulation produced no dermal irritation in the test animals or in the negative controls treated with the test substance. No dermal irritation was observed in the vehicle controls either. DNCB produced slight erythema in 2 negative controls by 48 hrs after treatment. Mild to severe erythema with necrosis, blanching or oedema was observed in the positive control animals (Table 26).

Table 267: Oxamyl: summary of dermal sensitisation findings (Buehler)

Response	IND1410-304[a]		Negative Control IND1410-304[b]		DNCB[b]		Vehicle Control		Positive Control	
	24 hr	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**
No reaction	20/20	20/20	10/10	10/10	9/10	8/10	10/10	10/10	0/5	0/5
Mild erythema	0/20	0/20	0/10	0/10	1/10	2/10	0/10	0/10	0/5	3/5
Moderate erythema	0/20	0/20	0/10	0/10	0/10	0/10	0/10	0/10	3/5	1/5
Severe erythema	0/20	0/20	0/10	0/10	0/10	0/10	0/10	0/10	2/5	1/5

[a] Induction and challenge
[b] Challenge only

Conclusions:

The skin sensitisation study HLR 179-88, originally submitted under EU Rev8 Point IIA 5.2.6 and conducted with test material oxamyl technical 42, was conducted under guideline U.S. EPA 81-6 (1984). A review of this study indicates that it meets the current EEC Method B.6, although the concentration of the active substance was reduced due to severe toxic effects (see results of the previous study). This study is considered valid.

RMS comments and conclusion for the renewal.

Together with the results of the previous supporting study, this is considered acceptable as a key study.

B.6.2.7 Phototoxicity

Study submitted to the EU for the first time in this submission.

B.6.2.7/01

Reference: CA 5.2.7/01	**Report**	Markell, L.K. (2015); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* 3T3 NRU phototoxicity test **DuPont Report No.:** DuPont-42100 **Guidelines:** OECD 432, 2004 **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-42100 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Oxamyl was assessed for phototoxicity in the presence of UVA light. Balb/3T3 cells, cultured in 96-well plates, were incubated with oxamyl at concentrations of 1000, 316.5, 100, 31.6, 10, 3.16, 1.0, and 0.316 μg/mL for the main experiments (three biological replicates) each one in technical replicates of six. The test substance was soluble at 1000 μg/mL, the maximum concentration specified by the test guideline. The stock solution vehicle was water, at a final concentration of 1.0% in these assays. A positive control plate was included with each independent experiment evaluating the test chemical, in order to demonstrate that the assay responded as expected. Eight concentrations of the positive control, chlorpromazine (CPZ), were tested, in the presence and absence of UVA irradiation. After approximately 22 hours in culture, cells were analysed for cytotoxicity by neutral red uptake (NRU).

Under the conditions of this study, no phototoxic effect was observed with oxamyl following UVA irradiation, as determined by the Mean Photo Effect (MPE). However, Photo-Irritation-Factor (PIF) values in two of the three biological replicates (Runs 2 and 3) were between 2 and 5 (indicating 'probable phototoxicity'): compounds in this range generally do not warrant further photosafety evaluations. Considering the overall results it can be concluded that no inherent toxicity or phototoxicity to Balb/3T3 cells was observed at any concentration of oxamyl in this assay.

Oxamyl was not phototoxic following UVA irradiation when tested at concentrations up to 1000 μg/mL.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-532
   Purity: 99.1%
   Description: Solid powder
   CAS #: 23135-22-0
   Stability of test compound: The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed
   Solvent used: Water
2. Control materials
   Negative (solvent) control/final concentration: Water, 1%
   Positive control: Chlorpromazine (CPZ), in 1% DMSO in HBSS to final concentrations of 0.032, 0.1, 0.32, 1, 3.2, 10, 31.6, and 100 μg/mL

3. Test system

A permanent mouse fibroblast cell line, Balb/3T3, clone A31, was obtained from American Type Culture Collection (ATCC®) (Manassas, Virginia, U.S.A., catalogue number CCL-163). The cells were stored frozen in liquid nitrogen. Balb/3T3 cells were started from frozen batches and were cultured for at least one passage prior to their use. Cells were checked regularly for the absence of mycoplasma contamination.
The cells were grown in media containing DMEM media base with 10% newborn bovine serum. Cells were incubated at approximately 37°C in an atmosphere of approximately 5% CO_2. Passage #69 was used for Run 1, and passage #70 was used for Run 2, and passage #67 was used for Run 3.

4. Dosimetry

The intensity of light (irradiance) was regularly checked before each phototoxicity test using a Honle Basic UV Meter and UVA sensor with spectrum detection (330–400 nm); maximum intensity 5 W/cm². The intensity was measured through the same type of 96-well plate lid as was used in the assay. The UV-meter was calibrated to zero by exposure to no UVA irradiation (covering sensor).
A dose of 10 J/cm^2 (as measured in the UVA range) was determined to be non-cytotoxic to Balb/3T3 cells and sufficiently potent to excite chemicals to elicit phototoxic reactions. The exposure time was calculated in the following way:

$$t\ (\text{min}) = \frac{\text{irradiation dose (J/cm}^2) \times 1000}{\text{irradiance (mW/cm}^2) \times 60}\ (1\text{J} = 1\ \text{Wsec})$$

The UVA irradiance during Run 1 was 3.96 mW/cm^2, and cells were irradiated for 42 minutes and 6 seconds. The UVA irradiance during Run 2 was 3.83 mW/cm^2, and cells were irradiated for 43 minutes and 31 seconds. The UVA irradiance during Run 3 was 4.17 mW/cm^2, and cells were irradiated for 39 minutes and 58 seconds. The light source was approximately 76 cm from the test plates during Run 1 and Run 2, and was 63.5 cm from the test plate during Run 3.
The UVA sensitivity of the cells was checked approximately every fifth passage for sensitivity to the light source by assessing their viability following exposure to increasing doses of irradiation. Several doses of irradiation were used in this assessment. Cells were seeded at the density used in the *in vitro*

3T3 NRU phototoxicity test and irradiated the next day. Cell viability was then determined 1 day later using Neutral Red uptake. It was demonstrated that the UVA dose of 10 J/cm ^{2} was sufficient to correctly classify the positive control chemical, while producing less than 20% cytotoxicity compared to the non-irradiated solvent control.

5. Culture preparation

Cell culture maintenance and stock dosing media contained DMEM media base with 10% calf bovine serum.
Cells from frozen stock cultures were seeded in culture medium at an appropriate density and subcultured at least once before they were used in the *in vitro* 3T3 NRU phototoxicity test.
Cells used for the phototoxicity test were seeded in culture medium at 1×10^4 cells per well, so that cultures did not reach over-confluence by the end of the test, *i.e.*, when cell viability was determined, approximately 48 hours after cell seeding.
For the test substance or positive control, cells were seeded identically in two separate 96-well plates, which were then taken concurrently through the entire test procedure under identical culture conditions except for the time period where one of the plates was irradiated (+Irr), and the other one was kept in the dark (-Irr).

6. Test compound preparation

Test substances were prepared fresh immediately prior to use.
The test substance was dissolved in water, which was present at a constant volume in all cultures: testing doses as well as the solvent controls contained 1% water in HBSS, non-cytotoxic at that concentration. Test substance concentrations were selected to avoid precipitation or cloudy solutions. The test substance was soluble in water with no precipitate observed when solubilized in 1% water in HBSS up to 1000 µg/mL. Vortex mixing was used to aid solubilisation.
The test substance also had an appropriate pH at 1000 µg/mL, which was selected as the upper limit concentration for this assay: the pH of the cell cultures after adding test chemical was in the range 6.5–7.8., meaning that toxicity was not induced by improper culture conditions (e.g highly acidic or alkaline pH).
The ranges of concentrations of a test substance in the presence (+Irr) and in the absence (-Irr) of light were adequately determined in dose range-finding experiments. The test substance was tested at the following concentrations: 1000, 316.5, 100, 31.6, 10, 3.16, 10, and 0.316 µg/mL solubilised in 1% water in HBSS during definitive experiments (Run 1, Run 2, and Run 3).

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

04-December-2014 to 20-March-2015

2. Day 1

A 100-µL volume of culture medium (DMEM + 10% Newborn Calf Serum) was dispensed into the peripheral wells of a 96-well tissue culture microtiter plate (= blanks). In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL in culture medium (= 1×10^4 cells/well) was dispensed. Two plates were prepared for each individual test substance concentration and for the positive control. Plates were labelled with the Work Request and Service Code for each assay, and a well map was included in the study records to identify the contents of each well.
The cells were incubated for 24 ± 2 hours at approximately 37°C and approximately 5% CO_2 in a humidified incubator. This incubation period allowed for cell recovery, adherence, and exponential growth.

3. Day 2

From the two plates prepared for each series of test substance concentrations and the controls, one was selected for the determination of cytotoxicity (-Irr, the control plate), and one (the UVA treatment plate) for the determination of photocytotoxicity (+Irr).
Prior to dosing, a mastermix of each concentration of test substance or positive control stock solution was prepared by adding 40 µL of the stock solution to 3.960 mL of HBSS with Ca^{2+}/Mg^{2+}. Also, a mastermix containing 40 µL of the solvent and 3.960 mL of HBSS was prepared. This solution was used to dose the solvent control wells. The final solvent concentration in all dosing solutions was 1.0%.

After a 24 ± 2-hour pre-incubation of plated cells, the plates were removed from the incubator and checked for attachment and morphology prior to dosing. The cell media was removed, dosing buffer (100 µL/well of the appropriate mastermix) was added, and the plates were returned to the incubator (37°C, 5% CO_2) and incubated for 1 hour.
To perform the +Irr exposure, the cells were irradiated at room temperature for approximately 30 to 50 minutes through the lid of the 96-well plate. The non-irradiated plates (-Irr) were kept at room temperature in a dark box for an incubation equivalent to the UVA light exposure time.
Test solution was decanted and carefully washed once with 150 µL of HBSS with Ca^{2+}/Mg^{2+}. The buffer was replaced with culture medium and incubated overnight (18–22 hours) at approximately 37°C and approximately 5% CO_2 in a humidified incubator.
A 40-µg/mL preparation of Neutral Red (NR) (3-amino-7-dimethylamino-2-methylphenazine hydrochloride, CAS number 553-24-2) in DMEM, 5% Newborn Calf Serum was made and incubated at approximately 37°C overnight for use on Day 3.

4. Day 3

Microscopic evaluation

Cells were examined for growth, morphology, and integrity of the monolayer using a phase contrast microscope. Changes in cell morphology and effects on cell growth were recorded.

Neutral Red Uptake Test

Media was removed, and the cells were washed with 150 µL of HBSS with Ca^{2+}/Mg^{2+}. The washing solution was removed. A 100-µL volume of 40 µg/mL NR in DMEM, 5% Newborn Calf Serum was added to each well and incubated at approximately 37°C and approximately 5% CO_2 in a humidified incubator for 3 hours ± 30 minutes.
After incubation, the NR medium was removed, and the cells washed with 150 µL of HBSS with Ca^{2+}/Mg^{2+}. HBSS was decanted and the excess removed by blotting.
A 150-µL volume of NR desorb solution (freshly prepared 49 parts water, 50 parts ethanol, and 1 part acetic acid) was added.
The microtiter plate was shaken gently on a microtiter plate shaker for approximately 10-15 minutes until NR had been extracted from the cells and had formed a homogeneous solution.
The optical density of the NR extract was measured at 540 nm in a spectrophotometer, using blanks as a reference. The data were saved in an appropriate electronic file format for subsequent analysis.

5. Data analysis

Absorbance values for both vehicle controls (-Irr and +Irr) were determined, and an average was calculated for each. Percent viability values were determined by dividing the absorbance of the treatment group by the mean absorbance of the vehicle control group and multiplying by 100. The average % control was determined for each concentration, along with standard deviation. These averages were graphed using a bar graph with the standard deviation represented by the error bars.
To enable evaluation of the data, a Photo-Irritation-Factor (PIF) or Mean Photo Effect (MPE) was calculated. Phototoxicity prediction software Phototox Version 2.0, ZEBET was used to determine both the PIF and MPE. The calculations performed by the software are described below.
For the calculation of photocytotoxicity metrics, the set of discrete dose-response values was approximated by an appropriate continuous dose-response curve (model). Fitting of the curve to the data was commonly performed by a non-linear regression method. To assess the influence of data variability on the fitted curve, a bootstrap procedure is recommended.

A PIF was calculated using the following formula:

$$PIF = \frac{IC_{50}\ (-Irr)}{IC_{50}(+Irr)}$$

For the MPE, the following description and calculation was applied.
The MPE is based on comparison of the complete concentration response curves. It is defined as the weighted average across a representative set of photo effect values.

$$MPE = \frac{\sum_{i=1}^{n} w_i \, PE_{c_i}}{\sum_{i=1}^{n} w_i}$$

The photo effect (PEc) at any concentration (C) is defined as the product of the response effect (REc) and the dose effect (DEc), *i.e.*, PEc = REc × DEc. The response effect (REc) is the difference between the responses observed in the absence and presence of light, *i.e.*, REc = Rc(-Irr) - Rc(+Irr). The dose-effect is given by:

$$DEc = \frac{C/C^* - 1}{C/C^* + 1}$$

where C* represents the equivalence concentration, *i.e.*, the concentration at which the +Irr response equals the -Irr response at concentration C. If C* could not be determined because the response values of the +Irr curve are systematically higher or lower than Rc(-Irr), the dose effect is set to 1. The weighting factors, wi, are given by the highest response value, *i.e.*, wi = MAX {Ri(+Irr), Ri(-Irr)}. The concentration grid Ci is chosen such that the same number of points falls into each of the concentration intervals defined by the concentration values used in the experiment. The calculation of MPE is restricted to the maximum concentration value at which at least one of the two curves still exhibited a response value of at least 10%. If this maximum concentration is higher than the highest concentration used in the +Irr experiment, the residual part of the +Irr curve is set to the response value "0." Depending on whether the MPE value is larger than a properly chosen cut-off value (MPE = 0.15) or not, the test substance is classified as phototoxic.

6. Evaluation criteria

The test data should allow a meaningful analysis of the concentration-response obtained in the presence and in the absence of irradiation, and, if possible, the concentration of test chemical by which cell viability was reduced to 50% (IC_{50}). If cytotoxicity was found, both the concentration range and the intercept of individual concentrations were set in a way to allow the fit of a curve to the experimental data.
For both clearly positive and clearly negative results, the primary experiment, supported by one or more preliminary dose range-finding experiment(s) was sufficient.
Equivocal, borderline, or unclear results should be clarified by further testing. In such cases, modification of experimental conditions should be considered. Experimental conditions that might be modified include the concentration range or spacing, the pre-incubation time, and the irradiation-exposure time. A shorter exposure time may be appropriate for water-unstable chemicals.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Oxamyl was present at acceptable concentrations in the dosing solutions (within 92.4, 91.6, and 92.1% of nominal concentrations 0.316, 10, and 1000 µg/mL, respectively, for samples formulated on 04-December-2014). Oxamyl was shown to be stable in the dosing solutions under the conditions of the study. Oxamyl was not found in the 0 mg/mL samples. The positive and solvent controls fulfilled the requirements for a valid test.

B. ASSAY PERFORMANCE

MPE and PIF values, and IC_{50} values of CPZ in the presence and absence of UVA irradiation (Table 27), were within the acceptable ranges as specified by the test guideline. In addition, these values were similar to the historical range for the positive control.

For the runs performed with the positive control CPZ and the test substance, oxamyl, the irradiated vehicle control showed a viability of more than 80%, when compared with the non-irradiated vehicle control for both experimental runs (Table 28).

Absorbance of the vehicle control was assessed to demonstrate that the cells seeded in well had grown with a normal doubling time during the 2 days of the assay. A test met the acceptance criteria if the mean OD_{540}

NRU of the untreated controls was ≥0.4. The runs for the positive control and oxamyl were slightly below the acceptance criteria; however, these values were within the historical range of assay performance for both irradiated and non-irradiated plates.

C. BALB/3T3 SENSITIVITY TO UVA IRRADIATION

The Balb/3T3 cells were treated with increasing intensities of UVA irradiation, and viability was compared to non-irradiated cells. 10 J/cm^2, the dose used for both CPZ and oxamyl treatment, showed a viability of more than 80% meeting the guideline quality criteria.

D. PHOTOTOXICITY

No phototoxic effect was observed with the test compound following UVA irradiation. Under the conditions of this study, the MPE for test compound was determined to be 0.014, 0.012, and 0.020 for Runs 1, 2, and 3, respectively. The PIF value for Run 1 was determined to be 1.850, also indicating that the test substance was not phototoxic. PIF values in Runs 2 and 3 were 2.3 and 4.2; however compounds in this range generally do not warrant further photosafety evaluations. (Table 27).

As expected, the positive control, CPZ, showed a substantial increase in toxicity following UVA irradiation compared to un-irradiated chlorpromazine, with PIF values in the range 28.4-46.4 and MPE between 0.35 and 0.47. (Table 27). The positive control was within acceptable ranges based upon OECD guidelines and provided confidence in the test system.

Controls: The positive and solvent controls fulfilled the requirements for a valid test.

Table 278 Summary of PIF and MPE results

	Run	**PIF[a]**	**MPE[b]**	**IC_{50} -Irr[c] (μg/mL)**	**IC_{50} +Irr[d] (μg/mL)**
CPZ[e]	1	28.444	0.351	30.168	1.062
	2	35.334	0.479	34.382	0.973
	3	46.389	0.451	29.752	0.645
Oxamyl	1	1.850	0.014	177.128	95.829
	2	4.222	0.012	345.743	82.253
	3	2.363	0.020	145.736	62.413

[a] Photo-irritation-factor
[b] Mean photo effect
[c] Non-irradiated cells
[d] Irradiated cells
[e] Chlorpromazine

Table 28 Cell viability after exposure to 10 J/cm^2

	Run	Percent control[a]	SEM
Oxamyl -Irr[b]	1	100.0%	0.015
Oxamyl +Irr[c]	1	105.5%	0.013
Oxamyl -Irr	2	100.0%	0.008
Oxamyl +Irr	2	94.0%	0.008
Oxamyl -Irr	3	100.0%	0.008
Oxamyl +Irr	3	102.3%	0.009
CPZ[d] -Irr	1	100.0%	0.012
CPZ +Irr	1	99.5%	0.017
CPZ -Irr	2	100.0%	0.012
CPZ +Irr	2	103.7%	0.013
CPZ –Irr	3	100.0%	0.012
CPZ +Irr	3	97.8%	0.024

[a] Percent viability was calculated by comparing the +Irr solvent control average absorbance (OD_{540} NRU) to the –Irr solvent control average absorbance (OD_{540} NRU).
[b] Non-irradiated cells
[c] Irradiated cells
[d] Chlorpromazine

III. CONCLUSION

Under the conditions of the study, the MPE for test compound was determined to be 0.014, 0.012, and 0.020 for Runs 1, 2, and 3, respectively, and the PIF of 1.850 was determined for Run 1. The PIF values for Runs 2 and 3 were 2.3 and 4.2; however, compounds in this range do not warrant further photosafety evaluations. This result suggests that following UVA irradiation, oxamyl was not phototoxic when tested at concentrations up to 1000 μg/mL.

RMS comments and conclusion for the renewal

The study is considered acceptable as a key study.

B.6.2.8 Summary of acute toxicity

Table 30 Summary of acute toxicity studies with oxamyl

Type of study	Purity	Species	Result	Reference[a]
Acute oral LD_{50}	98.1%	Rat	LD_{50} 2.5 mg/kg bw (female)	DuPont-26931
Acute dermal LD_{50}	97.1%	Rabbit	LD_{50} >5000 mg/kg bw (male and female)	HLR 114-88
Acute inhalation LC_{50} (4 hr)	98.1%	Rat	LC_{50} 56 mg/m^3 (equivalent to 0.056 mg/L)	DuPont-6331
Acute skin irritation	98.1%	Rabbit	Not irritating	DuPont-7060
Acute eye irritation	98.2%	Rabbit	Not irritating	DuPont-7059
Skin sensitisation	96.9%	Guinea pig	Study discontinued due to toxicity	DuPont-3021
Skin sensitisation	42%[b]	Guinea pig	Not a sensitiser	HLR 179-88
Phototoxicity	99.1%	Mouse fibroblast cell line, Balb/3T3, clone A31	Not phototoxic	DuPont-42100

[a] Summarised in Point CA 5.2 in this document.
[b] Oxamyl technical 42 (42% a.s. in cyclohexanone and water)

High purity oxamyl (~97–98%) has been evaluated in acute oral, inhalation, and dermal toxicity studies. The results indicate that oxamyl has high acute oral and inhalation toxicity, but relatively low acute dermal toxicity. High purity oxamyl was not irritating to either the skin or eyes of rabbits. Toxicity precluded testing of pure oxamyl for skin sensitisation in guinea pigs by both the Maximisation and Buehler methods when the test substance was administered at doses recommended by the test guidelines. However, negative results were obtained in a study with oxamyl technical (42%), which represented the approximate maximum concentration that could be evaluated without significant clinical signs of toxicity or mortality. Oxamyl was determined not to have the potential to be phototoxic.

B.6.3 Short-term toxicity

Oxamyl has been evaluated in several short-term toxicity studies that have included 90-day feeding studies in rats and dogs, one-year feeding studies in dogs, and short-term dermal exposure studies in rabbits. Summaries of these studies are presented below.

B.6.3.1 Oral 28-day study

It is not mandatory to conduct either 14-day or 28-day oral studies.

B.6.3.2 Oral 90-day study

Subchronic (90-day) feeding studies were conducted with oxamyl in rats and dogs.

Oral 90-day toxicity in the rat

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.3.2/01

Reference:	**Report:**	[redacted] [redacted] (1969); Ninety-day feeding study in rats with 1-(dimethylcarbamoyl)-N-(methylcarbamoyloxy)thioformimidic acid, methyl ester (IND-1410) **DuPont Report No.:** HLR 308-69 **Guidelines:** Not given GLP: no (not in place at the time the study was perfomed)
--		

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-7
   Purity: Not given

Deviations: Based on OECD test guideline 408 the following deviations were identified in the study protocol:-

- The age of the rats used and the weight variation within each sex were not specified.
- The frequency at which clinical observations were made is unknown.
- The frequency at which each animal was examined for signs of morbidity and mortality is unknown.
- Advanced post-mortem changes prevented any pathological evaluation of tissues from the premature mortalities.
- No ophthalmic investigations were conducted.
- No investigation was conducted at the end of the exposure period concerning sensory reactivity to various types of stimuli.
- Based on both OECD guideline 408 and Directive 87/302/EEC Part B, 90-Day Subchronic Oral Toxicity in Rodents, the haematological investigations did not include a platelet count or a measure of blood clotting time and potential. Plasma / serum sodium, potassium, glucose, total cholesterol, urea, blood urea nitrogen, creatinine, total protein and albumin were not determined.
- OECD guideline 408 requires the wet weights of the epididymes, uterus and ovaries which were not determined after necropsy. Relative organ weights were not determined.
- A histopathological examination was not performed on the parathyroid, oesophagus, ileum, aorta, female mammary gland, urinary bladder and skin as recommended in OECD guideline 408.

Directive 87/302/EEC Part B, 90-Day Subchronic Oral Toxicity in Rodents recommends a histopathological investigation of the jejunum, caecum, rectum and femur.

- No determination of effects on cholinesterase was performed in this study although OECD 408 recommends performing relevant determinations if it is known or suspected that cholinesterase metabolism is affected by treatment.

Materials & Methods:

Male and female weanling albino rats ([redacted]-CD strain) were examined for body weight gain and clinical signs of toxicity during the week before testing. They were subsequently divided into 4 groups of 16 males and 16 females per group. A haematological investigation (erythrocyte count, leucocyte count, haematocrit and haemoglobin concentration) was conducted prior to dosing on 6 male and 6 female rats randomly chosen from each group.

Oxamyl (IND-1410 technical; batch IND-1410-7; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), IND-1410-7 = D1410-7 which has a purity of ~100%) was administered for 91 to 95 days in the diet (Purina Laboratory Chow (GPLC) containing 1% added corn oil). This was freshly prepared each week and refrigerated until required. The average daily dose was 0, 3.92, 8.37 and 11.86 mg/kg bw/day (males) and 0, 4.30, 9.18 and 12.83 mg/kg bw/day (females) corresponding to nominal doses of 0 (control), 50, 100 and 150 ppm. The high dose groups initially received 500 ppm Oxamyl. These groups were placed on a control diet within four days of receiving test material. Three days later this was changed to 150 ppm and maintained at that level for the remainder of the study. When the first weeks dose is included, the top dose administered is 14.56 mg/kg bw/day (males) and 13.99 mg/kg bw/day (females).

Each animal was weighed twice per week and their weekly food intake calculated. The animals were routinely examined for abnormal behaviour or clinical signs of toxicity. Haematological, urine and blood alkaline phosphatase analyses were conducted on 10 males and 10 females from the control, 100 and 150 ppm groups after 1, 2 and 3 months. Plasma alanine aminotransferase was also determined at these time points in the control and high dose groups. At study termination, all but 6 males and 6 females from each group were sacrificed by chloroform administration. Following necropsy, individual organs were weighed and a number of tissue samples were preserved and stained for histological examination. The remaining 6 males and 6 females in each group were used for a reproduction study (evaluated in Section B.6.6.1.3 Reproduction and Developmental Toxicity (Annex IIA, 5.6)).

Findings:
Mortality:

One male rat in the 100 ppm group was found dead on day 30. Another high dose male rat was found dead on day 5. This was attributed to treatment with 500 ppm on days 1 – 4.

Clinical signs:

Male rats administered 500 ppm Oxamyl started to fasciculate after 2 days. Mild neurological symptoms indicative of cholinesterase inhibition such as ruffled fur, mild diarrhoea, fasciculations, bulging eyes, lacrimation, excessive food spillage and weight loss were recorded in both sexes at this dose after 4 days. These symptoms decreased in severity or disappeared altogether when a control diet was administered to the high dose group. Dosing recommenced after 3 days at 150 ppm. No clinical signs of toxicity were recorded at 50 ppm.

Body weight / body weight gain:

Lower body weight gains compared to control animals were recorded in males and females starting from 100 ppm (around 13% in bothe sexes at 100 ppm (Table 29). No estimate of statistical significance was provided

Table 291: 13-week body weight gains in male and female rats (grams)

	Control	**50 ppm**	**100 ppm**	**150 ppm**
Males	428	426	372 (-13.1%)	339 (-20.8%)
Females	202	195	175(-13.9%)	183 (-9.4%)

No estimate of statistical significance was provided

Food consumption / efficiency:

Total food consumption by male rats administered 50 ppm was significantly higher than in control rats while in male rats administered 150 ppm it was significantly lower than in control rats. There were no statistically significant differences with respect to food efficiency between test and control groups in females (Table 30).

Table 302: Total food consumption in male and female rats (grams) during weeks 1 – 13

	Control	**50 ppm**	**100 ppm**	**150 ppm**
Males	2171	2217*	2139	1887**
Females	1617	1586	1585	1445

*Statistically significant difference from control at $p < 0.01$
**Statistically significant difference from control at $p < 0.001$

Haematology:

The percentage increase in haematocrit in females with increasing dose recorded at the study start was no more observed at the 1, 2 or 3 month evaluations. No significance was attributed to this finding.

Urinalysis:

The results of the urinalysis for blood (Occultest ®), protein (Albustix ®) and sugar (Clinitest ®) indicate that positive samples have a higher incidence of blood and protein at the top dose ; the presence of blood was detected also in the mid-dose group (Table 31).

Table 313: Perturbations in urine parameters in male and female rats (number of abnormal specimens)

Dose	**Blood Occultest ®** No. positive	**Protein Albustix ®** No. ≥ 2+	**Sugar Clinitest ® No. ++**	**Total abnormal / 60 specimens**
Control	0	1	0	1
50 ppm	-	-	-	-
100 ppm	4	0	1	5
150 ppm	5	4	1	10

Clinical chemistry:

No treatment-related effect on alkaline phosphatase or alanine aminotransferase activity was recorded.

Organ weights:

A number of statistically significant or highly significant mean organ weight changes were recorded. Weight reductions were recorded in the heart (14% and 13%), kidney (12% and 15%), spleen (19% and 29%) and thymus (22% and 19%) in males administered 100 and 150 ppm, respectively. The reductions in the kidney and spleen weights were part of a dose- response trend. Liver weight in high-dose males was reduced by 21%. Female kidney and lung weights were reduced by 16% and 14%, respectively, at 100 ppm while female stomach weight was increased by 12% at 150 ppm. Female liver weight was reduced by 13% and 11% at 100 ppm and 150 ppm, respectively. When based on organ to body weight ratios, the differences in organ weight did not achieve statistical significance and probably reflected the reduced body weight gain in these groups (Table 32).

Gross pathology / histopathology:

No treatment-related pathological lesions were recorded in the high dose or control groups.

Table 324 Mean organ weights (grams) in male and female rats

Organ		Control	50 ppm	100 ppm	150 ppm
Heart	♂	1.54	1.64	1.32*	1.34*
	♀	0.95	0.96	0.83	0.94
Kidneys	♂	3.76	3.53	3.32*	3.19*
	♀	2.22	2.20	1.87**	2.13
Liver	♂	18.20	18.80	16.56	14.46*
	♀	10.03	10.66	8.73*	8.92*
Lung	♂	17.6	1.79	1.52	1.61
	♀	1.39	1.40	1.20*	1.46
Spleen	♂	0.90	0.86	0.73*	0.64**
	♀	0.60	0.65	0.58	0.58
Stomach	♂	1.89	2.06	1.92	1.71
	♀	1.36	1.41	1.39	1.52*
Thymus	♂	0.64	0.65	0.50*	0.52*
	♀	0.46	0.39	0.37	0.45

M, male; F, female
* Statistically significant difference from control at $p < 0.01$
** Statistically significant difference from control at $p < 0.001$

Conclusions:

The oral 90-day study HLR 308-69 was originally submitted under EU Rev8 Point IIA 5.3.2 and conducted with test material pure oxamyl (PAI). Guidelines were not given. A review of this study indicates that it only partially meets the current guideline B.26; deviations include age of the rats at study start was not specified in the report; ±20% weight variation of the rats on test Day 0 was not determined; an ophthalmological examination was not conducted; the following haematology/clinical chemistry parameters were not evaluated: clotting potential, electrolyte balance, carbohydrate metabolism, kidney function. The following tissues were not evaluated: aorta, oesophagus, skin, jejunum, ileum, caecum, rectum, urinary bladder, mammary gland, femur, exorbital glands.

One male mortality was recorded at 100 ppm. Statistically significant reductions in body weight gain and absolute organ weight were recorded in both sexes at 100 and 150 ppm. An increase in occult blood in the urine was recorded at these same two dose levels; an increase in urine protein level was also recorded at 150 ppm. Male food consumption was significantly reduced at 150 ppm. The percentage increase in haematocrit in females decreased with increasing dose though the statistical significance of this was not determined. Based on these findings the NOAEL is 50 ppm (equivalent to 3.92 mg/kg bw/day in males).

RMS comments and conclusion for the renewal.

Given the extent to which the protocol deviates from the recommendations of the current guidelines and in particular the absence of any investigation into the cholinesterase effects of the test compound, the results of this investigation are regarded as supplemental.

However, reconducting it, beside being not in line with the EU animal welfare policy, is unlikely to yield a significantly different result because **the mechanism of action of carbamates (cholinesterase inhibition) is well understood. Further, the most relevant effect is the acute neurotoxic effect, which is rapidly reversible and similar across species, as demonstrated by the rapid recovery of animals treated with 500 ppm, with evere cholinesterase effects during the first days of treatment, which recovered completely when administered control diet for three days.**

Oral 90-day toxicity in the mouse

A 90-day toxicity study in the mouse has not been conducted for oxamyl due to the fact that the mechanism of action of carbamates (cholinesterase inhibition) is well understood. Further, the most relevant effect is the acute neurotoxic effect which is rapidly reversible and similar across species. The conduct of additional subchronic toxicity studies, beside being not in line with the EU animal welfare policy, will not contribute to the understanding of the hazards or add relevant information to the risk assessment.

Oral 90-day toxicity in the dog

A 90-day toxicity study in the dog was provided and included in the first EU approval review conducted with oxamyl before any guideline was adopted and the put in place of the GLP system ([redacted] **1969: 13-week oral administration – dogs – insecticide 1410)**

Purebred young adult beagle dogs, 4 males and 4 females per dose group, were administered 0, 50, 100 and 150 ppm Oxamyl. The perturbations in the clinical, gross pathological, histopathological and organ weight parameters recorded were not considered to be compound-related. Therefore, the NOAEL was derived as 150 ppm (equivalent to 5.00 mg/kg bw/day in males), that is the highest dose tested.

Given the extent to which the protocol deviates from the recommendations of the current guidelines

- The frequency at which each animal was examined for signs of morbidity and mortality is unknown.
- No ophthalmic investigations were conducted.
- The haematological investigations did not include a platelet count or a measure of blood clotting time and potential.
- The wet weights of the gall bladder, ovaries, uterus, parathyroids, thymus and brain were not determined after necropsy. Relative organ weights were not determined.
- A histopathological examination was not performed on the cervical and lumbar spinal chord, parathyroid, thymus, oesophagus, salivary gland, trachea, aorta, accessory sex organs and the female mammary gland as recommended in OECD guideline 409.
- No determination of effects on cholinesterase was performed in this study although OECD 409 recommends performing relevant determinations on a case by case basis.

the results of this investigation were regarded as supplemental. Considering that no significant effect was observed, the results of this study were not reported in the DAR for the renewal, in view of the available one year dog study submitted and reviewed (HLR-381-90 summarized below) which provides enough subchronic toxicity information in the dog for this compound. The mechanism of action of carbamates (cholinesterase inhibition) is indeed well understood and the most relevant effect is the acute neurotoxic effect (no significant variation depending on the duration of the study) which is rapidly reversible and similar across species. The conduct of an additional subchronic study in the dog, beside being not in line with the EU animal welfare policy, would not contribute to the understanding of the hazards or add relevant information to the risk assessment.

Oral 1-year toxicity in the dog

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.3.2/02

Reference: --	**Report:**	[redacted] (1990); Chronic toxicity study with oxamyl (IN D1410-196) one-year feeding study in dogs (2 volumes) **DuPont Report No.:** HLR 381-90 **Guidelines**: U.S. EPA 83-1 (1982) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Deviations: Based on OECD test guideline 452 the following deviations were identified in the study protocol:-

- The low dose (50 ppm) elicited a toxic response although the guideline recommends that the low dose should not produce toxicity.
- The femur with joint was not preserved for histopathological evaluation although this is listed in the guideline.

I. MATERIALS & METHODS

All beagle dogs ([redacted]) were subjected to an ophthalmic and clinical pathology evaluation during a 44-day pre-test period. All dogs were treated for roundworm infection with Strongid T® (pyrantel pamoate) 8 days prior to the start of the study. On day 41 of the study, faecal samples showed no evidence of roundworm infection. On the basis of the pre-test evaluations, the dogs were divided into 4 dose groups, 5 males and 5 females per group. At the start of the study the mean age was 171 days and the mean group body weight was 8.9 and 7.1 kg for males and females, respectively.

Oxamyl technical (IN D1410; batch IN D1410-196; purity 97.1%) was administered in the diet at doses of 0, 50, 150 and 250 ppm corresponding to 0, 1.56, 4.60 and 8.00 mg/kg bw/day (males) and 0, 1.46, 4.50 and 7.84 mg/kg bw/day (females) for 1 year (estimated on the basis of the purity of the technical material). Oxamyl was mixed to homogeneity in Ground Purina® Certified Canine Diet #5007. The diet was freshly prepared each week and refrigerated until required. The homogeneity and stability of the Oxamyl concentration in the diet (including controls) and the concentration on study days 265, 293, 328 and 356 was verified. Water was supplied ad libitum. The purity of Oxamyl technical material was determined to be 100% and 99% during the pre-test determination and on study day 386, respectively.

Body weights and food consumption were determined for each animal on a weekly basis. Appearance and behaviour were investigated daily. Clinical signs of acute toxicity were recorded daily 3 hours after feeding for the first 3 months and weekly thereafter. Clinical signs of chronic toxicity were recorded weekly. An ophthalmic investigation was conducted on each animal in the pre-test period and on study day 358. Haematological, clinical chemistry and urinalysis investigations were performed during the pre-test period and on study days 81, 181, 264 and 356. Plasma and erythrocyte cholinesterase activity was determined during the pre-test period and on study days 7, 34, 88, 187, 270 and 363. On study days 382 – 386, surviving dogs were anaesthetized with thiamylal sodium and euthanized by exsanguination. Absolute and relative organ weights were determined and a histopathological examination of tissue samples performed. Cholinesterase activity was determined in sections of brains taken from the caudate nucleus, cerebellum/medulla and cerebrum.

II.RESULTS

Mortality:

One 250 ppm female dog was administered control diet from day 74 – 76 due to excessive weight loss. On day 77 her weight had increased and she was replaced on the original test diet. During the study, one control male received ampicillin for a swollen paw while another received ophthalmic ointment for a swollen eye. One 250 pm female was found dead on day 348. The cause of death could not be determined from the gross and histopathological examinations.

Body weight / body weight gain:

The group mean body weight of 250 ppm male dogs was significantly reduced ($p < 0.05$) on weeks 24, 26 and 48 – 54 with a reduction at study termination of 77% compared to the control value. Mean body weight gain, calculated over the entire exposure period, was significantly reduced ($p < 0.05$) in 250 ppm males only, being 19% of the control value. The body weight trend in the other groups was a reduction with increasing dose. This began approximately on study days 42 and 147 for the 150 and 50 ppm groups, respectively. No statistically significant reductions in body weight were recorded in females. The terminal female body weight reduction at 150 and 250 ppm was approximately 10% and 17%, respectively. The correlation of the time course for body weight reductions in both sexes with reductions in plasma cholinesterase activity suggests the body weight reductions are biologically significant (Table 33).

Table 335: Mean body weight gains (kg; mean ± SD) calculated over days 0 – 378 for male and female dogs

	Control	50 ppm	150 ppm	250 ppm
Male	3.6 ± 1.6	2.7 ± 0.7	2.8 ± 1.1	0.7 ± 1.0*
Female	4.1 ± 1.7	4.2 ± 1.3	3.0 ± 1.2	2.1 ± 0.4

* Statistically significant difference from control at $p < 0.05$

Food consumption:

Group mean food consumption in males, calculated over the entire exposure period, was reduced in a dose-dependent manner compared to controls and reached statistical significance at 250 ppm (89% of control value; $p < 0.05$). Group mean food consumption in females was also reduced at 150 and 250 ppm compared to controls but this reduction was not statistically significant (Table 34).

Table 346: Group mean daily food consumption (g; mean ± SD) calculated over days 0 – 378 for male and female dogs

	Control	50 ppm	150 ppm	250 ppm
Male	328.7 ± 4.5	326.4 ± 4.1	317.0 ± 15.0	291.8 ± 28.3*
Female	278.1 ± 35.2	282.6 ± 48.9	266.4 ± 29.5	258.4 ± 46.2

* Statistically significant difference from control at $p < 0.05$

Food efficiency:

Group mean food efficiency, calculated over the entire exposure period, was significantly reduced in 250 ppm males compared to controls (24% of control value; $p < 0.05$). Group mean food efficiency was significantly reduced during the first week in 150 and 250 ppm females (Table 35).

Table 357: Group mean daily food efficiency (g weight gained / kg food consumed; mean ± SD) calculated over days 0 – 378 for male and female dogs

	Control	50 ppm	150 ppm	250 ppm
Male	29 ± 13	22 ± 6	24 ± 10	7 ± 9*
Female	38 ± 11	39 ± 8	30 ± 10	22 ± 7

* Statistically significant difference from control at $p < 0.05$

Clinical signs:

Clinical signs of toxicity were categorised as acute (occurring within 3 hours of feeding) and chronic (occurring after 3 hours). The increased incidence of diarrhoea, vomiting, and mucoidal stool in 150 and 250 ppm males and/or females was not statistically significant but was consistent with symptoms of cholinesterase inhibition and was therefore considered to be biologically significant. There was a statistically significant increase in the number of males and females with tremors at 150 and 250 ppm. The trend toward a higher incidence of tremors was also statistically significant. Although this began at 50 ppm in females, it was not considered to be biologically significant due to the absence of other statistically significant clinical signs of toxicity including perturbations in plasma cholinesterase activity. However, the incidence of vomiting at 50 ppm was part of a statistically significant increasing trend and was recorded sooner than in the 150 ppm females. The incidence of diarrhoea at 50 ppm was similar to that at 150 ppm, being recorded from 2 weeks. The clinical signs of toxicity at 50 ppm are qualitatively similar to those recorded at 150 ppm which suggests that they are biologically significant (Table 36).

The clinical signs of toxicity recorded in the exposed groups during the chronic phase of the study were not significantly different from control values. However, the incidence of diarrhoea, soft stool, vomiting and bloody stool (females only) continued beyond the acute phase and, with the exception of vomiting, was evident at 50 ppm in both sexes.

Table 368: Clinical observations of acute (3 hours after feeding) toxicity in individual female dogs.

Observation	0 ppm					50 ppm					150 ppm					250 ppm				
Animal Number	2835	2833	2846	2842	2845	2840	2836	2850	2839	2848	2834	2844	2851	2841	2849	2831	2847	2852	2837	2832
Diarrhoea	√	√			√		√	√	√			√	√	√		√	√	√	√	√
Hyperactive										√						√	√			
Lethargy															√	√	√			√
Bloody stool																√		√		
Mucoidal stool																√	√	√	√	
Soft stool	√			√	√	√	√	√	√	√	√	√		√		√	√	√	√	√
Uneaten chow*	√	√	√	√	√	√	√	√		√	√	√	√	√	√	√	√	√	√	√
Vomiting**										√	√	√		√		√	√	√	√	√
Panting																√				
Tremors						√			√	√	√	√	√	√	√	√	√	√	√	√

* Includes incidences where no chow, little chow, half of the chow and most of the chow was consumed
** Mucus and/or chow were vomited

Ophthalmology:

Bilateral small optic discs were recorded in one male and one female approximately 5 weeks before the study commenced. These findings were judged insignificant and did not preclude these animals from the study. The final ophthalmoscopic examination on test day 358 recorded a krukenberg spindle in the eye of a control male but again this finding was judged insignificant and it was concluded that compound-related ocular effects did not occur.

Cholinesterase activity:

Group mean plasma cholinesterase activity in males and females was significantly reduced compared to controls from week 1 in the 150 and 250 ppm dose groups and in week 1 and months 6, 9 and 12 in 50 ppm males. A significant decrease in erythrocyte cholinesterase activity was recorded in 250 ppm males at the 6-month sampling. A decease also occurred at the 1-week sampling in 50 ppm females but this was not statistically significant (Table).

Table 39: Effects of Oxamyl on plasma and erythrocyte cholinesterase activity (group mean value; % of the control) in male and female beagle dogs.

Dose level	1-week		1-month		3-month		6-month		9-month		12-month	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Plasma cholinesterase												
0 ppm	100	100	100	100	100	100	100	100	100	100	100	100
50 ppm	**79**	92	81	84	82	89	**67***	92	**66***	99	**68***	92
150 ppm	**26***	**54***	**28***	**57***	**37***	**41***	25*	**60***	**30***	**51***	**52***	**63**
250 ppm	**35***	**53***	**52***	**42***	**52***	**36***	**36***	**45***	**49***	**71**	**42***	**55***
Erythrocyte cholinesterase												
0 ppm	100	100	100	100	100	100	100	100	100	100	100	100
50 ppm	90	**51**	103	96	114	124	88	105	82	90	104	122
150 ppm	**78**	83	87	**76**	**79**	90	83	90	81	82	103	101
250 ppm	**75**	74	85	84	99	92	**72***	81	87	93	94	102

* Statistically significant difference from control at $p < 0.05$
Biologically significant (≥ 20%) reductions in cholinesterase activity compared to controls

A statistically significant decrease in cholinesterase activity was recorded in the male caudate nucleus at all dose levels and in the cerebellum/medulla at 150 ppm. Biologically significant reductions (≥ 20%) were recorded in

the cerebellum/medulla of both sexes and in the female caudate nucleus at 150 and 250 ppm and in the male cerebrum from 50 ppm. These reductions in brain cholinesterase in both sexes were biologically significant (Table 37).

Table 37: Effects of Oxamyl on brain cholinesterase activity (group mean value; % of the control) in male and female beagle dogs.

Brain region	0 ppm		50 ppm		150 ppm		250 ppm	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Caudate nucleus	100	100	**69***	100	**53***	**76**	**70***	**70**
Cerebellum / medulla	100	100	83	103	**62***	**78**	**72**	**76**
Cerebrum	100	100	**77**	90	**54**	84	**66**	85

* Statistically significant difference from control at $p < 0.05$

Biologically significant (≥ 20%) reductions in cholinesterase compared to controls

Haematological, clinical chemistry and urinalysis:

Statistically significant perturbations of haematological, clinical chemistry and urinalysis parameters (compared to controls) were sporadic and not considered to be treatment-related.

Organ weight:

Statistically significant increases in mean relative organ weights were recorded in males only, specifically brain and kidney in 250 ppm males and heart in 50 ppm males. The absolute brain and kidney weights did not differ significantly between the groups and therefore the increase is probably due to the significantly decreased body weight in 250 ppm males. The increase in heart weight reflects the higher absolute heart weight in 50 ppm males. However, this was insignificant and a further increase with increasing dose was not evident. Spontaneous heterogeneity in canine heart size is proposed as the most likely explanation. Lung discolouration was increased in 2 and 3 males at 150 and 250 ppm, respectively, in a dose-dependent, but statistically insignificant manner. Terminal aspiration of ingesta was recorded in these animals during histopathological examination. No other treatment-related gross pathological effects were recorded.

Gross pathology / histopathology:

Histopathological findings in the test groups were not significantly different compared to controls. Minimal to mild multifocal basophilia / vacuolation in convoluted tubule epithelial cells was recorded in 3 males at 250 ppm. Minimal tubular dilation and tubular proteinaceous fluid were recorded in 2 and 3 males, respectively, at 250 ppm. These findings were not recorded in the other test and control groups. Minimal chronic renal multifocal inflammation was increased in the 150 and 250 ppm males compared to controls. The study author's claim that such findings represent regenerative changes in the kidney. This implies that more significant kidney damage occurred earlier in the study. However, these findings are not corroborated by gross pathology, blood urea nitrogen or urinalysis findings. Foreign material in the airways and alveoli of the lungs (aspiration of ingesta) was recorded in 1 male at 50 ppm, 2 males and 1 female at 150 ppm and 4 males and 2 females at 250 ppm. Vomiting was recorded in all of these animals to varying degrees. One male in the 50 ppm group vomited on day 187 of the study only. Aspiration of ingesta was not recorded in all animals even in the high dose groups where vomiting was most frequent. These findings were attributed to the emetic properties of Oxamyl.

III. CONCLUSIONS

The supplementary studies on the active substance study HLR 381-90, originally submitted under EU Rev8 Point IIA 5.3.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 83−1 (1982). A review of this study indicates that it partially meets the current guideline (Directive 87/302/EEC Part B 30, Chronic Toxicity Test—Non-Rodent); deviations include the rib with joint was collected and evaluated instead of the femur with joint and adrenals and ovaries were not weighed at necropsy. Although a toxic response was documented at the lowest administered dose, for the most part the study protocol adhered to the recommendations of the guideline and is therefore acceptable.

Decreases in body weight and body weight gain beginning at 50 ppm in males and at 150 ppm in females correlated with significant decreases in plasma cholinesterase activity and were therefore biologically significant. Significant reductions in food consumption and efficiency were also recorded in males at 250 ppm. A statistically significant trend toward a higher incidence of tremors was observed in both sexes at 150 and 250 ppm within 3 hours of dosing. However, this began at 50 ppm in females and was accompanied by other clinical signs of cholinesterase inhibition which, although not as pronounced as at the higher doses, were nonetheless biologically significant. A number of these manifestations persisted for more than 3 hours after dosing. Significant decreases in brain cholinesterase activity were recorded in males from 50 ppm. Biologically significant reductions in brain cholinesterase activity in females were recorded at 150 and 250 ppm. **A NOAEL for male and female dogs cannot be established, the lowest dose can be considered as a LOAEL (corresponding to approximately 1.5 mg/kg bw per day in both sexes).**

RMS comments and conclusion for the renewal

Although a toxic response was documented at the lowest administered dose, for the most part the study protocol adhered to the recommendations of the guideline and is therefore acceptable as a supporting study. NOAEL was not determined in this study. However, reconduct seems unnecessary when the results are combined with the results from HLO 555-90, providing a complete understanding of oxamyl repeated toxicity in dogs. This study is considered valid when combined with HLO 555-90.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.3.2/03

Reference: --	**Report:**	[redacted]. (1991); 52-week dietary toxicity study with IND-1410 (oxamyl) in male dogs **DuPont Report No.:** HLO 555-90 **Guidelines:** U.S. EPA 83-1 (1982) GLP: YES

1. Test material: Pure oxamyl (P+AI)
   Lot/Batch #: IN-D1410-196
   Purity: 98%

I. MATERIALS & METHODS

Male beagle dogs ([redacted]) were subjected to an ophthalmic, physical, clinical and clinical pathology evaluation during a 22-day pre-test period. The dogs were divided into 5 dose groups of 5 animals each. The age of the dogs at the commencement of the study was 4 – 5 months and they weighed 6.5 – 8.9 kg.

Oxamyl technical (IND-1410; batch IN-D1410-196; purity 97.1%, 98% by analysis) was administered in the diet (Ground Purina® Certified Canine Diet #5007) at doses of 0, 12.5, 20, 35 and 50 ppm corresponding to 0, 0.372, 0.577, 0.930 and 1.364 mg/kg bw/day for 1 year. Dietary samples were taken directly from the mixing container on weeks 1, 4, 13, 27, 40 and 53 and were shipped in dry ice to the sponsor for routine analysis. The diet was freshly prepared each week and refrigerated until required. Water was supplied *ad libitum*.

The dogs were observed twice daily for mortality, moribundity and clinical signs of toxicity. Body weights and food consumption were recorded before treatment, every week up to weeks 15 and 14 of treatment respectively and thereafter once every 4 weeks until necropsy. Ophthalmic investigation were conducted on each animal in the pre-test period, during weeks 26 and 39 and just before necropsy. Haematological and clinical chemistry investigations (including plasma and erythrocyte cholinesterase activity measurements) were performed during the pre-test period and during weeks 13, 26, 39 and 53. After 52 weeks of treatment, surviving dogs were anaesthetized with sodium pentobarbital, euthanized by exsanguination and necropsied. The external surface of the body was subjected to a macroscopic examination. Absolute and relative organ weights and organ-to-brain weight ratios were determined and a histopathological examination on tissue samples performed. Brain cholinesterase activity was determined in the caudate nucleus, cerebellum/medulla and cerebrum.

II. RESULTS

Mortality:

There were no treatment-related mortalities.

Body weight / body weight gain:

At study termination, group mean body weights and body weight gains were increased in all test groups compared to controls but not in a dose-dependent or statistically significant manner (Table 38).

Food consumption / efficiency:

Group mean food consumption in the test groups was not significantly different from controls. Group mean food efficiency was not determined (Table 38).

Table 38: Group mean cumulative body weight gains (kg; mean ±SD) and group mean food consumption (kg / animal / week) on week 51

	Control	12.5 ppm	20 ppm	35 ppm	50 ppm
Body weight gain	4.8 ± 0.5	6.3 ± 1.1	5.3 ± 1.2	5.4 ± 1.0	5.7 ± 1.0
Food consumption	2.2 ± 0.4	2.3 ± 0.2	2.3 ± 0.5	2.1 ± 0.2	2.2 ± 0.3

Ophthalmology:

No visible lesions were recorded at any of the ophthalmic examinations.

Haematology / clinical chemistry:

Although statistical significance was not achieved, the trend for plasma cholesterol was for an increase in concentration with increasing dose at weeks 13, 26, 39 and 53. However, since this trend was also recorded one week before the start of treatment the finding is not biologically significant. No other haematological or clinical chemistry perturbations were recorded.

Cholinesterase activity:

Erythrocyte cholinesterase was significantly decreased compared to controls in the high dose group on week 53. However, this was an isolated finding and was specifically due to low cholinesterase readings in 2 animals. It was not part of a dose-response trend. There were no treatment-related perturbations in plasma or brain cholinesterase activities (Table 39).

Table 392: Effects of Oxamyl on plasma and erythrocyte cholinesterase activity (group mean values; mUnits / mL blood)

	Week -2	Week -1	Week 13	Week 26	Week 39	Week 53
Plasma cholinesterase						
Control	1670	1646	1404	1416	1408	1499
12.5 ppm	1637	1520	1333	1289	1277	1381
20 ppm	1647	1582	1344	1347	1387	1429
35 ppm	1612	1503	1250	1214	1120	1222
50 ppm	1805	1681	1426	1374	1401	1492
Erythrocyte cholinesterase						
Control	1818	1783	1876	1539	1888	1981
12.5 ppm	1935	1982	1946	1538	1818	1783
20 ppm	1783	1737	1830	1527	1935	2063
35 ppm	1748	1853	1760	1422	1632	1888
50 ppm	1445	1620	1305	1200	1550	1550*

* Statistically significant difference from control at $p < 0.05$

Organ weight / pathology:

Absolute organ weights and organ-to-body and organ-to-brain weight ratios were unaffected by treatment. Macroscopic investigations recorded no treatment-related anomalies.

III. CONCLUSIONS

This study on the active substance study HLO 555-90, originally submitted under EU Rev8 Point IIA 5.3.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 83–1 (1982). A review of this study indicates that it partially meets the current guideline (USEPA 83-1 (1982), Directive 87/302/EEC Part B. 30, Chronic Toxicity Test—Non-rodent.); deviations include:

- Only male dogs were tested
- Glucose and urinalysis endpoints were not evaluated
- Adrenals and ovaries were not weighed at necropsy
- Microscopic evaluation of tissues was not conducted.

However, this study was conducted to determine a NOEL for cholinesterase inhibition in male dogs, as this was not determined in HLR 381-90. The deviations in this current study were met in HLR 381-90, with the exception of the organ weight deviations listed above. The combination of the two studies allows to determine a NOEL/NOAEL in dogs for cholinesterase inhibition. Therefore this study is supplemental to the previous 1-year oral toxicity study in dogs ([redacted] 1990) and with this purpose is considered acceptable.

Results indicate that there is an almost complete lack of effect of test material at the doses examined in this study (the highest dose tested was the lowest in the previous study at which some effects were observed). The only statistically significant finding was a decrease in erythrocyte cholinesterase on week 53 in dogs administered 1.36 ± 0.26 mg/kg bw/day. This contrasts with the statistically significant decreases in plasma cholinesterase from 6 months and in caudate nucleus cholinesterase at termination in dogs administered 1.56 ± 0.14 mg/kg bw/day in the previous study. The decrease in erythrocyte cholinesterase recorded here was a sporadic finding and, although >20%, was not considered biologically significant. Therefore the NOAEL in this study is 50 ppm (1.36 mg/kg bw/day). The combination with the previous study according to which effects were observed at this same dose, suggest the opportunity to define an **overall NOAEL** at 35 ppm, (corresponding **to 0.930 mg/kg bw per day**).

RMS comments and conclusion for the renewal

This study is supplemental to the previous 1-year oral toxicity study in dogs ([redacted] 1990) and with this purpose is considered acceptable. The combination of the two studies allows to determine a NOAEL in dogs for cholinesterase inhibition.

B.6.3.3 Other routes

28- and 90-Day inhalation toxicity (rodents)

Due to the high acute oral and inhalation toxicity of oxamyl and rapid reversibility of nonlethal exposures, this study is not required. Furthermore, the conduct of a 28-day or 90-day inhalation toxicity test was not considered as relevant since oxamyl has a very low vapour pressure (5×12^{-5} Pa).

Percutaneous 28-day toxicity (rodents)

Two 21-day repeated dose dermal toxicity studies have been conducted in rabbits with oxamyl. The toxicological properties of this active substance (acute toxicity, rapid reversibility of effects, and lack of cumulative toxicity) suggest that testing for longer periods of time (28- or 90-days) is not necessary to adequately assess the hazards by dermal exposure.

A 21-day dermal toxicity study in rabbits ([redacted] 1988) was included in the first EU approval submission. The study has some limitations the most relevant of which are

- Premature mortalities among high-dose males, so that the number of males in the recovery group was less than the recommended minimum of 5 animals.

- A non-porous (plastic wrap) gauze dressing was used whereas the guideline recommends a porous gauze dressing. Since the test sites were wrapped with an impervious (plastic film) wrap, this could have enhanced test substance absorption.

Five animals of each sex in the low and mid dose groups and 10 animals of each sex in the high dose group were treated topically at nominal doses of 0, 2.5, 50 and 250 mg/kg bw. These corresponded to actual test material administration of 0, 5.9, 117.4 and 582.6 mg/kg bw (males) and 0, 5.7, 116.4 and 580.5 mg/kg bw (females). Cholinesterases effects were observed at the mid and the high dose.A NOAEL was identified as the lowest nominal dose (2.5 mg/kg bw) ; however due to the discrepancies between the nominal and the actual treatment dose, giving rise to uncertainties in the NOAEL derivation, an additional study was carried out, which is described in the following.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.3.3/01

Reference: --	**Report:**	[redacted] (1999); Oxamyl technical: 21-day repeated dose dermal toxicity study in rabbits **DuPont Report No.:** DuPont-1599 **Guidelines:** OECD 410 (1981), 59 NohSan No. 4200 (1985), U.S. EPA 82-2 (1982) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

The study was conducted to clarify the NOEL from a previous 21-day dermal toxicity study in rabbits ([redacted] 1988

Deviations: Based on OECD test guideline 410 the following deviations were identified in the study protocol:-

- The body weight range at the start of the study (1584.1 – 2082.2 g) was lower than the 2-3 kg recommended in the guideline.
- Haematology and clinical chemistry parameters (other than cholinesterase measurements) were not investigated during this study.
- Only brain and tissues with gross lesions were collected.

I. MATERIALS & METHODS

Young adult male and female HM:(New Zealand white)fBR rabbits ([redacted]) were quarantined for 8 days and were approximately 10 weeks old and weighed 1584.1 – 2082.2 g at the commencement of the study. The females were nulliparous and non- pregnant. The rabbits were chosen on the basis of body weight gain, clinical evaluation and disease-free status and were assigned to treatment groups so that there was no statistically significant difference in group mean plasma cholinesterase activity within a sex. The animals were administered approximately 125 grams Certified High Fibre Rabbit LabDiet® (PMI Nutrition International Inc.) per day and water was available *ad libitum*.

Two pilot studies were conducted; one to compare the sensitivity of the assay used for cholinesterase activity with that used in study HLR 523-88 reported previously and the other to determine the time of peak inhibition of cholinesterase activity. In the first pilot study, 1 male and 1 female rabbit per dose group were topically exposed to 25 or 50 mg/kg bw Oxamyl. After treatment, swabs of the test sites were taken to determine if test material

had been completely removed. One hour after treatment, 0.5 mL of blood was collected from the jugular vein of each rabbit following which the animals were sacrificed and the brains removed. Plasma, erythrocyte and brain cholinesterase activities were determined. In the second pilot study, 8 rabbits (sex unstated) were topically exposed to 150 mg/kg bw test material. 24 hours previously, a blood sample was taken for baseline plasma and erythrocyte cholinesterase determinations. Further determinations were made at 3, 6, 6.5, 7, 8 and 9 hours post application after which the animals were sacrificed.

One day prior to the initiation of the main study, the dorsal and trunk hair of each rabbit was shaved. Based on the results of study HLR 523-88 reported previously (B.6.3.3.1), doses of 0, 25, 40, 50 and 75 mg/kg bw/day Oxamyl technical (DPX-D1410; batch D1410-196; purity 96.9%) were selected. The dose was applied to a 25 cm2 area (approximately 1% of the total body surface area) of 6 male and 6 female rabbits. A similar sized group acted as a control and was similarly administered deionised water. The animals were fitted with plastic collars to prevent ingestion of the test material or interference with the dressing. Test material was prepared immediately prior to use. It was mixed with distilled water to form a paste and spread evenly over the exposed skin and the test site occluded with a porous gauze dressing and adhesive bandages. The doses were adjusted based on the test material purity of 96.9%. The homogeneity of the paste was not determined. After treatment for 6 hours, the dressing was removed, the application site washed with warm water and Ivory® soap and patted dry. The animals were exposed daily for 21 days except one male rabbit, which replaced another male killed in extremis on day 2, was exposed for 20 days. The animals were reshaved during the study to facilitate the evaluation of dermal effects.

One day prior to the commencement of treatment, approximately 0.5 mL of blood was collected from the jugular vein of each rabbit. Baseline plasma and erythrocyte cholinesterase activity was determined. The animals were also observed for clinical signs of cholinergic toxic effects to determine their baseline behaviour. Blood was sampled in the same manner each day after treatment. Animals were observed daily after treatment for clinical signs (including cholinergic clinical signs) of toxicity and dermal irritation. If positive cholinergic toxicity was recorded, the same animal was observed the following morning prior to treatment.

The rabbits were weighed at 3 – 4 day intervals and at sacrifice. Group mean food consumption and food efficiency were estimated weekly.

On day 21, a blood sample (0.5 – 1 mL) was taken from the auricular artery of each animal 7 hours post dosing. All animals were terminated by barbiturate anaesthesia followed by exsanguination. The brains were collected, weighed and frozen at –70°C and later analysed for cholinesterase activity. Other gross lesions were collected and preserved. Plasma, erythrocyte and brain cholinesterase activities were determined.

II. RESULTS

Mortality:

There were no treatment-related mortalities.

Body weight / body weight gain:

There were no statistically significant differences in group mean body weight or body weight gain compared to controls in either sex. Male rabbits in the 40 mg/kg bw/day dose group lost, on average, 130 grams body weight over the exposure period compared to an average weight gain of 17 grams in the control group males. However, this difference was not significant and was considered unrelated to treatment. Body weight losses were also occasionally recorded in females in all but the 25 mg/kg bw/day dose group. Again there was no significant difference between the exposed groups and the control group and no dose-response trend was evident.

Food consumption / efficiency:

There were no treatment-related effects on food consumption. Fluctuations in food efficiency in both sexes were not significantly different from controls and were attributable to the fluctuations in body weight gain.

Clinical signs:

Possible clinical signs of toxicity, before and after daily exposure, generally did not show a dose-response relationship. These included potential manifestations of cholinergic perturbation such as diarrhoea and

hyperactivity. The frequency of occurrence of these manifestations was low and they were also recorded during the baseline evaluation. Pupillary constriction was recorded in one male in the 50 and 100 mg/kg bw/day dose groups and in one high dose and one 25 mg/kg bw/day dose group female. This was recorded once in the these animals and in each case only a single eye was affected. This manifestation was considered to be an artefact of the washing procedure rather than a treatment-related effect. The incidence of mild erythema in males was greater in all treatment groups than in controls reaching statistical significance at 25 and 75 mg/kg bw/day. However, it was not dose-related and occurred sporadically in the affected animals during the test period. Oedema, eschar, scratching and sores were also documented following daily exposure. The frequency of these manifestations was low, statistically insignificant compared to controls and not dose-related. No biological significance was attributed to the skin irritation.

Cholinesterase activity:

In the first pilot study, inhibition of plasma cholinesterase occurred at 50 mg/kg bw. Results from the second pilot study indicated that maximum cholinesterase inhibition occurred 7 hours post application (1 hour after cessation of treatment). Statistically and biologically significant inhibition of plasma cholinesterase activity was recorded in high-dose females with a group mean inhibition of 29% compared to controls. Four high-dose females had plasma cholinesterase activities < 80% of the control value as did two 40 mg/kg bw/day females and a single female in each of the 25 and 50 mg/kg bw/day dose groups. A relationship between inhibition magnitude and dose was not evident in either sex. No statistically significant difference between control and treated erythrocyte cholinesterase activity was recorded in either sex. A biologically significant decrease in activity (24% compared to controls) was recorded in high-dose females. Three of these animals recorded decreases of ≥20% as did two females in the 25 mg/kg bw/day group and a single female in each of the 40 and 50 mg/kg bw/day groups. Although the trend in males was toward increasing inhibition of erythrocyte cholinesterase with increasing dose, the magnitude of the inhibition was not biologically significant at any dose and did not correlate with inhibition of either plasma or brain cholinesterase. Group mean brain cholinesterase activity in males was increased compared to controls in all except the top dose group where a statistically insignificant decrease of 5.9% was recorded. Group mean brain cholinesterase activity in females was decreased in a dose- dependent manner; the decrease of 10.7% compared to controls recorded at the high dose was statistically significant. One high dose female recorded a > 20% inhibition of brain cholinesterase activity (Table 40).

Table 403: Effects of Oxamyl on plasma, erythrocyte and brain cholinesterase activity (group mean % of the control, mean ± SD) in male and female rabbits. Cholinesterase activity was measured as units / litre of blood (plasma and erythrocyte) or units / gram of tissue (brain)

Dose (mg/kg bw/day)	Plasma cholinesterase		Erythrocyte cholinesterase		Brain cholinesterase	
	Male	Female	Male	Female	Male	Female
25	93 ± 16	106 ± 23	120 ± 20	88 ± 20	105 ± 6	99 ± 6
40	82 ± 27	95 ± 31	97 ± 26	98 ± 24	107 ± 3	96 ± 8
50	93 ± 18	100 ± 25	99 ± 22	94 ± 20	102 ± 8	96 ± 5
75	81 ± 34	**71 ± 14***	83 ± 33	**76 ± 22**	95 ± 8	89 ± 6*

* Significantly different from control at $p < 0.05$

Biologically significant (≥ 20%) reductions in cholinesterase activity compared to controls

Organ weight:

Treatment-related gross lesions or effects on brain weight were not recorded.

III. CONCLUSIONS

The repeated dermal toxicity study DuPont-1599, originally submitted under EU Rev8 Point IIA 5.3.3 and conducted with test material pure oxamyl (PAI), was conducted under guideline OECD 410 (1981), 59 NohSan No. 4200 (1985), U.S. EPA 82-2 (1982). A review of this study indicates that it partially meets the current EEC Method B.9. Deviations include: 1) animals were dosed for 21 days instead of 28 days, 2) only tissues with gross lesions were collected, 3) haematology and clinical chemistry parameters (other than cholinesterase measurements) were not investigated during this study, and 4) the body weights of the rabbits were outside the

range testing guideline suggested. The study was conducted to clarify the NOEL from study HLR 523-88 discussed above.

Statistically and/or biologically significant inhibition of plasma, erythrocyte or brain cholinesterase activity was not recorded in males at any dose level. Biologically significant inhibition of plasma and erythrocyte cholinesterase activities was recorded in females at 75 mg/kg bw/day and reached statistical significance in the case of plasma and brain cholinesterase. Manifestations of cholinergic perturbation such as diarrhoea and hyperactivity were recorded. Other clinical signs of toxicity did not achieve statistical or biological significance. Based on the statistically and biologically significant inhibition of cholinesterase at the high dose, the NOAEL for female rabbits is 50 mg/kg bw/day.

RMS comments and conclusion for renewal

The study is considered acceptable as a key study.

B.6.3.4 Summary of short-term toxicity

Results of short-term toxicity studies with oxamyl are summarised in Table 41.

It has to be underlined that the standard of these available studies varied considerably. Some of them were dated: they were not conducted in compliance with GLP principles and deviated substantially from the recommendations of current EU /OECD guidelines (deviations are listed above). They were therefore considered as supporting studies or not used at al for the evalutation.

Table 41 Summary of short-term toxicity studies with oxamyl

Type of study	Species	Dose range tested	NOAEL	LOAEL	Target organ(s) and effects	Reference[a]
Oral, 13 weeks	Rat	0, 50, 100, 500/150 ppm (equivalent to 0, 3.9, 8.4, 11.9 mg/kg bw/day for males and 0, 4.3, 9.2, 12.8 mg/kg bw/day for females)	50 ppm (3.9 and 4.3 mg/kg bw/day, males and females, respectively)	100 ppm (8.4 and 9.2 mg/kg bw/day males and females, respectively)	↓ body weight gain, urine blood, ↓ absolute organ weight	HLR 308–69 Supporting
Oral, 13 weeks	Dog	0, 50, 100, 150 ppm (equivalent to 0, 1.5, 2.9, 5.0 mg/kg bw/day for males and 0, 1.3, 2.6, 4.2 mg/kg bw/day for females)	>150 (HDT)[b] (>4.2 mg/kg bw/day)	>150 (HDT)[b] (>4.2 mg/kg bw/day)	No treatment-related effects	HLO 328-69[c] Study with limitations not used for the evaluation
Oral, 12 months	Dog	0, 50, 150, 250 ppm (equivalent to 0, 1.56, 4.60, 8.0 mg/kg bw/day for males, and 0, 1.46, 4.50, 7.84 mg/kg bw/day for females)	Not detected	50 ppm (lowest dose tested) (1.56 mg/kg bw/day)	↓ plasma and brain cholinesterase activity and clinical signs	HLR 381–90 To be evaluated combined with HLO 555-90
Oral, 12 months	Dog	0, 12.5, 20, 35, 50 ppm (males only) (equivalent to 0, 0.372, 0.577, 0.93, 1.364 mg/kg bw/day)	50 ppm (HDT) (1.36 mg/kg bw/day)(males) **35 ppm (0.930 mg/kg bw/day) rounded to 1 mg/kg bw/day** when evaluated in combination with HLR381-90	>50 ppm (HDT)[b] (1.36 mg/kg bw/day) (males)	No treatment-related effects	HLO 555-90 To be evaluated combined with HLR 381-90
Dermal, 21 days	Rabbit	Nominal :0, 2.5, 50, 250 mg/kg bw/day (occlusive, non-porous dressing) Actual: 0, 5.9, 117.4 and 582.6 mg/kg bw	2.5 mg/kg bw/day	50 mg/kg bw/day	↓ plasma, erythrocyte and brain cholinesterase activity	HLR 523–88[c] Study with limitations not used for the evaluation
Dermal, 21 days	Rabbit	0, 25, 40, 50, 75 mg/kg bw/day (porous, semi-occlusive dressing)	**50 mg/kg bw/day**	75 mg/kg bw/day	↓ plasma, erythrocyte and brain cholinesterase activity among females	DuPont-1599 Key study

[a] Summarised in Point CA 5.3 in this document, except where noted.
[b] HDT=Highest dose tested.
[c] Study submitted in the EU Dossier in 2001 and included in the first EU approval review. Now cited in Reference Lists "Documents Not Submitted and Not Relied Upon." Included for comparison purposes.
[d] 50 ppm is a threshold dose; therefore, the NOEL for male dogs is based on the daily intake value of 1.36 mg/kg bw/day established in the second 12-month feeding study (HLO 555-90).

In a 13-week rat study, decreased body weight gain; decreased kidney, heart, thymus, spleen, liver, and lung weights; and increased stomach weights were noted in males and/or females at the mid- and/or high-dose level. At the high dose, clinical signs of toxicity (fasciculations, ruffled fur, mild diarrhoea, bulging eyes, and lacrimation), decreased food consumption, and an increased incidence of protein and blood in the urine were observed. In a 13-week dog study, affected by a number of limitations in the study design, when compared with the current reuest of the guidelines for this kind of studies, no treatment-related effects were noted at any dose level up to the highest dose tested of 150 ppm. It was therefore not considered valid in the present evaluation However, since 1-years dog studies are available it was considered not appropriate to ask for the re-conduct of the study, also considering that the mechanism of action of carbamates (cholinesterase inhibition) is well understood and the most relevant effect is the acute neurotoxic effect (e.g. there are no significant variation depending on the duration of the study) which is rapidly reversible and similar across species. The conduct of an additional subchronic study in the dog, beside being not in line with the EU animal welfare policy, would not contribute to the understanding of the hazards or add relevant information to the risk assessment.

The results of one oral 12-month dog study showed significant and biologically relevant dose-dependent effects on body weight, body weight gain, food consumption and food efficiency, and clinical signs of toxicity related to cholinesterase inhibition in males and/or females at the mid- and high-dose levels (150 and 250 ppm, respectively). Body weight effects and plasma and brain cholinesterase inhibition were also observed in males at the lowest dose tested (50 ppm; equivalent to 1.56 mg/kg bw/day): being consistent with the mechanism of action of the a.s. and being part of the dose-dependentce of the effects they were considered relevant and therefor a NOAEL could not be derived. For this reason, a second oral 12-month study was specifically designed to establish a NOEL in male dogs, by using 4 doses (plus the control) ≤ 50 ppm. No treatment-related findings, including cholinesterase inhibition were noted even at the highest dose tested (50 ppm , equivalent to 1.36 mg/kg bw/day). Based on the results of these two studies, since at 50 ppm controversial results were obtained (significant quite relevant brain cholinesterase inhibition was observed in the first study when male dogs consumed a diet of 50 ppm at 1.56 mg/kg bw/day, whereas no toxicity was observed in the second study when male dogs consumed a similar diet at 1.36 mg/kg bw/day) it is consider appropriate to set **an overall subchronic oral NOAEL** at 35 ppm, corresponding to 0.930 mg/kg bw/day, rounded to **1 mg/kg bw/day**.

In a 21-day dermal toxicity study in rabbits, plasma, RBC, and/or brain cholinesterase activities were decreased at the mid- and high-doses (nominal value 50 and 250 mg/kg bw/day, respectively). Mild hyperglycemia and an accumulation of an eosinophilic material in the duodenal submucosa were also noted at the high dose. However a high incidence of mortality was observed in the high dose group, and the quantification of the treatment dose was questionable: the actual treatment was considered to be almost 2-fold higher than the nominal one. In addition, the test sites were wrapped with an impervious (plastic film) wrap, which would have enhanced test substance absorption. For this reason it was not considered valid in the present evaluation. A second 21-day study was conducted to more precisely define the NOEL in rabbits by the dermal route of exposure, in which the test sites were wrapped with a porous, semi−occlusive (gauze) wrap as requested by the test guideline. Although the treatment has a shorter duration with respect to the requested 28 days, the mechanism of action of carbamates (cholinesterase inhibition) is well understood and the most relevant effect is the acute neurotoxic effect (e.g. there are no significant variation depending on the duration of the study) which is rapidly reversible and similar across species. The conduct of an additional subchronic dermal toxicity study in the rabbit or in the rat, beside being not in line with the EU animal welfare policy, would not contribute to the understanding of the hazards or add relevant information to the risk assessment. In this study, plasma, RBC, and brain cholinesterase activities were decreased in females at the high dose of 75 mg/kg bw/day. No treatment-related decreases in plasma, RBC, and brain cholinesterase activities were noted in females at dose levels of 50 mg/kg bw/day and below and in males at any dose level (up to 75 mg/kg bw/day). In the second study, no treatment-related effects were noted in males and females at **50 mg/kg bw/day**, which is therefore the **subchronic dermal NOAEL**.

B.6.4 Genotoxicity

A battery of *in vitro* and *in vivo* tests was conducted to determine the genotoxic potential of oxamyl.

B.6.4.1 *In vitro* studies

Genotoxicity data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the article reviewed can be found in Appendix 1.

Bacterial assay for gene mutation

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.4.1/01

Reference: --	**Report:**	Gladnick, N.L. (1999); Oxamyl technical: Bacterial reverse mutation test in *Salmonella typhimurium* and *Escherichia coli* **DuPont Report No.:** DuPont-3084 **Guidelines:** 59 NohSan No. 4200 (1985), EEC Method B.14. (1992), U.S. EPA 870.5100 (1997), EEC Method B.13. (1992), OECD 471 (1997) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

I. MATERIALS AND METHODS

The mutagenic potential of Oxamyl technical (batch DPX-D-1410-196; purity 96.9%) was investigated using *Salmonella typhimurium* strains TA97a, TA98, TA100, TA1535, and *Escherichia coli* strain WP2 uvrA (pKM101) in the presence and absence of an exogenous metabolic activation system (Aroclor-induced rat liver S9 fraction, 4.0 mg/mL protein). The test was performed in two independent trials using the plate incorporation method (3 replicates were used per trial, with and without S9 mix, for each strain and dose). The exposure concentrations of test substance used were 0, 5 (Trial 1 only), 10, 50, 100, 500, 1000, 2500, and 5000 µg/plate. The highest dose level was set based on the lack of evidence of toxicity and on OECD Test Guideline 471. Dimethylsulphoxide (DMSO) was used as the test substance solvent and the negative control. The test substance was administered to the test system as a 50 mg/mL solution in DMSO.

Concurrent strain specific positive controls were also included in each trial. 2-aminoanthracene and 9,10-dimethyl-1,2-benzanthracene were used with S9 mix and 2- nitrofluorene, N-ethyl-N-nitro-N-nitrosoguanidine, sodium azide, ICR-191 acridine mutagen were used without S9 mix. Treatment solutions, control solutions and the S9 mixture were not analysed for concentration, uniformity or stability.

A test substance was classified as positive if the mean number of revertants in any strain at any test substance concentration was at least twice the mean of the concurrent negative control and there was a concentration-related increase in the mean number of revertants per plate in that same strain.

II. RESULTS

The number of revertants at all concentrations of the test substance was similar to concurrent controls with and without metabolic activation (Table 42). There was no evidence of test substance precipitation or toxicity. All positive controls exhibited more than a three fold increase over the respective mean of the concurrent negative control for each tester strain. All tester strains exhibited appropriate phenotypic characteristics and the mean number of revertants per plate scored for the negative solvent controls was within the prescribed acceptable range.

Table 425: Summary of average revertants per plate

Compound	CONC. µG/PLATE	TA97a		TA98		TA100		TA1535		WP2 *uvr*A (PKM101)	
		Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II	Trial I	Trial II
Mean Revertants/Plate without Activation[a]											
Oxamyl	0	153	135	27	19	136	97	22	14	167	114
	5	147	ne	30	ne	132	ne	19	ne	166	ne
	10	148	148	23	20	136	104	20	14	156	109
	50	152	161	25	19	151	109	19	15	163	112
	100	147	147	24	17	137	108	21	14	145	126
	500	154	129	26	18	145	108	18	13	142	108
	1000	140	138	30	19	133	117	20	10	153	103
	2500	145	126	25	18	123	96	23	11	157	89
	5000	130	139	25	17	116	90	16	8	125	88
NaAZ	2	ne	ne	ne	ne	1977	1949	2314	2045	ne	ne
ICR-191	2	2377	2209	ne	ne	ne	ne	ne	ne	ne	ne
2NF	25	ne	ne	1667	1428	ne	ne	ne	ne	ne	ne
ENNG	2	ne	ne	ne	ne	ne	ne	ne	ne	1657	1391
Mean Revertants/Plate with Activation[a]											
Oxamyl	0	130	145	34	25	128	108	18	11	193	117
	5	125	ne	33	ne	124	ne	13	ne	189	ne
	10	136	144	32	25	149	103	15	14	180	122
	50	146	176	34	18	154	114	15	13	187	133
	100	140	174	37	28	154	118	15	14	185	150
	500	133	163	35	23	147	128	17	12	209	145
	1000	127	154	27	27	130	107	15	11	174	146
	2500	119	144	31	22	135	106	17	11	180	130
	5000	115	129	25	18	129	107	14	12	173	105
2AA	2	ne	ne	317	328	1557	419	96	69	ne	ne
DMBA	20	1620	1434	ne	ne	ne	ne	ne	ne	1925	1561

[a] average of 3 replicates per trial
ne = not evaluated
NaAZ = sodium azide; ICR-191 = ICR-191 acridine; 2NF = 2-nitrofluorene; ENNG = N-Ethyl-N-nitro-N-nitrosoguanidine; 2AA = 2-aminoanthracene; DMBA = dimethylbenz(a)anthracene

III. CONCLUSIONS

The *in vitro* study DuPont-3084, originally submitted under EU Rev8 Point IIA 5.4.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline 59 NohSan No. 4200 (1985), EEC Method B.14. (1992), U.S. EPA 870.5100 (1997), EEC Method B.13. (1992), OECD 471 (1997). A review of this study indicates that it fully meets the current guideline B. 13/14.

Under the conditions of the study, **Oxamyl was negative for mutagenic activity** in both non- activated and S9-activated test systems at doses of up to 5000 µg/plate in all 5 strains of bacteria tested in two independent trials. These results suggest that Oxamyl does not cause gene mutations in bacterial (prokaryotic) cells.

RMS comments and conclusion for renewal

This study is considered valid as a key study

Chromosomal abnormalities in mammalian cells

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.4.1/02

Reference: --	**Report:**	Gudi, R., Schadly, E.H. (2000); Oxamyl technical: *In vitro* mammalian chromosome aberration test **DuPont Report No.:** DuPont-2936 **Guidelines:** EEC Method B.10. (1992), 59 NohSan No. 4200 (1985), U.S. EPA 870.5375 (1996) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Deviations: Based on OECD test guideline 473, no guideline deviations were identified

I.MATERIALS AND METHODS

The clastogenic potential of Oxamyl (batch DPX-D-1410-196; purity 96.9%) was investigated in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL) both with and without an exogenous metabolic activation system (Aroclor 1254-induced rat liver S9 fraction). A preliminary toxicity test was performed (using a reduction in mitotic index as the endpoint) to establish the dose range for testing in the initial chromosome aberration assay. An independent repeat assay was also conducted.

The preliminary toxicity assay was conducted at concentrations of 0.22 - 2200 µg/mL (highest concentration equivalent to 10 mM) both in the presence (4-hour treatment time) and absence (4- and 20-hour treatment times) of S9 metabolic activation.

Based on the findings of the preliminary study, the doses chosen for the initial chromosome aberration assay were 0, 10, 25, 50, 100, 200, 300, 400, 500, and 600 µg/mL in the non-activated assay and 0, 100, 200, 300, 400, 500, 600, and 800 µg/mL in the S9-activated assay.

For the independent repeat assay, the concentrations used were 0, 50, 75, 150, 200, 250, 300, and 400 µg/mL in the non-activated assay and 0, 50, 100, 200, 300, and 400 µg/mL in the S9- activated assay. Positive and negative controls were tested concurrently. Mitomycin C (MMC) was used as the positive control in the non-activated study at final concentrations of 0.13 and 0.25 µg/mL and cyclophosphamide (CP) was used as the positive control in the S9- activated study at final concentrations of 25 and 50 µg/mL. The solvent vehicle for the test article (water) was used as the solvent control.

In the initial assay, cells were treated with non-activated and S9-activated test substance for four hours. In the independent repeat assay, cultures were exposed continuously to non- activated test substance for 20 hours while cultures exposed to S9-activated test substance were treated for 4 hours. Two hours prior to scheduled cell harvest at 20 hours after treatment initiation, Colcemidwas added to the cultures (0.1 µg/mL) in order to arrest the cells in metaphase.

For all assays, the maximum concentration selected for cytogenetic evaluations was one that induced an approximate 50% reduction in the mitotic index. In addition, two lower concentrations were evaluated together with the concurrent negative (solvent) and positive controls.

Cells were evaluated for toxicity (mitotic inhibition) and then for structural and numerical chromosome aberrations. The test substance was considered to induce a positive response if (a) the percentages of cells with aberrations were increased in a concentration-responsive manner with one or more concentrations being statistically elevated relative to the concurrent solvent control ($p < 0.05$) or (b) a reproducible and significant

increase in the percentage of cells with aberrations occurred at a single concentration relative to the concurrent solvent control.

II.RESULTS

No statistically significant increases in structural chromosome aberrations were observed in either trial at any of the test substance concentrations evaluated (Table 43). In addition, no statistically significant increases in numerical chromosome aberrations (polyploidy or endoreduplication) were observed. In the initial chromosome aberration assay, toxicity (mitotic inhibition) was approximately 53% at 50 µg/mL in the non-activation assay and also 53% at 300 µg/mL in the S9-activation assays. In the independent repeat assay, toxicity was approximately 46% at 200 µg/mL in the 20-hour non-activation assay and 53% at 300 µg/mL in the 4-hour S9 activation assay.

The percentage of structurally damaged cells in the positive controls in all cases was significantly increased compared to solvent controls, thereby verifying the validity of the test.

Table 436: Summary of chromosome aberration data

Treatment	S9 Activation	Treatment Time (hours)	Mitotic Index	Cells Scored	Aberrations per cell[1] (mean ± SD)	Cells with Aberrations (%)	
						Numerical	Structural
Initial Assay							
Water	-	4	5.5	200	0	0	0
Oxamyl							
10 µg/mL	-	4	4.6	200	0	0	0
25 µg/mL	-	4	4.9	200	0.010 ± 0.100	0.0	1.0
50 µg/mL	-	4	2.6	200	0.010 ± 0.100	0.0	1.0
MMC							
0.25 µg/mL	-	4	4.0	200	0.115 ± 0.355	0.0	11.0*
Water	+	4	5.7	200	0.020 ± 0.140	0.0	2.0
Oxamyl							
100 µg/mL	+	4	3.4	200	0.010 ± 0.100	0.0	1.0
200 µg/mL	+	4	3.1	200	0.010 ± 0.100	0.0	1.0
300 µg/mL	+	4	2.7	200	0.050 ± 0.218	0.0	5.0
CP							
25 µg/mL	+	4	0.6	200	0.175 ± 0.475	0.0	15.0*
Independent Repeat Assay							
Water	-	20	11.6	200	0.005 ± 0.071	0.0	0.5
Oxamyl							
50 µg/mL	-	20	10.2	200	0.010 ± 0.100	0.0	1.0
150 µg/mL	-	20	7.0	200	0.000 ± 0.000	0.0	0.0
200 µg/mL	-	20	6.3	200	0.015 ± 0.122	0.0	1.5
MMC							
0.13 µg/mL	-	20	8.5	200	0.130 ± 0.379	0.0	11.5*
Water	+	4	10.4	200	0.005 ± 0.071	0.0	0.5
Oxamyl							
100 µg/mL	+	4	7.1	200	0.010 ± 0.100	0.0	1.0
200 µg/mL	+	4	5.5	200	0.020 ± 0.140	0.5	2.0
300 µg/mL	+	4	4.9	200	0.020 ± 0.140	0.0	2.0
CP							
25 µg/mL	+	4	2.6	200	0.130 ± 0.352	0.0	12.5*

[1]Severely damaged cells were counted as 10 aberrations
* Statistically significant difference from negative control at $p \leq 0.01$ (Fishers Exact Test) MMC = mitomycin C; CP = cyclophosphamide

III. CONCLUSIONS

The *in vitro* study DuPont-2936, originally submitted under EU Rev8 Point IIA 5.4.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline EEC Method B.10. (1992), 59 NohSan No. 4200 (1985), U.S. EPA 870.5375 (1996). A review of this study indicates that it meets the current guideline B.10.

Oxamyl was negative for structural and numerical chromosome aberrations in non-activated and S9-activated test systems in the *in vitro* mammalian cytogenetics test using human peripheral blood lymphocytes indicating that Oxamyl does not induce structural chromosome aberrations in cultured mammalian somatic cells.

RMS comments and conclusion

This study is considered valid as a key study.

Mammalian cell assay for gene mutation (*e.g.*, CHO/HGPRT or mouse lymphoma mutation assay)

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.4.1/03

Reference: --	**Report:**	San, R.H., Clarke, J.J. (2000); Oxamyl technical: *In vitro* mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay **DuPont Report No.:** DuPont-2937 **Guidelines:** U.S. EPA 870.5300 (1996), Directive 87/302/EEC Part B (1987), U.S. EPA 84-2 (1982) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

Deviations: Based on OECD test guideline 476 the following deviations were identified in the study protocol:

- The cells used for the independent repeat assay were subcultured more than 4 times.

I. MATERIALS AND METHODS

Oxamyl Technical (batch DPX-D-1410-196; purity 96.9%) was evaluated for mutagenic activity in vitro at the HGPRT locus in Chinese hamster ovary (CHO) cells. The test article was soluble in sterile distilled water at a concentration of 220 mg/mL. A preliminary toxicity assay, both in the presence and absence of an exogenous metabolic activation system (Aroclor-induced rat liver S9 fraction), was conducted up to the limit concentration of 2200 µg/mL (10 mM). Based on the results from this assay, the concentrations were determined for the initial and independent repeat mutagenicity assays.

In the initial assay, duplicate flasks of exponentially growing CHO-K1-BH4 cells were exposed to the test substance at concentrations of 0, 50, 150, 200, 250, 300, and 500 µg/mL in the non-activated system and 0, 150, 200, 250, 300, and 400 µg/mL in the S9-activated system. In the independent repeat assay, cells were exposed to 0, 50, 100, 150, 200, 250, and 300 µg/mL in the non-activated system and to 0, 150, 200, 250, 275, 300, and 350 µg/mL in the S9-activated system. In both assays, the cells were treated with test substance for 5 hours at 37 ±1°C. Positive and negative controls were tested concurrently. Ethyl methanesulfonate (EMS) and benzo(a)pyrene (B(a)P) were used as positive controls for the non-activated and activated test systems, respectively. The solvent vehicle for the test article (water) was used as the solvent control. Following both mutagenesis assays, the cells were then independently subcultured for assessment of cytotoxicity (cloning

efficiency) and for expression and selection of the 6- thioguanine (TG, 2-amino-6-mercaptopurine)-resistant phenotype. Again, positive and negative controls were tested concurrently.

Cytotoxicity was defined as a cloning efficiency of ≤50% of the concurrent vehicle controls. The mutagenesis assay was considered positive when a dose-dependent increase in mutation frequencies occurred with at least 2 consecutive doses having mutation frequencies of greater than 40 mutants per 106 clonable cells.

II. RESULTS

The pH of the cultures was neutral and no test substance precipitate was observed at any dose level in the exposure matrix. The osmolality of the solvent control and top-dose test solutions was 286 and 290 mmol/kg, respectively. None of the treated cultures in either the initial or independent repeat assay exhibited mutant frequencies of > 40 mutants per 106 clonable cells. Single elevated responses were observed in the independent repeat assay at concentrations of 250 and 200 g/mL in the non-activated and S9-activated cells, respectively, but these responses were not considered significant as there was no clear dose-response relationship and the responses were not reproducible.

III. CONCLUSIONS

The *in vitro* study DuPont-2937, originally submitted under EU Rev8 Point IIA 5.4.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 870.5300 (1996), Directive 87/302/EEC Part B (1987), U.S. EPA 84-2 (1982). A review of this study indicates that it partially meets the current guideline B.17 with only one minor deviation; the cells used for the independent repeat assay were subcultured more than 4 times. Because the solvent and positive control mutant frequencies were within the specified limits, the deviation for OECD test guideline 476 was not believed to have affected the integrity or conclusions of the study.

Oxamyl was negative in the non-activated and S9-activated test systems **in the CHO/HGPRT mutation assay** indicating that Oxamyl does not cause forward gene mutations in this gene locus in Chinese hamster cells.

RMS comments and conclusion for the renewal
This study is considered valid as a key study.

DNA interaction or damage (*e.g., In vitro* unscheduled DNA synthesis)

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.4.1/04

Reference: --	**Report:**	Vincent, D.R. (1987); Assessment of IN D1410-196 in the *in vitro* unscheduled DNA synthesis assay in rat primary hepatocytes (revision 1) **DuPont Report No.:** HLR 719-82, Revision No. 1 **Guidelines:** U.S. EPA 84-2 (1982) GLP: YES

1	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	97.1%

Deviations: Based on OECD test guideline 482 the following deviations were identified in the study protocol:-

- Only 25 nuclei, instead of the recommended minimum of 50 nuclei, were scored for unscheduled DNA synthesis (UDS) in all of the trial 2 cultures and in the duplicate cultures in trial 1.

I. MATERIALS AND METHODS:

Two independent assays were performed to assess the ability of Oxamyl (batch IND1410-196; purity 97.1%) to induce UDS in rat hepatocytes. Freshly isolated hepatocytes from livers of young male Sprague-Dawley rats were treated with Oxamyl in phosphate buffered saline at 7 different dose levels ranging from 1 x 10-5 to 10 mM (equivalent to 0.002 to 2193 µg/mL). The cultures were incubated in culture medium containing [3H]-thymidine at 37°C for 18 hours. Negative (solvent) and positive (7,12 dimethylbenz[a]anthracene (DMBA)) control substances were also tested. Duplicate cultures were used in all cases. After the incubation period, slides of the cultures were prepared for autoradiographic analysis. Nuclear and mean cytoplasmic grain counts were recorded (25-50 nuclei per slide) and the net nuclear grain count was determined. The test compound was considered to elicit a positive response when the average net nuclear grain count was at least five in both trials for any of the concentrations tested. The test compound was considered to be cytotoxic when fewer than 25 hepatocytes were available for scoring per slide for any of the concentrations tested.

II. RESULTS

None of the concentrations of Oxamyl tested gave a nuclear grain count of at least five and no significant increase in the mean net nuclear grain count occurred at any concentration when compared to the negative controls. Cytotoxicity was not observed in trial 1 but was observed at 5.0 and 10.0 mM in trial 2. Data from the positive and solvent controls in the two trials were considered acceptable.

Table 447: Unscheduled DNA Synthesis assay: Trial 1 and 2

Concentration mM (µg/mL)	**Nuclei/slide scored**	**Average Net Grains Per Nucleus ± SD**	
		UDS assay-Trial 1	**UDS assay-Trial 2**
Vehicle control (PBS)	50	0.7 ± 8.8	
	25	0.0 ± 10.2	-3.3 ± 6.9 and -5.2 ± 9.0
1 x 10^{-5} (0.002)	50	-2.0 ± 5.2	
	25	-5.6 ± 7.8	-6.0 ± 6.0
1 x 10^{-4} (0.02)	50	-3.7 ± 11.7	
	25	-0.6 ± 13.3	-1.6 ± 6.4
1 x 10^{-3} (0.2)	50	-0.3 ± 7.1	
	25	-3.2 ± 10.5	-5.4 ± 7.8
1 x 10^{-2} (2.19)	50	-8.2 ± 10.0	
	25	-7.0 ± 10.1	-3.5 ± 7.4
0.1 (21.9)	50	3.9 ± 19.1	
	25	-8.8 ± 27.8	-5.6 ± 9.3
1.0 (219)	50	-1.1 ± 3.6	
	25	-1.9 ± 4.7	-4.9 ± 6.3
5.0 (1097)	50		
	25		*
10.0 (2193)	50	-1.7 ± 2.1	
	25	-8.9 ± 5.0	*
Positive control (DMBA)	50	124.8 ± 89.4	
	25	109.5 ± 26.0	102.7 ± 39.0
	25	116.9 ± 49.3	129.0 ± 63.4

* Microscopic examination of the coverslips revealed very few nuclei. The number of silver grains in background areas was insufficient to generate a calibration curve. The nuclei that were on the coverslip did not have an increased incidence of UDS. DMBA = 7,12 dimethylbenz[a]anthracene

III. CONCLUSIONS

The *in vitro* study HLR 719-82, Revision No. 1, originally submitted under EU Rev8 Point IIA 5.4.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 84-2 (1982). A review of this study indicates that it partially meets the current guideline B.18; deviations is related to the fact that in some cases 25 (instead of 50 nuclei were scored). However, since the rest of genotoxicity data indicates oxamyl is not likely to have genotoxic potential, an additional study is unlikely to yield a significantly different

result. It is consider that this study can adequately completes the understanding of the genotoxic potential of oxamyl when considered in the context of the weight of evidence.

Under the conditions of this study, **Oxamyl did not induce unscheduled DNA damage and repair** in rat hepatocytes *in vitro*.

RMS comments and conclusion for renewal **The study is consider acceptable.**

B.6.4.2 *In vivo* studies in somatic cells

Study submitted in the EU Dossier in 2003 and included in the first EU approval review.

B.6.4.2/01

Reference: --	**Report:**	[redacted] (2002); Oxamyl (DPX-D1410) technical (98% w/w): Mouse bone marrow micronucleus assay **DuPont Report No.:** DuPont-10618 **Guidelines:** 59 NohSan No. 4200 (1985), U.S. EPA 870.5395 (1998), OECD 474 (1997), EEC Method B.12. (1992) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.2%

I. MATERIALS AND METHODS:

Crl:CD-1®(ICR)BR mice were chosen on the basis of adequate body weight, freedom from clinical signs of disease or injury and a body weight ±20% of the mean within a sex. Based on the results of a range-finding study, the mice (approximately 7 weeks old;) were administered a single 10 mL aliquot of a freshly-prepared aqueous solution of Oxamyl (batch DPX-D1410-196; purity 98.21%) by oral gavage at final dose concentrations of 0, 1, 2 and 3 mg/kg bw. Ten mice/sex/group were dosed. In the 3 mg/kg bw group, 14 mice/sex/group were dosed to offset unexpected mortality and ensure a minimum of 5 animals /sex for assay.

At least 5 animals/sex/group were sacrificed at 24 and 48 hours. Five animals/sex were administered the 10 mL of positive control (20 mg/kg bw cyclophosphamide) and were sacrificed at 24 hours. All animals were housed individually and maintained on a diet of PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and water supplied ad libitum. Contaminant levels in food and water and sanitation were periodically checked. Oxamyl stability and dose concentration verification was performed using HPLC with UV detection.

Body weights were determined prior to treatment and prior to sacrifice at which times the animals were also individually handled and examined for abnormal behaviour and appearance. Clinical signs of toxicity were recorded at 15, 30, 45 (3 mg/kg bw and positive control groups only), 60, 120 and 180 minutes after dosing and once a day on test days 1 and 2. Mice were sacrificed by CO_2 asphyxiation and bone marrow samples extracted. At least 3 bone marrow smears were prepared from each animal. Only cells with good morphology were counted. 2000 PCEs per animal were evaluated for the presence of micronuclei. Since the scoring unit was the micronucleated cell, PCEs with > 1 micronucleus were scored as a single micronucleated PCE. The number of PCEs among 1000 erythrocytes was also recorded for each animal.

II. RESULTS

Dose concentration and stability:

Analytical results of the dietary samples showed that the test substance was uniformly mixed, was at the target level (±12% of nominal) and was stable at room temperature for 5 hours. Vehicle control was Oxamyl-free.

Body weight:

No significant changes in body weight were recorded in either sex.

Mortality:

One high-dose male mouse was found dead 15 minutes after dose administration.

Clinical signs:

All clinical signs were recorded on the day of dosing with the exception of enophthalmus in low-dose females which manifested 24 hours after dosing. No clinical signs were recorded in the negative or positive control groups. The abnormal posture and lethargy recorded in the mid- and/or low-dose groups were transient. All clinical signs had resolved by 2 hours after dosing.

Table 458: Clinical observations in male and female mice

	Males				Females			
Dose level (mg/kg bw):-	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Animal number:-	10	10	10	14	10	10	10	14
Enophthalmus	0	0	1	1	0	2	0	0
Wet perineum	0	0	2	2	0	0	0	1
Low carriage	0	0	0	1	ne	ne	ne	ne
Abnormal posture	0	1	7	1	0	0	6	13
Laboured breathing	0	0	0	5	0	0	0	3
Eye discharge	0	0	0	2	0	0	0	3
Clear mouth discharge	0	0	0	5	0	0	0	6
Tremors	0	0	0	13	0	0	0	14
Lethargy	0	1	4	13	0	0	3	12
Laying on stomach	0	0	0	10	0	0	0	10

ne: not evaluated

Micronucleus evaluation:

The incidence of micronucleated PCEs was significantly increased in female mice in the 2 and 3 mg/kg bw dose groups at the 48-hour time point only. However, this was not considered to be biologically significant because the observed incidence was within the laboratory historical negative control range and could be attributed to the lower concurrent negative control MNPCE frequency compared to the 24-hour female and both male negative control values. Also, the actual frequency values in the 2 and 3 mg/kg bw dose females were similar in magnitude to the 24-hour female and the 24- and 48-hour male frequency values.

There were no treatment-related effects on the PCE/total erythrocytes frequency. However, a significant increase in the PCE frequency, and therefore the PCE/NCE ratio, was recorded in females at 2 mg/kg bw at the 48-hour sacrifice.

The incidence of micronucleated PCEs was significantly increased in cyclophosphamide- treated mice of both sexes. No significant decrease in the PCE/total erythrocytes frequency was recorded indicating that cyclophosphamide did not induce bone marrow toxicity.

Table 469: Micronucleus evaluation for male and female mice (n = 5 for all means)

Dose level (mg/kg bw):-	Evaluation time	0	1	2	3	Positive Control
Males						
PCEs/1000 erythrocytes	24	562 ± 89	575 ± 64	622 ± 96	589 ± 27	551 ± 102
	48	573 ± 60	489 ± 81	547 ± 100	574 ± 91	ne
PCE/NCE ratio	24	1.37 ± 0.58	1.40 ± 0.39	1.80 ± 0.76	1.44 ± 0.17	1.32 ± 0.51
	48	1.39 ± 0.39	1.00 ± 0.32	1.29 ± 0.47	1.46 ± 0.66	ne
MNPCE/2000 PCEs	24	5 ± 2	7 ± 2	6 ± 3	8 ± 5	15 ± 6[a]
	48	6 ± 1	7 ± 6	7 ± 4	9 ± 5	ne
Females						
PCEs/1000 erythrocytes	24	642 ± 95	630 ± 71	660 ± 59	562 ± 98	631 ± 49
	48	528 ± 56	620 ± 94	661 ± 59[b]	529 ± 62	ne
PCE/NCE ratio	24	1.93 ± 0.65	1.79 ± 0.59	2.01 ± 0.49	1.36 ± 0.47	1.75 ± 0.36
	48	1.15 ± 0.29	1.76 ± 0.64	2.03 ± 0.48[b]	1.26 ± 0.32	ne
MNPCE/2000 PCEs	24	7 ± 2	8 ± 4	8 ± 3	6 ± 2	15 ± 4[b]
	48	4 ± 2	5 ± 2	9 ± 2[bc]	8 ± 3[bc]	ne

ne: not evaluated

[a] Statistically significant difference from control at $p < 0.05$ by Dunn's test

[b] Statistically significant difference from control at $p < 0.05$ by Dunnett/Tamhane-Dunnett test

[c] Statistically significant difference from control at $p < 0.05$ using trend test (Jonckheere-Terpstra)

The *in vivo* studies in somatic cells study DuPont-10618, originally submitted under EU Rev8 Point IIA 5.4.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline 59 NohSan No. 4200 (1985), U.S. EPA 870.5395 (1998), OECD 474 (1997), EEC Method B.12. (1992). A review of this study indicates that it meets the current guideline B.12.

No biologically relevant increases in the incidence of micronucleated polychromatic erythrocytes in mouse bone marrow were recorded. **Oxamyl did not induce chromosome damage in vivo**.

RMS comments and conclusion for renewal

The study is consider acceptable.

***In vivo* unscheduled DNA synthesis or a mouse spot test**

Neither the *in vitro* unscheduled DNA synthesis or gene mutation tests were positive; therefore, no additional *in vivo* testing of unscheduled DNA synthesis or a mouse spot test are needed.

B.6.4.3 *In vivo* genotoxicity in germ cells

At the present time, the conduct of an *in vivo* study in germ cells is not triggered, as oxamyl has produced negative results in a battery of *in vitro* and *in vivo* genotoxicity tests.

B.6.4.4 Summary of genotoxicity testing

A summary of the results of genotoxicity testing is given in Table .

Table 50 Summary of *in vitro* and *in vivo* genotoxicity studies with oxamyl

Type of study	Organism/Cells	Concentration range tested	Result	Reference[a]
Gene mutation assays				
Reverse mutation	*Salmonella typhimurium Escherichia coli*	5–5000 μg/plate (+/-S9)	Negative	DuPont-3084
Mammalian cell gene mutation	Chinese hamster ovary cells	50–500 μg/mL (-S9) 150–400 μg/mL (+S9)	Negative	DuPont-2937
Chromosome aberration assay				
In vitro cytogenetics	Human peripheral blood lymphocytes	10–600 μg/mL (-S9) 50–800 μg/mL (+S9)	Negative	DuPont-2936
DNA damage and repair assay				
In vitro UDS	Rat primary hepatocytes	1×10^{-5}–10 mM (0.002–2193 μg/mL)	Negative	HLR 719-82, Revision No. 1
***In vivo* cytogenetics assay**				
In vivo micronucleus	Mouse	1, 2, 3 mg/kg bw	Negative	DuPont-10618

[a] Summarised in Point CA 5.4 in this document.

The mutagenic and DNA damaging potential of oxamyl was studied in several *in vitro* test systems using bacterial and mammalian cells. Oxamyl did not show any evidence of gene mutations, chromosome aberrations, or DNA damage and repair. Additionally, an *in vivo* test for chromosome damage in mice produced negative results. Based on these data, oxamyl does not pose a mutagenic or genotoxic concern.

B.6.5 Long-term toxicity and carcinogenicity

The chronic toxicity and/or carcinogenicity of oxamyl were evaluated in rats, mice, and dogs. Summaries of these studies are provided below. The 1-year oral toxicity study in dogs (considered as a subchronic study based on dog life-span) is summarised under Point CA 5.3.2.

B.6.5.1 Long-term oral toxicity and carcinogenicity in rats

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.5.1/01

Reference:	**Report:**	[redacted] (1991); Combined chronic toxicity/oncogenicity study with oxamyl (IN D1410-196) long-term feeding study in rats **DuPont Report No.:** HLR 278-91 **Guidelines:** U.S. EPA 83-5 (1982) GLP: YES
--		

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Deviations: Based on OECD test guideline 453 the following deviations were identified in the study protocol:-

- Body weight range (± 20% overall mean weight on test Day 0) was not determined.
- Ten animals/sex/concentration were evaluated for chronic toxicity at the one year interim sacrifice instead of 20 animals/sex/concentration.
- Adrenals and ovaries were not weighed at necropsy.
- The list of tissues evaluated for microscopic pathology does specify the regions of the brain or spinal cord that were evaluated, as specified in test guidelines.
- The survival rate in control, 25 and 50 ppm males and control and 25 ppm females at terminal sacrifice was <50%.

I. MATERIALS & METHODS:

After quarantine, 310 Crl:CD®BR rats [redacted] of both sexes were chosen on the basis of adequate body weight gain and an absence of ophthalmological abnormalities, injury or clinical signs of disease. Oxamyl (batch D1410- 196; purity 97.1%) was administered in the diet which consisted of irradiated Purina® Certified Rodent Chow® #5002 meal. Diets were prepared weekly and refrigerated until use. Tap water was provided ad libitum. Food and water were periodically analysed for contaminants and the concentration and homogeneity of Oxamyl in the diet was confirmed.

The rats were 38 days old at the start of the study. Each sex was divided into 5 groups of 62 animals per group with no statistically significant difference in group mean body weight within a sex. The dose range investigated was 0, 25, 50, 100 and 150 ppm (w/w adjusted for purity) based on the results of a previous non-guideline 2-year study and a 2-week probe study. These doses corresponded to a mean Oxamyl intake of 0.992, 1.97, 4.19, and 6.99 mg/kg bw/day in males and 1.32, 2.69, 6.73, and 11.1 mg/kg bw/day in females.

Body weights, food consumption and food efficiency were determined every week for the first 13 weeks and every second week thereafter.

Each animal was examined daily for clinical signs of toxicity. An ophthalmological examination was conducted on days –3, 358 and 728. Haematological, clinical chemistry (including plasma and erythrocyte cholinesterase activity) and urinalysis parameters were determined at 1 (cholinesterase activity only), 3, 6, 12, 18 and 24 months. The rats were fasted for 16 hours before blood sampling from the orbital sinus. Urine was collected during the fasting period. Ten rats/sex/group were used for the 1, 3, 6 and 12 month analyses after which they were sacrificed. Ten rats/sex/group were also used for both the 18 and 24-month analyses. All remaining animals were sacrificed at 24 months.

All rats found dead, sacrificed in extremis or sacrificed as scheduled were necropsied. Individual organs were isolated and weighed and a number of tissue samples were preserved for histological examination. Brains were collected at both scheduled sacrifices and brain acetylcholinesterase activity was determined.

II. RESULTS

Mortality

A statistically significant trend toward enhanced survival was recorded in 100 and 150 ppm males and in females from 50 to 150 ppm. This was correlated with the lower body weights recorded at these dose levels throughout the treatment period (Table 47).

Body weight:

Group mean body weights of 100 ppm males were significantly lower than control values (7 – 14%) from test day 7 – 470 and in 150 ppm males (13 – 22%) from test day 7 – 680. Group mean body weight decreases of 8 – 12% and 9 – 30% were recorded for 100 ppm and 150 ppm females, respectively, from test days 7 – 722. Sporadic significant decreases in body weight in both sexes at 25 and 50 ppm were considered unrelated to treatment. Sporadic increases and decreases in body weight gain were recorded in both sexes at all dose levels. Group mean male and female body weight gain was statistically significantly decreased at 100 and 150 ppm

over the interval 0 – 91 days and 0 – 372 days. A statistically significant decrease was also recorded at 100 and 150 ppm for females over the interval 0 – 722 days (Table 48).

Table 47: Mortality recorded over the 0 – 694 day interval

Dose (ppm)	Male					Female				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Found dead	20	24	21	12	9	20	14	15	8	11
Sacrificed *in extremis*	13	5	11	8	8	10	18	8	8	4
Total unscheduled deaths	***33***	***29***	***32***	***20***	***17****	***30***	***32***	***23***	***16****	***15****
Interim sacrifice	10	10	10	10	10	10	10	10	10	10
% Survival	37	44	38	62[#]	67[#]	42	38	56[#]	69[#]	71[#]

* Statistically significant difference from control by Fisher's Exact test with a Bonferroni correction ($p \leq 0.05$)
[#] Statistically significant trend by Cochran-Armitage test at $p \leq 0.05$

Table 482: Terminal mean body weights (grams) and interim and terminal mean body weight gains in male and female rats (grams)

Dose (ppm)	Male					Female				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Mean body weight **Day 722**	760	743 (-2)	877 (+15)	765 (+1)	666 (-12)	555	606 (+9)	506 (-9)	471* (-15)	389* (-30)
Mean body weight gain Day 0 – 372	681	659 (-3)	687 (+1)	612* (-10)	510* (-25)	330	322 (-2)	317 (-4)	240* (-27)	209* (-37)
Mean body weight gain Day 0 – 722	616	604 (-2)	734 (+19)	622 (+1)	519 (-16)	425	468 (+10)	375 (-12)	338* (-20)	258* (-39)

Values in parentheses are the % change in body weight / body weight gain compared to control
* Statistically significant difference from control at $\alpha = 0.05$ using one-way ANOVA and Dunnett's test

Food consumption / efficiency:

A slight increase in food consumption was recorded in males at 100 and 150 ppm while a slight decrease was recorded in females at the same dose levels, neither difference achieving statistical significance. Food efficiency in 150 ppm males was reduced over the 0 – 91, 0 – 327 and 0 – 722 day intervals. A reduction in food efficiency was also recorded in 100 ppm males from day 0 – 91. Food efficiency in 150 ppm females was reduced over the 0 – 91, 0 – 372, 372 – 722 and 0 – 722 day intervals while a reduction was also recorded in 100 ppm females from days 0 – 91 and 0 – 372. Reductions in food efficiency did not reach statistical significance (Table 49).

Table 493: Terminal mean daily food consumption (grams) and mean food efficiency (mg weight gain / g food consumed) in male and female rats

Dose (ppm)	Male					Female				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Food consumption Day 0 – 722	28.1	27.4	27.9	26.9	26.4	21.6	21.5	21.3	23.4	23.4
Food efficiency Day 0 – 722	30	30	36	32	27	27	30	24	20	15

Clinical observations:

Several clinical signs of treatment-related toxicity were recorded. The incidence of hyper-reactivity in male and female rats dosed at 100 and 150 ppm was statistically significantly increased compared to controls. In addition, the incidence of swollen legs or paws was also significantly increased in 100 and 150 ppm males and 150 ppm females. The incidence of sore skin was significantly increased in 100 and 150 ppm females as was the incidence of alopecia in 150 ppm females. This was recorded earlier in both 100 and 150 ppm females than in

the control group. These observations did not correlate with any pathological findings at the interim or terminal sacrifices. Significant differences were recorded for several other clinical signs but these were isolated incidences and were not considered to be treatment-related. The incidence of grossly observable masses was significantly increased in 100 ppm males and significantly decreased in 150 ppm females compared to controls. However, within either sex, for any given site, no significant inter-group differences were recorded in the incidence of masses (Table 50).

Table 504: Selected clinical observations in male and female rats (total number of animals)

Dose (ppm)	Male					Female				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Alopecia	39 (316)	44 (225)	39 (288)	46 (267)	45 (133)	37 (232)	40 (218)	27 (274)	46 (77)	58*# (70)
Diarrhoea	35 (442)	31 (442)	31 (442)	23 (428)	20*# (449)	4 (512)	9 (638)	7 (638)	7 (623)	8 (509)
End of tail missing	0	0	1 (722)	2 (712)	3 (498)	1 (526)	0	0	1 (734)	4 (526)
Hyperreactive	17 (260)	23 (204)	20 (204)	32* (176)	39*# (147)	11 (260)	14 (288)	13 (260)	31* (232)	45*# (133)
Skin sore	44 (386)	44 (386)	44 (378)	50 (330)	51 (344)	24 (479)	23 (456)	26 (533)	38* (463)	51*# (288)
Stained fur	26 (575)	20 (624)	24 (654)	26 (617)	31 (540)	26 (541)	29 (582)	25 (582)	21 (526)	39 (386)
Swollen legs / paws	7 (568)	13 (623)	13 (652)	18* (638)	21*# (456)	3 (487)	2 (437)	6 (534)	5 (487)	14*# (484)
Wet inguen	13 (554)	6 (645)	11 (638)	10 (694)	12 (663)	12 (568)	13 (602)	10 (632)	1* (400)	9 (414)
Wet perineum	5 (623)	7 (596)	10 (633)	8 (708)	9 (673)	13 (582)	13 (602)	10 (615)	2* (323)	9 (379)
Number with masses	32 (316)	30 (379)	36 (379)	46* (316)	39 (414)	38 (470)	34 (489)	35 (428)	31 (526)	23*# (386)

Values in parentheses are the median number of test days on which the observations were first recorded
* Statistically significant difference compared to controls at $\alpha = 0.05$
# Statistically significant trend by Cochran-Armitage test at $p \leq 0.05$

Ophthalmology:

A number of ophthalmological anomalies were recorded at the interim and terminal examinations, the greatest incidence occurring at the latter examination at all dose levels in both sexes. With the exception of pale ocular fundi in males at the terminal examination, these anomalies did not show a dose-response trend. However, the occurrence of pale ocular fundi was not corroborated histopathologically. Since no statistically or biologically significant reduction in erythrocyte, haemoglobin or mean corpuscular haemoglobin count was recorded in either sex at any dose level at the 12- or 24-month haematological evaluations, anaemia can be precluded as a possible cause of the pale ocular fundi. Instead, it may have been a consequence of retinal thinning and the dose-response trend a consequence of the differing rates of survival at each dose level. Pale ocular fundi are a spontaneous ophthalmic lesion in the rat. An increased incidence of retinal photoreceptor cell atrophy was recorded during a microscopic evaluation of 150 ppm female ocular tissue. This was thought to be a consequence of reduced nutritional status (Table 51).

Haematology:

At the 3-, 6- and 12-month evaluations, statistically significant lower erythrocyte levels, mean corpuscular volume and mean corpuscular haemoglobin were recorded in 150 ppm males. The reductions in erythrocyte count were dose related at 6 months only. A significant reduction in leucocyte (monocyte) count was recorded in 50, 100 and 150 ppm males at 24 months. However, these reductions were not part of a dose-response trend and the values were within the range of biological variation.

Table 515: Incidence of eye lesions after approximately 12 and 24 months in male and female rats (% of total surviving animals)

Dose (ppm)	Male					Female				
	0	**25**	**50**	**100**	**150**	**0**	**25**	**50**	**100**	**150**
Interim (Day 358)	5 (61)	9 (58)	7 (59)	6 (62)	3 (59)	5 (59)	5 (61)	3 (60)	2 (62)	10 (62)
Terminal (Day 728)	8 (12)	42 (12)	39 (18)	19 (27)	19 (31)	6 (18)	7 (14)	14 (22)	6 (32)	21 (34)
Pale ocular fundi (Day 728)	0 (12)	8 (12)	11 (18)	11 (27)	13 (31)	6 (18)	7 (14)	14 (22)	3 (32)	9 (34)

Values in parentheses are the numbers of surviving animals on the day of examination

Clinical chemistry:

Serum sodium was significantly increased at 18 months in 50 – 150 ppm males and in 50 – 150 ppm females at 12 and 18 months. Serum chlorine was significantly increased in these groups at 6 and 12 months and also in males at 18 months while serum potassium was significantly increased in 100 and 150 ppm males at 18 months and in 50 – 150 ppm females at 12 months. Creatine kinase activity was significantly reduced in 150 ppm females at the 6-, 12- and 24-month evaluations and in 100 ppm females at 24 months. Significantly lower serum bilirubin was recorded in 100 and 150 ppm females at 6, 12 and 18 months and also in 50 ppm females at 6 months. All of these values were within the range of biological variation associated with the corresponding control values and were not considered to be biologically significant. Other statistically significant deviations in clinical chemistry parameters recorded in both sexes were sporadic and not biologically significant.

Statistically significant reductions in plasma cholinesterase were recorded in all male dose groups and in 100 and 150 ppm females at the 1-month sampling time and in 100 and 150 ppm males at 6, 12 and 18 months. Biologically significant plasma cholinesterase inhibition (≥20%) was recorded in 25 – 150 ppm males at 6 and 12 months, 50 – 150 ppm males at 18 months and 150 ppm males at 24 months. There was no clear time-related trend in plasma cholinesterase inhibition in males. There was a dose-related trend in plasma cholinesterase inhibition in males at 18 months and in females at 1 month. Other statistically or biologically significant reductions in plasma or erythrocyte acetylcholinesterase activity in either sex were sporadic. The toxicological significance of the reductions in activity in females is questionable (Table 52). There were no statistically or biologically significant reductions in brain acetylcholinesterase activity.

Urinalysis:

Urine volume was reduced in a dose-dependent and statistically insignificant manner in males at 24 months. The incidence of proteinuria in 100 and 150 ppm males and 25 – 100 ppm females showed an increasing trend with time. However, no dose-response relationship was evident and values in test animals were not significantly different from controls.

Gross pathology and organ weights:

Mean absolute liver weight was significantly reduced in 150 ppm males and 100 ppm females while mean relative heart and brain weights were significantly increased in 150 ppm males and females at the interim sacrifice. Mean relative heart and brain weights were also significantly increased in 100 ppm females and mean relative kidney weight was significantly increased in 150 ppm females, again at the interim sacrifice. A significant reduction in mean absolute kidney weight and a significant increase in mean absolute brain weight was recorded in 150 ppm males at the terminal sacrifice. The latter increase was also recorded in 100 and 150 ppm females at this sacrifice as was a significant increase in relative brain weight. Mean relative liver and kidney weights were significantly increased in 100 and 150 ppm females while mean relative heart weight was significantly increased in 150 ppm females at terminal sacrifice. The relative organ weight changes were primarily a consequence of significantly reduced final body weights at the higher doses. There were no histopathological findings to corroborate any of the organ weight changes (Table 53).

Table 526: Effects of Oxamyl on plasma and erythrocyte cholinesterase activity (group mean % ± SD of the control mean) in male and female rats

Dose	Sex	1-month	3-month	6-month	12-month	18-month	24-month
Plasma cholinesterase							
0	♂	100 ± 16	100 ± 41	100 ± 47	100 ± 50	100 ± 34	100 ± 16
	♀	100 ± 25	100 ± 38	100 ± 30	100 ± 23	100 ± 47	100 ± 50
25	♂	84 ± 10*	90 ± 25	**72 ± 20**	**65 ± 19***	86 ± 29	104 ± 38
	♀	93 ± 14	105 ± 30	128 ± 29	130 ± 44	94 ± 37	84 ± 28
50	♂	86 ± 14*	91 ± 27	**80 ± 28**	**76 ± 28**	**78 ± 30**	91 ± 53
	♀	84 ± 35	82 ± 45	104 ± 30	102 ± 45	82 ± 26	**70 ± 25**
100	♂	85 ± 14*	**78 ± 17**	**66 ± 16***	**67 ± 16***	**63 ± 19***	96 ± 43
	♀	**62 ± 22***	83 ± 25	114 ± 23	84 ± 16	88 ± 44	**80 ± 37**
150	♂	**65 ± 34***	82 ± 12	**70 ± 14***	**61 ± 20***	**52 ± 19***	**73 ± 31**
	♀	**31 ± 14***	94 ± 28	115 ± 25	109 ± 44	94 ± 22	**80 ± 29**
Erythrocyte acetylcholinesterase							
0	♂	100 ± 6	100 ± 15	100 ± 9	100 ± 7	100 ± 8	100 ± 13
	♀	100 ± 14	100 ± 18	100 ± 8	100 ± 15	100 ± 11	100 ± 8
25	♂	103 ± 14	121 ± 12*	104 ± 9	98 ± 16	108 ± 11	90 ± 6
	♀	93 ± 7	109 ± 12	90 ± 25	109 ± 13	110 ± 15	98 ± 5
50	♂	92 ± 6*	118 ± 8*	103 ± 9	102 ± 10	105 ± 11	91 ± 8
	♀	103 ± 18	107 ± 12	**79 ± 12** *	110 ± 13	112 ± 8	109 ± 17
100	♂	102 ± 8	106 ± 10	104 ± 9	106 ± 9	102 ± 11	94 ± 18
	♀	101 ± 12	117 ± 21	83 ± 15	115 ± 20	112 ± 19	108 ± 23
150	♂	91 ± 18	121 ± 19*	109 ± 5	101 ± 9	115 ± 33	92 ± 18
	♀	106 ± 10	106 ± 7	102 ± 17	113 ± 21	103 ± 9	102 ± 8

* Significantly different from control at 5% level by Dunnett or Mann-Whitney U criteria
Biologically significant (≥ 20%) reductions in cholinesterase activity compared to controls

Table 537: Statistically significant absolute and relative organ weight changes in male and female rats at the interim and terminal sacrifices (% of control)

	Weight	Male				Female			
		25ppm	50ppm	100ppm	150ppm	25ppm	50ppm	100ppm	150ppm
Interim sacrifice									
Body weight		89	101	94	83*	89	99	71*	77*
Liver	Absolute	97	108	93	82*	89	95	75*	82
Kidneys	Relative	105	103	98	111	103	95	118	123*
Heart	Relative	104	97	98	117*	102	97	121*	120*
Brain	Relative	111	100	105	126*	109	98	139*	136*
Terminal sacrifice									
Body weight		93	109	103	87	108	95	81*	66*
Liver	Relative	82	79	90	91	93	116	121*	134*
Kidneys	Absolute	94	91	96	82*	105	111	98	98
	Relative	100	76	88	88	95	117	121*	147*
Heart	Relative	91	90	91	101	88	107	116	136*
Brain	Absolute	99	104	105	106*	104	104	109*	108*
	Relative	105	92	100	122	92	108	133*	159*

* Significantly different from control at $p < 0.05$ by Dunnett's Test;

Histopathology:

At the interim sacrifice the only gross morphological finding in males was a statistically significant increase in the incidence of skin ulcers or erosion at 100 ppm. This finding was not part of a dose-response trend. A number

of statistically significant increases and especially decreases in gross morphological findings were recorded at the terminal sacrifice. Some of these findings were associated with a dose-response trend. A decreasing incidence with increasing dose, with or without statistical significance at the middle and higher doses, was evident for a number of findings in males and/or females. These include periocular chromodacryorrhea, skin staining, stomach ulcer and erosion, large aorta, nasal discharge and staining, kidney deformity and discoloration, small spleen, fluid vagina, mammary gland masses, brain compression, pituitary masses, large parathyroid and pancreatic nodules. These findings are also age- and stress-associated and corroborate the reduced body weight and enhanced survival in both sexes at the higher doses.

Microscopic observations:

At the interim sacrifice, a significantly increased incidence of splenic extramedullary haematopoiesis and red pigment in 150 ppm males was recorded. The incidence of pituitary hyperplasia (pars distalis) was significantly reduced in these animals compared to controls. The incidence of chronic glomerulonephropathy showed a dose-dependent decrease in males which reached statistical significance at 100 and 150 ppm. At the terminal sacrifice, several increased incidences of microscopic observations were documented in a number of tissues in both sexes including liver, rectum, spleen, bone marrow, limbs, thymus, lungs, local lymph nodes, ovaries and uterus. A dose-response trend was evident in most cases with the magnitude of the increase reaching statistical significance at the higher doses. Similarly, decreased incidences of microscopic observations, sometimes associated with a dose-response trend, were documented in liver, aorta, nose, kidney, spleen, adrenals, brain and eyes. These observations may have been age-related due to the increased survival with increasing dose or they may be related to the lower body weight recorded in 150 ppm males and females and 100 ppm females. The incidence of photoreceptor cell atrophy in females at the terminal sacrifice was 66%, 69%, 66%, 66% and 93% at 0, 25, 50, 100 and 150 ppm, respectively. Statistical significance was reached at 150 ppm. This may be a consequence of a deficiency in taurine due to the lower food efficiency at 150 ppm in females. The incidence of subacute ocular anterior chamber and corneal inflammation in females was decreased in a dose response manner reaching statistical significance at 150 ppm.

The incidence of males or females with pituitary masses at the terminal sacrifice decreased with increasing dose reaching statistical significance at 150 ppm. Mammary gland masses were similarly reduced in females. No significant difference was recorded between control and test groups (both sexes) in terms of the incidence of primary benign / malignant tumours or in terms of the total number of benign / malignant tumours recorded per group. Statistically significant increases or decreases in the total number of secondary tumours were recorded at certain dose levels in both sexes at the interim and terminal sacrifices. However, the total number of animals involved was not significantly different from control values.

III. CONCLUSIONS

The long-term toxicity and carcinogenicity study HLR 278-91, originally submitted under EU Rev8 Point IIA 5.5.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 83-5 (1982). A review of this study indicates that it partially meets the current guideline (B.33.Combined chronic toxicity/carcinogenicity test); deviations included:

- Body weight range (± 20% overall mean weight on test Day 0) was not determined.
- Ten animals/sex/concentration were evaluated for chronic toxicity at the one year interim sacrifice instead of 20 animals/sex/concentration.
- Adrenals and ovaries were not weighed at necropsy.
- The list of tissues evaluated for microscopic pathology does specify the regions of the brain or spinal cord that were evaluated, as specified in test guidelines.
  The survival rate in control, 25 and 50 ppm males and control and 25 ppm females at terminal sacrifice was <50%.

However, reconduct is unlikely to yield a significantly different result because no evidence of chronic toxicity attributed to test substance exposure was observed at the one year evaluation or in the animals evaluated for carcinogenicity.. In addition, 10 animals/sex/concentration is the required number specified for the one year interim sacrifice for this type of study in the OPPTS 870.4300 test guideline. Therefore, the number of animals evaluated at one year is considered to have been adequate to assess chronic toxicity.
No adrenal or ovarian microscopic pathology was observed. Therefore the lack of organ weight is not considered to have impacted evaluation of test substance-related effects in these organs.

Common practice at the time of study conduct was to examine multiple sections of brain and spinal cord, as well as any gross lesions that were observed. No test substance-related pathology findings were reported in either brain or spinal cord, so it is considered that these tissues were adequately evaluated for toxicity and carcinogenicity.
Although survival at the end of the study was not >50% in all test groups, it was considered adequate to evaluate the potential for carcinogenicity. Indeed, the low survival rate in control, 25 and 50 ppm males and control and 25 ppm females at terminal sacrifice was not considered to have adversely influenced the outcome of this study. The strain of rat used is noted for its poor survival rate. Survival in the top 2 male dose groups and top 3 female dose groups at the end of the study all exceeded 50% of the 50 animals required for assessment of carcinogenicity. All test groups retained >50% survival through at least test day 652 (males) or 666 (females).

Body weight was significantly reduced in females at 100 and 150 ppm. Body weight gain was also significantly reduced in these groups at the interim and terminal sacrifices and also in males at these dose levels at the interim sacrifice. The incidence of perturbations in several clinical parameters was also increased in both sexes at 100 and 150 ppm. Statistically significant perturbations in absolute and relative organ weight in both sexes at 100 and 150 ppm can be considered to be a consequence of body weight changes. There were no statistically or biologically significant reductions in brain acetylcholinesterase activity at any dose level. Statistically and/or biologically significant inhibition of plasma cholinesterase was recorded in males at doses ≥ 25 ppm at 1, 6 and 12 months and at doses of ≥50 ppm at 1, 6, 12 and 18 months. Inhibition at 25 and 50 ppm was not associated with other treatment-related toxicity. Biologically significant inhibition was recorded in females at doses of ≥50 ppm at 24 months. However, the toxicological relevance of plasma cholinesterase inhibition is questionable, in the absence or erythrocyte acetylcholinesterase inhibition. For this reason it iwas decided that the **chronic oral NOAEL for non neoplastic effects in males and females is 50 ppm (1.97 and 2.69 mg/kg bw/day, respectively). Oxamyl was not oncogenic in this study.**

RMS comments and conclusion for renewal **The study is considered acceptable as a key study.**

B.6.5.2 Carcinogenicity study in the mouse

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.5.2/01

Reference: --	**Report:**	[redacted] (1981); Long term feeding study in mice with oxamyl **DuPont Report No**.: HLO 252-81 **Guidelines:** Not given GLP: YES *Plus its amendment* [redacted] (1990); Long term feeding study in mice with oxamyl **DuPont Report No**.: HLO 252-81, Amendment No. 1 **Guidelines:** Directive 87/302/EEC Part B, Carcinogenicity Test**.** GLP:Yes

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: Not given
   Purity: 97.1%

Deviations: Based on OECD test guideline 453 the following deviations were identified in the study protocol:-

- Body weight range (±20% overall mean weight on test Day 0) was not determined.

- Diet analysis data not reported
- Differential WBC counts were not evaluated in animals in deteriorating health or in control and high dose animals at end of study (24 months).
- Haematological determinations were performed on 10 animals/sex/group instead of the recommended 20 animals/sex/group.
- No urinalysis or clinical chemistry determinations were performed.
- The salivary glands, peripheral nerves and sternum were not preserved for microscopic analysis from terminal sacrifice animals while, in addition to these tissues, the spinal chord and seminal vesicles were not collected from animals found dead or sacrificed moribund.
- Animals that died during the first few weeks of the study received a gross necropsy and tissue collection but tissues were not evaluated microscopically.
- The high dose group had its dietary concentration lowered during week 6 due to unexpected high mortality. Extra animals were added to the study ~weeks 4–5 to ensure adequate group numbers.
- Survival in all groups at terminal sacrifice was 35 – 47%

I. MATERIALS & METHODS

Oxamyl (DPX-D1410 technical; batch not provided; purity 97.1%) was administered to male and female weanling [redacted]CD®-1 mice ([redacted]) by dietary admixture in Purina Laboratory Rodent Chow® #5001 (meal). Water and chow were provided ad libitum throughout the study. Diets were prepared weekly and refrigerated until use. Enhanced grinding and mixing of test material was performed after the initial high mortality to ensure homogeneity of the dietary admixture and to avoid occasional acute exposures.

Animals were selected for treatment based on their eating habits, body weight gain and clinical signs of toxicity recorded during the 10 day observation period. They were divided into treatment groups based on body weight. Oxamyl homogeneity and stability was confirmed periodically. Three groups of mice, 80 per sex per group, received either 0, 25, 50 or 100 ppm Oxamyl (equivalent to 0, 4.2, 8.7, and 13.5 mg/kg bw/day in males and 0, 5.2, 10.8, and 16.8 mg/kg bw/day in females) every day for 101 weeks. These dose levels were selected on the basis of an 8-week range finding study. After 6 weeks, the highest dose was reduced to 75 ppm. The mean top dose administered during the first 6 weeks was 29.7 and 37.4 mg/kg bw/day in males and females, respectively, while the mean top dose during weeks 7 – 101 was 11.5 and 14.3 mg/kg bw/day, respectively. A further 22 mice were added 20 – 31 days after the commencement of the study mostly to the high dose groups.

All animals were observed twice daily for mortality and clinical signs of toxicity including behavioural changes. The animals were palpated once weekly for the presence of masses. A haematological evaluation was carried out on 10 randomly-selected animals of each sex after 1, 3, 6, 12 and 18 months and prior to termination. Urine and faeces were collected one week prior to termination for residue analysis.

Body weights, food consumption and food efficiency were measured once weekly up to week 29, once every fortnight from weeks 31 – 53 and once per month from weeks 57 – 105.

Surviving animals at the end of the treatment period and those that were moribund or experienced sudden large weight loss during the study were sacrificed by CO_2 asphyxiation and a gross necropsy performed. Absolute and relative organ weights were determined for all animals at termination. Tissues were preserved for histopathological examination except for 27 animals that died during the first few weeks of treatment or were found to be in an advanced state of post-mortem autolysis (5 animals) or were accidentally omitted from the evaluation (1 animal). Tissue residue analysis was performed on selected pooled tissue samples after necropsy.

II. RESULTS

Mortality:

High mortality in the mid and high dose groups in the first few weeks of the experiment was treatment-related and probably due to an acute exposure. Treatment-related increases in mortality were not recorded over the remainder of the treatment period. The overall increase in mortality with increasing dose is due to the initial increased trend up to week 6 (Table 54).

Table 548: Mortalities recorded over weeks 0 – 6, 7 – 105 and 0 – 105.

Dose ppm	Male				Female			
	0	**25**	**50**	**100/75***	**0**	**25**	**50**	**100/75**
N	80	80	80	80	80	80	80	80
Extra**	0	0	1	8	0	1	4	8
0 – 6	1 (1)	2 (3)	3 (4)	15 (17)	0	3 (4)	9 (11)	12 (14)
7 – 105	41 (52)	43 (55)	48 (62)	41 (56)	46 (57)	44 (56)	44 (59)	45 (59)
0 – 105	42 (53)	45 (56)	51 (63)	56 (64)	46 (57)	47 (58)	53 (63)	57 (65)

* The dose for the first 6 weeks was 100 ppm. This was changed to 75 ppm for the remainder of the study
** Extra animals added up to week 4 of treatment
Values shown are the total number of mortalities include those animals found dead and those that were moribund when sacrificed. Values in parentheses are the percentages of the total number of animals (including extra additions).

Body weight:

The body weights of 50 ppm treated males were significantly reduced compared to controls on week 1 and also from week 8 – 81 ($p < 0.01 – 0.05$). Body weights of high dose males were highly significantly reduced ($p < 0.01$) from week 2 – 24 and occasional significant reductions ($p < 0.05$) were recorded thereafter. Significant reductions in female body weights were recorded in the mid and high dose groups from weeks 8 – 15 ($p < 0.01 – 0.05$) with occasional reductions in 50 ppm females thereafter. No dose-related trend in body weight gain was evident when calculated for the periods 0 – 6, 0 – 105 and 6 – 105 weeks (Table 55).

Table 559: Group mean body weights and body weight gains (grams) in male and female mice

Dose (ppm)	Male				Female			
	0	**25**	**50**	**100/75**	**0**	**25**	**50**	**100/75**
Mean body weight Week 6	29.7	30.8* (+4)	29.7 (0)	28.1** (-5)	24.2	24.9 (+3)	23.8 (-2)	23.6 (-2)
Mean body weight gain# Weeks 0 – 6	10.8	11.9 (+10)	10.0 (-7)	9.1 (-16)	7.6	8.3 (+9)	7.2 (-5)	7.0 (-8)
Mean body weight Week 105	38.4	38.1 (-1)	36.6 (-5)	38.2 (-1)	35.0	36.4 (+4)	34.2 (-2)	35.9 (+3)
Mean body weight gain# Weeks 0 – 105	19.5	19.2 (-1)	17.7 (-9)	19.3 (-1)	18.4	19.8 (+8)	17.6 (-4)	19.3 (+5)
Mean body weight gain# Weeks 6 – 105	8.7	7.3 (-16)	6.9 (-21)	10.1 (+16)	10.8	11.5 (+6)	10.4 (-3)	12.3 (+14)

Values in parentheses are the % change in body weight / body weight gain compared to control
* Significantly different from controls at $p < 0.05$ using Dunnett's test
** Significantly different from controls at $p < 0.01$ using Dunnett's test
# Body weight gain was not calculated in the original report and therefore no statistical evaluation was provided

Food consumption / efficiency:

With the exception of weeks 12 and 19, food consumption in high dose males was significantly lower than controls from weeks 11 – 84 ($p < 0.01$). No clear trend in statistically significant perturbations in food consumption was evident in the other dose groups in either sex. No treatment-related effects on food efficiency were recorded.

Clinical observations:

No clinical signs of toxicity attributable to treatment were recorded. Dose-related increasing or decreasing trends were evident for a number of clinical signs. However, the differences between test and control values did not reach statistical significance and the findings were not corroborated histopathologically (Table).

Table 60: Selected clinical observations in male and female mice (total number of observations)

Dose (ppm)	Male				Female			
	0	**25**	**50**	**100 (75)**	**0**	**25**	**50**	**100 (75)**
Alopecia	148 (3)	273 (6)	753 (6)	572 (7)	1140 (11)	351 (6)	315 (3)	49 (5)
Scruffy coat	547 (9)	216 (8)	167 (3)	172 (5)	374 (15)	90 (9)	352 (7)	302 (4)
Distended abdomen	675 (20)	539 (21)	388 (20)	273 (10)	391 (21)	669 (18)	307 (12)	339 (8)
Lethargy	7 (3)	ND	6 (2)	1 (1)	11 (7)	25 (5)	27 (4)	95 (6)
Bleeding vagina	ND	ND	ND	ND	118 (3)	114 (3)	33 (3)	12 (1)
Pale extremities	12 (1)	14 (3)	1 (1)	ND	63 (9)	58 (6)	24 (1)	5 (2)

Values in parentheses are the numbers of affected animals
ND; no data

Haematology:

A number of statistically significant ($p < 0.05$) or highly significant ($p < 0.01$) observations were recorded. At the 4 week test period, a decrease in erythrocyte count, haemoglobin concentration and haematocrit was recorded in 25 ppm males (erythrocyte count only) and high dose males. An increase in mean corpuscular haemoglobin was recorded in 25 and 50 ppm males. Haematocrit and mean cell volume were increased in 50 ppm females at weeks 13 (mean cell volume only) and 26 while mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were increased in 50 ppm and high dose males at 26 weeks. The erythrocyte count was increased while mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were decreased in high dose and 25 ppm males, respectively, at week 52. White blood cell count was decreased in 25 ppm males at week 104. Although a treatment-related effect on red cell mass is suggested during week 4, a number of factors mitigate against this conclusion. Of these findings, only the increase in mean corpuscular haemoglobin concentration in 50 ppm and high-dose males at week 26 was dose-related. Most of the significantly different values recorded were within the normal range (±95% confidence limits) of the historical controls for that laboratory. Values outside these ranges were sporadic and often associated with concurrent control values that were outside the historical control ranges as well.

PROJECT NO.: WIL-77033 *** HEMATOLOGY VALUES--SUMMARY OF MEANS ***
CLIENT: E.I. DUPONT DE NEMOURS LONG TERM FEEDING STUDY IN MICE WITH OXAMYL WEEK 4
CLIENT STUDY NO.: 10,963

	SEX	MALE				FEMALE			
	DOSE GRP	1	2	3	4	1	2	3	4
WBC	THOUS/UL	8.80	7.64	10.74	8.13	7.87	7.64	9.03	9.33
RBC	MIL/UL	9.17	8.40**	8.67	8.41**	8.72	8.54	8.69	8.66
HGB	G/DL	16.22	15.83	16.24	15.45*	16.39	16.12	16.30	16.49
HCT	%	50.68	47.82	49.67	47.08*	49.70	48.23	49.49	49.34
MCV	CUBIC U	55.32	57.03	57.45	55.99	57.03	56.59	56.96	57.38
MCH	UUG	17.72	18.91*	18.74*	18.41	18.81	18.91	18.76	19.18
MCHC	G/DL	32.08	33.14	32.70	32.91	33.00	33.43	32.94	33.42

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST
** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .01 LEVEL USING DUNNETT'S TEST

UNIT CODE: THOUS/UL = THOUSANDS/MICROLITER, MIL/UL = MILLION/MICROLITER, G/DL = GRAMS/DECILITER
CUBIC U = CUBIC MICRONS, UUG = MICRO MICRO GRAM

PROJECT NO.: WIL-77033 *** HEMATOLOGY VALUES--SUMMARY OF MEANS ***
CLIENT: E.I. DUPONT DE NEMOURS LONG TERM FEEDING STUDY IN MICE WITH OXAMYL WEEK104
CLIENT STUDY NO.: 10,963

	SEX	MALE				FEMALE			
	DOSE GRP	1	2	3	4	1	2	3	4
WBC	THOUS/UL	10.07	2.79**	6.28	6.73	5.07	3.87	5.45	4.53
RBC	MIL/UL	8.80	7.85	7.43	8.83	7.80	7.36	7.52	8.31
HGB	G/DL	14.76	14.44	13.25	15.12	14.18	13.53	13.42	14.81
HCT	%	42.41	39.15	37.80	42.86	41.15	39.01	39.17	42.42
MCV	CUBIC U	48.18	51.76	51.94	48.54	52.76	53.32	52.91	51.27
MCH	UUG	16.94	17.38	18.25	17.16	18.23	18.49	18.27	17.90
MCHC	G/DL	35.01	35.56	35.13	35.41	34.52	34.68	34.55	34.93

* = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .05 LEVEL USING DUNNETT'S TEST
** = SIGNIFICANTLY DIFFERENT FROM CONTROL GROUP AT .01 LEVEL USING DUNNETT'S TEST

UNIT CODE: THOUS/UL = THOUSANDS/MICROLITER, MIL/UL = MILLION/MICROLITER, G/DL = GRAMS/DECILITER
CUBIC U = CUBIC MICRONS, UUG = MICRO MICRO GRAM

Gross pathology and organ weights:

Mean absolute liver weight was significantly reduced in 50 ppm males. This isolated finding was not considered to be biologically significant. Mean relative kidney weight was highly significantly increased in high-dose males. However, in the absence of any histopathologically corroborative findings, this was not considered to be biologically significant. No statistically significant histopathological findings were recorded with the exception of a decrease in the incidence of chronic interstitial nephritis with increasing dose in terminal sacrifice males ($p < 0.05$) in all dose groups compared to controls.

Tissue masses were recorded in all groups with the exception of high dose females. A decrease in the number of affected animals and in the number of masses recorded with increasing dose was evident in females. The duration of the masses ranged from 3 to 281 days. There were no statistically significant differences in the incidence of masses in exposed groups compared to controls.

There were no statistically significant treatment-related increases in the incidence of neoplasia in any tissue examined. Several dose-related decreases in tumour incidence were recorded in both sexes. A number of tumour incidences showed an increasing trend with dose in males or females in specific tissues (Table 56).

Table 56: Tumour incidence in specific tissues in male and female mice (total number of animals affected)

		Male				Female			
		0	**25**	**50**	**100/75**	**0**	**25**	**50**	**100/75**
Found dead & Sacrificed moribund									
Adrenal gland	Nonthymic lymphoma	0	1	0	3	4	1	1	0
Lymph node (mandibular)	Nonthymic lymphoma	1	1	2	4	6	4	3	0
Lymph node (mesenteric)	Nonthymic lymphoma	0	2	3	4	5	3	1	0
Terminal Sacrifice									
Liver	Hepatoma	4	5	4	8	0	0	0	0
Lung	Adenoma	7	7	5	6	3	10	5	11

Differences from the control values were not statistically significant

Benign and malignant lung (bronchiolar-alveolar) tumours are thought to represent different stages of the same morphologic continuum and, along with certain liver tumours, are often difficult to classify as benign or malignant. Combining malignant and benign tumours of common histomorphogenic origin can reduce the effects of variability in diagnoses, allowing for more accurate comparisons across studies and to historical data. Rodent lymphoid neoplasms are often disseminated affecting multiple organs and in many instances the primary site of origin cannot be determined. It can be difficult to ascertain if a given occurrence of a lymphoid neoplasm in a specific organ is primary or metastatic. Consequently, tumour incidences for the respective tumour types were also tabulated in accordance with the recommendations of the National Toxicology Program (USA) which recommend that the benign and malignant forms of tumours of common histomorphogenic origin be combined for analysis (Table 57). Contemporary historical control data was not available. Historical tumour incidences for [redacted] CD-1 mice from 2-year studies conducted from 1978-1984 (the period during which this study was performed) were supplied as an appropriate reference population for this study.

Table 572: Total tumour incidence (found dead, sacrificed moribund and terminal sacrifice) of lung, liver, and lymphoid neoplasms in male and female mice (total number of animals affected)

	Male					Female				
Dose (ppm):-	**0**	**25**	**50**	**100/75**	**Historical Control** [b]	**0**	**25**	**50**	**100/75**	**Historical Control** [b]
Number of animals [a]	**76**	**78**	**79**	**74**		**79**	**79**	**75**	**77**	
Lymphoma	10 (13)	9 (12)	6 (8)	6 (8)	(7:4-11)	18 (23)	18 (23)	8 (11)	6 (8)	(13:7-25)
Pulmonary adenoma / adenocarcinoma	14 (18)	12 (15)	7 (9)	9 (12)	(20:11-34)	4 (5)	12* (15)	8 (11)	15* (19)	(20:6-39)
Hepatocellular adenoma / adenocarcinoma	8 (11)	8 (10)	9 (11)	10 (14)	(13:7-21)	0 (0)	1 (1)	0 (0)	0 (0)	(2:0-4)

Values in parentheses are the tumour incidences expressed as a percentage of the total number of animals
[a] Group numbers represent animals on study beyond week 6
[b] Historical control values are mean percentage occurrence of tumour type: occurrence range of tumour type
* Significantly different from controls by Fisher's exact test

There were no compound-related or statistically significant increases in liver and lymphoid tumours in either sex or in lung tumours in male mice. Increases in the incidences of these tumour types were similar to, or less than, historical control incidences. A statistically significant increase in lung tumours was present in 25 and 100/75 ppm females. However, these increases were not considered to be treatment related as no dose-response relationship was evident and the incidence was less than the overall historical control group mean. Also, there was no evidence of increased progression of benign to malignant lung tumours in treated groups and no increases in lung tumour incidence were documented in male mice. The tumour incidence in concurrent controls was less than in historical controls. Furthermore, lung (bronchiolar-alveolar) tumours are common spontaneous lesions in mice of this strain and age, particularly in studies of 2-years duration.

Although the cause of the unscheduled deaths was not determined, disseminated lymphoma (irrespective of subtype) can be presumed to have contributed to these deaths in both treated and control animals.

The total number of males with malignant tumours showed a decreasing trend with dose. Whatever the sacrifice time, the incidence of malignant tumours at the high dose was lower than in the controls in both sexes while the incidence of benign tumours in the terminally sacrificed animals was higher than in controls. The total incidence of benign tumours in females was increased in the dose groups compared to controls (Table 58).

Table 583: Tumour incidence in mice: combined data from animals found dead, sacrificed moribund and sacrificed at study termination

	Male				Female			
	0	**25**	**50**	**100/75**	**0**	**25**	**50**	**100/75**
Number of animals with tumours	35 (44)	36 (45)	28 (35)	30 (34)	32 (40)	43 (53)	29 (34)	29 (33)
Number of animals with multiple tumours	17 (21)	17 (21)	8 (10)	15 (17)	16 (20)	23 (28)	15 (18)	14 (16)

Number of animals with malignant tumours	16 (20)	15 (19)	9 (11)	9 (10)	21 (26)	28 (35)	13 (15)	13 (15)
Number of animals with benign tumours	20 (25)	23 (29)	22 (27)	21 (24)	11 (14)	22 (27)	18 (21)	18 (20)

Values in parentheses are the tumour incidences expressed as a percentage of the total number of animals

III. CONCLUSIONS

The long-term toxicity and carcinogenicity study HLO 252-81 and its Amndement No. 1 were originally submitted under EU Rev8 Point IIA 5.5.1 and conducted with test material pure oxamyl (PAI). Guidelines were not given. A review of this study indicates that it only partially meets the current guideline (B.32. Carcinogenicity test); deviations included:

- Body weight range (±20% overall mean weight on test Day 0) was not determined.
- Diet analysis data not reported, although it was indicated to have been conducted and reported separately by the sponsor
- Differential WBC counts were not evaluated in animals in deteriorating health or in control and high dose animals at end of study (24 months). However, WBC differential counts were evaluated at earlier time points, including 18 months (usual duration of mouse carcinogenicity study), and did not demonstrate any evidence of test substance-related effects on this parameter.
- Animals that died during the first few weeks of the study received a gross necropsy and tissue collection but tissues were not evaluated microscopically. However, it is reasonable to think that those animals have not been exposed long enough to develop test substance-related carcinogenic effects. Most of these early deaths were in the high dose group. This group had its dietary concentration lowered during week 6 due to unexpected high mortality. Extra animals were added to the study ~weeks 4–5 to ensure adequate group numbers. No test substance-related carcinogenicity was observed in animals retained for the full study length and/or their natural life span. Therefore the lack of evaluation of tissues from early deaths is not expected to have impacted evaluation of the carcinogenic potential of the test substance. Premature mortalities were a consequence of acute effects of Oxamyl and once the highest dose was reduced no further treatment-related effects on mortality were recorded.

For these reasons, the deviations from current guidelines cited above were not considered to have adversely influenced the outcome of this study. Therefore there is no need to reconduct such a kind of study.

The body weights of 50 ppm males were significantly reduced from week 8 – 81 while food consumption in high dose males was significantly reduced from week 11 – 84. Female body weights were significantly reduced in the 50 ppm and high dose groups from weeks 8 – 15 with further reductions in the 50 ppm group thereafter. Haematology findings are suggestive of a treatment-related effect on erythrocyte mass in high dose males at week 4. The **NOAEL for non neoplastic effects** in males and females is **25 ppm (4.2 mg/kg bw/day** in males and 5.2 mg/kg bw/day in females).

There were no compound-related or statistically significant increases in liver and lymphoid tumours in either sex or in lung tumours in male mice. Increases in the incidences of these tumour types were similar to, or less than, historical control incidences. A statistically significant increase in lung tumours was present in 25 and 100/75 ppm females. However, these increases were not considered to be treatment related as no dose-response relationship was evident and the incidence was less than the overall historical control group mean. Also, there was no evidence of increased progression of benign to malignant lung tumours in treated groups and no increases in lung tumour incidence were documented in male mice. The tumour incidence in concurrent controls was less than in historical controls. Furthermore, lung (bronchiolar-alveolar) tumours are common spontaneous lesions in mice of this strain and age, particularly in studies of 2-years duration. **Oxamyl was not oncogenic in this study.**

RMS comments and conclusion for renewal

This study is considered valid.

Study submitted in the EU Dossier in 2003 and included in the first EU approval review.

B.6.5.2/02

Reference: --	**Report:**	[redacted] (**1972**); Long term feeding study in rats and dogs with 1-(dimethylcarbamoyl)-N- (methylcarbamoyloxy)-thioformimidic acid, methyl ester (IND-1410) **DuPont Report No**.: **HLR 37-72** **Guidelines:** Not given (No one was in place at the time the study was carried out **GLP:Yes**

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: Not given
   Purity: 95.0%

Deviations: Based on OECD test guideline 453 the following deviations were identified in the study protocol:-

Rat:
- ± 20% weight variation on test day 0 was not determined.
- 36 animals of each sex were used per dose group, 6 animals for interim sacrifice and 30 animals for terminal sacrifice whereas the guideline recommends at least 50 animals for the terminal sacrifice alone.
- Survival in 1 male and 1 female control group at terminal sacrifice was < 50%.
- Blood clotting potential was not evaluated during the haematological investigations.
- Clinical chemistry evaluations were limited to liver function assays and the determination of blood acetylcholinesterase and aliesterase activity.
- The parathyroid, thymus, oesophagus, jejunum, ileum, rectum, skin and femur were not preserved for histopathological analysis.

Dog:
- Group mean body weights, food consumption, absolute and relative organ weights were not determined.
- Observations for clinical signs of toxicity were not performed.
- The statistics used in the evaluation of data were not described. The statistical significance of the differences between test and control group data values was frequently omitted.

I. MATERIALS & METHODS

Rat:

Weanling albino male and female rats (ChR-CD) were administered Oxamyl (DPX-D1410 technical; batch D1410-15; purity 95.0%) by dietary admixture at doses of 0, 50, 100 and 150 ppm for 2 years. These doses corresponded to a mean Oxamyl intake of 0, 2.3, 4.9, and 7.4 mg/kg bw/day for males and 0, 2.8, 6.1, and 9.3 mg/kg bw/day for females. The diet consisted of Purina Laboratory Chow (GPLC) containing 1% added corn oil. Diets were prepared freshly each week and refrigerated until used. Two control groups were used.

After a 15-day pre-test observations period, animals were chosen for the study based on their body weight gain and the absence of respiratory disorders and other clinical signs of disease. They were divided into 5 groups of 36 male and 36 female rats per group so that the average body weight of each group, within a sex, was approximately the same.

During the study the animals were regularly examined for clinical signs of toxicity. Body weights and food consumption were determined for each animal once a week up to 7 months, every fortnight from 7 to 12 months and monthly during the second year.

During the pre-test period, 6 male and 6 female rats were selected from each group for a haematological (erythrocyte, haemoglobin, hematocrit, and leukocyte count) evaluation. During the test period, peripheral blood was taken from 5 rats of each sex from the control groups and 10 rats of each sex from the 100 and 150 ppm groups at 1, 3, 6, 9, 12, 18 and 24 months. Urinalysis was performed on animals used for haematological evaluation. Urine specimens were collected for analysis 24 hours prior to blood sampling.

A clinical chemistry (alkaline phosphatase and alanine aminotransferase activities) evaluation was conducted on blood samples taken from the tail of 10 male and 10 female randomly- selected rats from the control, 100 ppm and 150 ppm groups (these animals were not those used for the haematological evaluations). The assays were performed at 1, 3, 6, 9, 12, 18 and 24 months.

Cholinesterase determinations were made on blood from the tails of 10 rats of each sex from the control and high dose groups on days 4 and 8 and after 1, 6, 12 and 24 months. The 100 ppm group was also investigated at 1 and 6 months. Aliesterase activity was determined in all of these animals at 1, 12 and 24 months.

At 1 year, an interim sacrifice by chloroform exposure reduced the size of each group to 30 animals. The animals were necropsied, absolute and relative organ weights recorded and a gross pathological and histopathological evaluation was conducted. The same procedure was followed for the remaining animals at the terminal (2-year) sacrifice. The tissues of animals that died during the study or were sacrificed in extremis were preserved for histopathological evaluation. Microscopic analysis of tissues from the control and 150 ppm groups was performed and, where a positive finding was recorded, in the 50 and 100 ppm groups as well. Urine and faeces were collected 24 hours prior to the interim and terminal sacrifices. These were pooled on the basis of sex and group and dispatched for residue analysis along with pooled samples of blood, liver, kidney, fat, muscle, brain and testes

Dog:

Beagle dogs aged 1 – 2 years were administered Oxamyl (DPX-D1410 technical; batch D1410-15; purity 95.0%) by dietary admixture at doses of 0, 50, 100 and 150 ppm for 2 years. These doses corresponded to a mean Oxamyl intake of 0, 1.3, 2.8 and 4.3 mg/kg bw/day in males and 0, 1.2, 3.0 and 4.2 mg/kg bw/day in females. Four dogs of each sex were allocated to each dose group. The diet consisted of Wayne Dog Food (Krums®) and was prepared freshly each week and refrigerated until used.

During the study the animals were examined daily for clinical signs of toxicity. Body weights and food consumption were determined for each animal once a week.

Three haematological, clinical chemistry and urinalysis evaluations were performed on each animal prior to the study and after 1, 2, 3, 6, 9, 12, 15, 18, 21 and 24 months of the study. Blood cholinesterase and aliesterase activity was measured prior to the study and at 1, 3, 12, 18 and 24 months.

After one year of the study an interim sacrifice of 1 male and 1 female dog from the control and high dose groups was performed. Sacrifice was carried out by electrocution and the animals necropsied. After 2 years all surviving animals were sacrificed in a similar manner. A gross pathological evaluation was conducted on every animal and organ weights recorded. Tissue specimens were preserved for microscopic investigation from control and high-dose animals only (liver, kidney and testis were examined microscopically from 50 and 100 ppm animals).

Samples of urine and faeces were collected 24 hours prior to the terminal sacrifice and dispatched for residue analysis together with samples of liver, kidney, muscle, fat, spleen, brain, blood and testes.

II. RESULTS

Rat

Mortality:

During the first year of the study, 4 male and 5 female rats died or were sacrificed in extremis; 3 control group females, 2 rats of each sex at 50 ppm, and 1 male each in the 100 and 150 ppm dose groups. These mortalities were not attributed to treatment. Over the entire treatment period, the incidence of animals found dead or killed *in extremis* was lower at 100 and 150 ppm than in the control groups (Table 64)

Table 64. Male and female rat mortalities recorded over the two year study period

	Male					Female				
Dose (ppm) :-	**0**	**0**	**50**	**100**	**150**	**0**	**0**	**50**	**100**	**150**
Killed *in extremis*	8	5	9	4	2	10	14	11	6	4
Found dead	8	9	8	6	3	6	7	4	4	3
By interim sacrifice; day 364	6	6	4	5	5	4	5	4	6	6
By terminal sacrifice; day 728	14	16	15	21	26	16	10	17	20	23

Body weight:

The body weights of 100 and 150 ppm males and females were consistently lower than control animals throughout the treatment period ($p < 0.05$). A dose-related decrease in body weight and body weight gain was recorded in both sexes at the interim sacrifice and in females at the terminal sacrifice. A decreasing trend in body weight and body weight gain with increasing dose was recorded in terminal sacrifice males (Table 65)

Table 595 Group mean body weights and body weight gains (grams) in male and female mice

	Male					Female				
Dose (ppm)	**0**	**0**	**50**	**100**	**150**	**0**	**0**	**50**	**100**	**150**
Mean body weight Interim: day 364	744	738	725 (-2)	697* (-6)	626* (-15)	446	469	440 (-4)	345* (-25)	328* (-28)
Mean body weight gain[#] Interim: day 364	604	596	583 (-3)	557 (-7)	487 (-19)	328	350	319 (-6)	226 (-33)	209 (-38)
Mean body weight Terminal: day 728	799	806	744 (-7)	750* (-7)	675* (-16)	640	635	588 (-8)	460* (-28)	415* (-35)
Mean body weight gain[#] Terminal: day 728	659	664	602 (-9)	610 (-8)	536 (-19)	522	516	467 (-10)	341 (-34)	296 (-43)

Values in parentheses are the % change in body weight / body weight gain compared to the mean control value
* Body weights at the 100 and 150 ppm dose levels were significantly lower than controls at $p < 0.05$.
[#] No statistical evaluation of body weight gain data was provided

Food consumption / efficiency:

Food consumption in high dose animals was slightly lower compared to controls. The differences were not statistically significant. No treatment-related effects on food efficiency were recorded.

Clinical observations:

Clinical signs of toxicity attributable to treatment were not recorded. The incidence of several clinical signs was the same across control and treatment groups.

Haematology:

No treatment-related effects on group mean haematological measurements were recorded. Decreases in erythrocyte count and haemoglobin concentration with time were observed in both sexes in the control and test groups and were probably age-related. A dose-related increase in leucocyte count was evident in females from months 1 to 9. However, the increase at 100 and 150 ppm was not significantly different from control values and this trend was not evident at 18 or 24 months.

Urinalysis:
No treatment-related effects on urinalysis measurements were recorded. Increases in urine volume and proteinuria with time in both sexes were probably age-related effects.

Clinical chemistry:
No treatment-related effects on alkaline phosphatase and alanine aminotransferase activities were recorded.

Mean blood cholinesterase activity at 150 ppm was significantly lower than in controls ($p < 0.05$) in males and females on days 8 and 4 of the study, respectively. These were isolated findings and were not corroborated by clinical signs of cholinesterase inhibition. Aliesterase activity at 150 ppm was consistently greater than in controls in both sexes. A time-related decrease in activity was also recorded in all groups investigated (Table 66)

Table 606. Blood cholinesterase and aliesterase activity (group mean values) in male and female rats

Activity	Dose (ppm)	Male						Female					
		Days		Months				Days		Months			
		4	8	1	6	12	24	4	8	1	6	12	24
Cholinesterase*	0	8.8	6.6	6.6	6.2	9.0	7.2	8.8	7.5	8.0	8.1	9.5	7.2
	100	-	-	6.8	7.5	-	-	-	6.3	7.7	7.7	-	-
	150	9.3	4.4	6.9	6.5	9.3	7.4	7.1	7.0	7.6	7.2	8.3	8.3
Aliesterase[#]	0	-	-	54	-	26	16	-	-	42	-	37	37
	100	-	-	-	-	-	-	-	-	-	-	-	-
	150	-	-	62	-	41	29	-	-	61	-	53	39

* Units of μM acetylcholine hydrolysed / 5 minutes / mL blood
[#] Units of μM CO_2 / 10 minutes / 50 mg blood; diethylsuccinate substrate

Gross pathology and organ weights and histopathology:

No treatment-related effects on absolute or relative organ weights were recorded in either sex at the interim or terminal sacrifices. A decreasing trend in absolute organ weight with increasing dose was evident for most organs in both sexes. However, this paralleled a decreasing trend in body weight with increasing dose. No trend in relative organ weight was evident and statistical significance in absolute or relative organ weight differences was not achieved.
Histopathological findings were not considered treatment-related. The incidence of pituitary and adrenal tumours in males was increased at 150 ppm compared to the control group incidences. No statistical or biological significance was attributed to this finding and the incidence of these tumours at 50 and 100 ppm was not determined. The degree of histopathological change associated with each tumour incidence was slight (Table 61).

Table 617 Selected tumour incidences (total number of animals with tumours out of the total number of animals examined) in male and female rats

	Dose (ppm)									
	Males					Females				
Tumour type	**0**	**0**	**50**	**100**	**150**	**0**	**0**	**50**	**100**	**150**
Pituitary	7/27 (26)	8/30 (27)	NE		10/29 (34)	15/29 (52)	17/30 (57)	NE		11/29 (38)
Adrenal	1/28 (4)	2/30 (7)	NE		4/30 (13)	3/29 (10)	1/30 (3)	NE		1/29 (3)

Values in parentheses are the percentage of the total number of animals examined with tumours NE; Not evaluated

Subsequently, an additional microscopic examination of tissues was conducted to include all tissues from the low and intermediate groups that were not examined in the original report (Pathology report no. 33-80). In addition, slides from tissues in the control and high dose groups were re-examined to confirm the original diagnosis. The tumour incidences for the pituitary and adrenal glands in male rats is presented in Table 62. Based on this supplemental review it was concluded that there were neither compound-related pathological changes nor carcinogenic effects observed in the treated rats.

Table 628 Incidences of primary pituitary and adrenal tumours in male rats (total number of animals with tumours out of the total number of animals examined): Supplemental Pathology Report 33-80

	Males				
Tumour type	**0**	**0 ppm**	**50**	**100**	**150**
Pituitary (Chromophobe	7/1	8/	6/	7/	8/
Adrenal (Pheochromocy	1/2	3/3	6/	2/	4/30 (13)

	Males				
Tumour type	**0 ppm**	**0 ppm**	**50**	**100**	**150**
Pituitary (Chromophobe adenoma)	7/18 (39)	8/	6/	7/	8/
Adrenal (Pheochromocytoma)	1/27 (4)	3/3	6/	2/	4/30 (13)

* Adrenal tumours in two animals of the IA control males were unspecified as to type. Because there were no other type of adrenal tumour noted in the male rats across all dose groups, it can be assumed that those tumours were also pheochromocytomas

Dog

Mortality:
No mortalities were recorded during the study.

Body weight:
No statistically significant differences in body weight were evident in the test groups compared to controls. Body weight gain was not determined.

Food consumption / efficiency:
Although food consumption on an individual animal basis varied from week to week, no treatment-related effects on food consumption were recorded. Food efficiency was not determined.

Clinical observations:
Clinical signs of toxicity were not recorded.

Haematology:
Occasional dose-related perturbations in haematological parameters were recorded. However, these effects were inconsistent and statistical significance was not achieved. Therefore, they were not considered treatment-related.

Urinalysis:
No treatment-related effects on urinalysis measurements were recorded.

Clinical chemistry:
Male blood cholesterol concentration was consistently elevated at 150 ppm throughout the test period. Female blood cholesterol concentration was also elevated except on month 24. Alkaline phosphatase activity was also consistently greater in 150 ppm males compared to controls from 1 month and in 150 ppm females at all but three of the evaluations. These parameters exceeded twice the standard deviation of the pre-test mean value (all dogs) most often in 150 ppm males. These effects, although suggestive of an effect on the liver, are not corroborated by gross or histopathological findings. Perturbations in other parameters occurred in control and test groups but were sporadic and unrelated to treatment. Blood cholinesterase and aliesterase activity was not affected by treatment.

Gross pathology, organ weights and histopathology:

No 'meaningful' differences between test and control group absolute or relative organ weights were recorded. The incidence of interstitial nephritis in females increased 0, 0, 1, 2 in the control, 50, 100 and 150 ppm dose groups. However, no statistical significance was attributed to this increase and it was not considered to be biologically significant.

III.CONCLUSIONS

Rat:

The body weights of 100 and 150 ppm males and females were significantly lower than controls throughout the treatment period. The mean blood cholinesterase activity at 150 ppm was significantly lower than that of controls in males and females on days 8 and 4 of the study, respectively. The NOAEL, based on decreased body weight, is 50 ppm (equivalent to 2.3 mg/kg bw/day in males and 2.8 mg/kg bw/day in females). Oxamyl was not oncogenic in rats in this study.

Dog:

Blood cholesterol concentration and alkaline phosphatase activity in the 150 ppm groups frequently exceeded the range established during the pre-test period in all dogs. Cholesterol levels in both sexes were greater at 150 ppm compared to controls throughout most of the test period. Based on this, the NOAEL is 100 ppm (equivalent to 2.8 mg/kg bw/day in males and 3.0 mg/kg bw/day in females). Oxamyl was not oncogenic in dogs in this study

RMS comments and conclusion for renewal

The study is dated and has a number of deviation from the currently adopted test guidelines. It can be accepted only as a supporing study.

B.6.5.3 Mechanism of action and supporting data

No additional studies were deemed necessary.

Oxamyl showed no evidence of oncogenicity in the long-term rat and mouse studies. The overall weight of the evidence suggests that oxamyl does not present a concern for carcinogenicity. Therefore, no study or supporting data on the mechanism of action was considered relevant.

B.6.5.4 Summary of long-term toxicity and carcinogenicity

Results of relevant long-term toxicity and carcinogenicity studies with oxamyl are summarised below.

Table 69 Summary of chronic toxicity studies for oxamyl

Type of study	Dose range tested	NOAEL (non neoplastic effects)		LOAEL (non neoplastic effects)		Target organ(s) and effects	Reference[a]
		ppm	mg/kg/d	ppm	mg/kg/d		
Oral (Feeding), 2-year Rat	0, 25, 50, 100, 150 ppm (equivalent to 0, 0.992, 1.97, 4.19, 6.99 mg/kg bw/day for males and 0, 1.32, 2.69, 6.73, 11.1 mg/kg bw/day for females)	50	m: 1.97 f: 2.69	100	m: 4.19 f: 6.73	Lower body weight, lower body weight gain and plasma cholinesterase inhibition at 100 ppm and higher	HLR 278–91
Oral (Feeding), 2-year Mouse	0, 25, 50, 100/75 ppm (equivalent to 4.2, 8.7, 13.5 mg/kg bw/day for males and 5.2, 10.8, 16.8 mg/kg bw/day for females)	25	m: 4.2 f: 5.2	50	m: 8.7 f: 10.8	Decreased body weights	HLO 252–81 HLO-81 Amendment No.1
Oral (Feeding), 2-year Rat and Dog	Rat: 0, 50, 100, 150 ppm (equivalent to 0, 2.3, 4.9, 7.4 mg/kg bw/day for males and 0, 2.8, 6.1, 9.3 mg/kg bw/day for females)	50	m: 2.3 f: 2.8	100	m: 4.9 f: 6.1	Decreased body weights	HLR 37–72 Only supporting
	Dog: 0, 50, 100, 150 ppm (equivalent to 1.3, 2.8, and 4.3 mg/kg bw/day for males and 1.2, 3.0, and 4.2 mg/kg bw/day for females)	100	m: 2.8 f: 3.0	150	m: 4.3 f: 4.2	Increased alkaline phosphatase and cholesterol levels	

[a] Summarised in Point CA 5.5 in this document, except where noted.

Oxamyl showed no evidence of oncogenicity in the long-term rat and mouse studies. The overall weight of the evidence suggests that oxamyl does not present a concern for carcinogenicity.

Oxamyl did not exhibit evidence of cumulative toxicity in chronic toxicity studies in rats, mice, or dogs. In one chronic toxicity/oncogenicity study in rats, decreased body weights, body weight gains, and food efficiency were noted in males and females at the mid- and high-doses (100 and 150 ppm), together with an increased incidence of several clinical signs of toxicity were recorded in one or both sexes at 100 and 150 ppm including hyperreactivity, swollen legs or paws, sore skin and alopecia. No pathological findings were recorded that corroborated these signs. In addition, plasma cholinesterase activity was inhibited in males at several sampling intervals and in females at one month at the mid- and high-doses, not associated with other treatment-related toxicity. No effects on rat brain or erythrocyte acetylcholinesterase activities were recorded. In another long-term rat study, decreased body weight was noted in males and females at the mid- and high-doses. Blood cholinesterase activity was decreased in males and females at the high-dose level. Oxamyl was not oncogenic in rats in long-term feeding studies. The incidence of adrenal tumours (pheochromocytoma) and pituitary

tumours (chromophobe adenoma) in male rats in the long-term study, was not significantly increased in the treated groups compared to controls nor was a dose-response trend evident.

In a mouse oncogenicity study, significant reductions in body weight in 50 ppm males and females and 75 ppm females were recorded associated with decreased food consumption, increased RBC count, and decreased corpuscular haemoglobin concentration levels were observed at the high-dose level. The combined incidences of lymphoma and pulmonary and hepatocellular adenoma / adenocarcinoma did not show a dose-related increase except in females where the incidence of pulmonary adenoma / adenocarcinoma was increased at the low and high doses compared to concurrent controls. However, as no dose- response relationship was evident and the incidence was less than in historical controls, they were considered unrelated to treatment.

In a two-year study in dogs, increased alkaline phosphatase and cholesterol levels were noted at the high-dose level. Oxamyl was not oncogenic in dog in this study.

Based on the results of chronic feeding studies in rats and mice, oxamyl is not a carcinogen, and the lowest NOAEL of approximately 1.97 mg/kg/day is based on lower body weights and body weight gain as well as plasma cholinesterase inhibition observed with dietary administration of 4.19 mg/kg bw/day and higher in rats. It is also evident that the duration of the study does not affect the reference value and that differences among species are limited.

B.6.6 Reproductive toxicity

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

Two multi-generation studies and a single-generation study were conducted in rats to assess the reproductive toxicity of oxamyl. Developmental studies in the rat and rabbit were also conducted. Summaries of these studies are provided below.

B.6.6.1 Generational studies

Two-generation reproductive study in the rat

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.6.1/01

Reference: --	**Report:**	[redacted] (1990); Reproductive and fertility effects with oxamyl (IN D1410) multi-generation reproduction study in rats **DuPont Report No.:** HLR 423-90 **Guidelines:** U.S. EPA 83-4 (1982) GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Deviations: Based on current OECD guideline 416 the following deviations were identified in the study protocol.

- Food consumption was not measured during lactation.
- Organs other than reproductive were not weighted (brain, liver, kidneys, spleen, pituitary, thyroid and adrenals).
- Histopathology was not conducted on cervix. Detailed histopathology was not conducted on the testes or on the ovary.

- Sperm analysis was not carried out.
- Oestrus cycle length and normality was not evaluated.
- Individual pups were not weighed at birth (but were weighed on day 4 and day 21 post- partum). Only litters were weighed at birth and on days 7 and 14.
- Physical development of pups (e.g. ear and eye opening, tooth eruption and hair growth, sexual maturation parameters and functional investigations) were not assessed.

I. MATERIALS & METHODS:

Groups of 30 male and 30 female Sprague Dawley rats each received Oxamyl (IN D1410; batch IND-1410-196; purity 99%) in the diet at concentrations of 0, 25, 75, and 150 ppm for approximately 70 days pre-mating and during mating, gestation and lactation. F1 weanlings (30/sex/group; 1 rat/sex/each litter where possible) were randomly selected and continued on their respective diets. These F1 animals were bred to produce F2 offspring when all had been on test at least 105 days.

On day 0 postpartum, live and dead pups in each litter were counted; live pups were weighed collectively by sex. On day 4 postpartum, pup counts and weights were determined, and the litters were culled randomly to 8 (4/sex) where possible. Litter counts and weights were also determined after culling on day 4 and on days 7 and 14 postpartum. On day 21 postpartum (weaning), individual pup weights were taken. On days 0, 4, 7, 14, and 21, offspring were individually examined for abnormal behaviour and appearance.

Clinical signs of toxicity were evaluated in all P1 and F1 rats weekly. All P1 and F1 rats were weighed weekly during premating. During gestation and lactation, females were weighed on days 0, 7, 14, and 21 of each period while males were weighed weekly. Food consumption was determined weekly throughout premating for P1 and F1 rats. Food consumption of pregnant rats was also recorded on days 0, 7, and 14 of gestation. All parental rats (P1 and F1) were evaluated for gross lesions. Tissues from the control and 150 ppm P1 and F1 parental rats were examined histologically including testes, epididymus, prostate, seminal vesicles and coagulating gland from males, ovaries, uterus and vagina from females and pituitary and all gross lesions from both sexes. Twenty F1 (from those not continuing to the next generation) and F2 weanlings per sex per group were also necropsied. Reproductive function indices were calculated for P1 and F1 parental rats. Mean Oxamyl intake was calculated and is presented in Table below.

Table 70: Mean intake of Oxamyl in male and female rats of the P_1 and F_1 generations

Dosage	Generation	Pre-mating growth period (mg/kg bw/day)		Gestation period (mg/kg bw/day)
		Males	Females	Females
25 ppm	P_1	1.43	1.74	1.74
	F_1	1.97	2.28	1.77
75 ppm	P_1	4.22	5.69	5.41
	F_1	6.17	7.48	5.43
150 ppm	P_1	8.74	12.8	12.2
	F_1	14.5	18.8	14.4

Analysis showed that the test substance was homogenously distributed, stable for at least 14 days and within 83% and 106% of nominal.

II. RESULTS

Clinical signs (P1 and F1):

A significant upward trend in the incidence of hyperreactivity in F1 males and females (premating) was observed which was statistically significant in males at 150 ppm. During the premating and/or gestation periods, 75 and 150 ppm F1 females showed a statistically significant dose-related increase in alopecia.

Body weights (P1 and F1):

In the 150 ppm group, three F1 males and one P1 female were found dead during the study (not considered to be treatment related). Mean body weight was consistently decreased in P1 males throughout the premating and post

mating periods beginning from day 7, in both 75 and 150 ppm groups (approximately 7% and 15%, respectively). Mean body weight gain was significantly reduced for the premating period (33%) and the post mating period (29%) in the 150 ppm males. Mean body weight gain was also reduced in 75 ppm males for the overall premating period.

Body weight was reduced in F1 males at 75 and 150 ppm throughout the entire premating and post mating periods (approximately 13% and 30%, respectively). Body weights of F1 males at ≥75 ppm on day 0 of F1 reflect the lower body weights of the male F1 pups on day 21 of weaning. Mean body weight gains of F1 males were significantly reduced at 150 ppm for the overall premating and mating periods (30% and 38% less than controls respectively). Mean body weight gains were significantly reduced from ≥75 ppm for the overall premating period (13% less than controls).

Mean body weight and mean body weight gains were significantly reduced from 75 ppm during the entire premating period (days 0-70) in P1 females. Mean body weight was reduced in 75 and 150 ppm F1 females from days 0-105 (reflecting the reduced weight of weanlings females on day 21). There was a significant reduction (5%) in mean body weight at 25 ppm on day 7. Otherwise weights were not affected at this dose level. Overall body weight gain was reduced from ≥75 ppm for the entire premating period.

Mean body weight was reduced in the P1 and F1 females in both 75 and 150 ppm groups (10% and 17% respectively). A reduction in body weight gain of 26% and 23% was recorded during the gestation periods 14-21 days and 0-21 days, respectively, in 150 ppm P1 females. A reduction in body weight gain was also recorded during the gestation periods 0-7, 14-21 and 0-21 days in F1 females. During the lactation period, mean body weights were decreased in the P1 and F1 females compared to controls (by approximately 8% and 15%, respectively). Mean body weight gains were significantly increased from ≥75 ppm in both F1 and P1 for the entire lactation period.

Food consumption and food efficiency:

In 75 ppm and 150 ppm males, significant reductions in food consumption and food efficiency were noted in P1 (9% and 12%, respectively) and F1 animals (10% and 14%, respectively) during premating. Significant reductions in food consumption were noted in F1 and P1 females at 150 ppm throughout most of the premating period. Food consumption was significantly increased at 150 ppm in the F1 females. Food efficiency was significantly decreased in 150 ppm P1 and F1 females during gestation.

Gross pathology, organ weights, histopathology (P1 and F1):

No test substance-related gross pathological lesions were observed at necropsy in parental rats. Mean relative testes weight in 75 and 150 ppm P1 and F1 males was statistically significantly increased. This was attributed to the lower mean body weights seen in these animals when compared to controls. Absolute testes weights were not different from controls. No other changes in mean organ weights were apparent at any dietary concentration. No treatment-related microscopic findings were noted in P1 or F1 adult males and females.

Reproductive parameters:

There were no statistically significant treatment-related effects on mating, fertility, and gestation length in the P1 and F1 rats (Table). The mating index ranged from 96.7% to 100% for treated animals in the P1 generation, compared to 96.7% in controls. Fertility index ranges from 79.3 to 90% in treated animals compared to 89.7% in controls. Likewise in the F1 generation, the mating and fertility parameters were not different from controls. The low fertility index in the F1 control was within the reported control range of 46.6% to 86.2% over the previous four years. Gestation lengths were not different from controls in either generation.

Table 71: Summary of reproductive parameters

Generation	Dose levels (ppm)	Mating index (%)	Fertility index (%)	Gestation length (days)
P_1	0	96.7	89.7	22.4
	25	96.7	79.3	22.7
	75	100	90.0	22.4
	150	100	86.7	22.7
F_1	0	76.7	69.6	22.7
	25	90.0	81.5	22.5
	75	96.7	72.4	22.3
	150	96.7	69.0	22.6

Offspring parameters; litter size and pup survival:

In the 150 ppm F1 and F2 generations, statistically significant decreases were recorded in the mean number of pups (males and females combined) born per litter or born alive per litter and in the number of pups alive on day 4 preculling. In the F2 offspring, treatment-related reduction in 4-day survivability was considerably more marked than in the F1 offspring (Table and Table).

A significant treatment-related reduction in F1 and F2 male and female pup mean body weights occurred at ±75 ppm at all time points throughout most of the lactation period. Sexes were analysed separately and in combination (Table).
A significant increase in the number of pups observed with no milk spot was noted in the F2 offspring at 150 ppm and was considered related to low pup weight. There were no compound-related lesions identified at necropsy of either F1 or F2 weanings.

Table 72: Summary of litter size data

Indices	P_1 Pups				F_2 Pups			
	0 ppm	25 ppm	75 ppm	150 ppm	0 ppm	25 ppm	75 ppm	150 ppm
	Mean number of pups/litter				**Mean number of pups/litter**			
Born	13.9	13.4	14.5	11.7*	13.5	14.4	12.4	11.8
Born Alive	13.4	13.2	14.3	11.2*	12.7	14.1	12.4	10.7
Day 4 Preculling	13.3	12.8	14.0	8.6*	12.2	13.5	11.1	5.3*
Day 4 Postculling	7.7	7.9	7.9	7.2	7.7	8.0	7.4	5.9*
Day 7	7.7	7.9	7.8	7.1	7.7	8.0	7.0	5.9*
Day 14	7.7	7.9	7.8	7.1	7.7	8.0	7.0	5.6*
Day 21	7.7	7.9	7.8	7.1	7.7	8.0	7.0	5.6*
	Mean number of male pups/litter				**Mean number of male pups/litter**			
Born	6.5	6.4	7.5	6.1	6.7	6.9	6.2	5.3
Born Alive	6.4	6.4	7.4	5.8	6.4	6.6	6.2	4.8*
Day 4 Preculling	6.4	6.3	7.3	4.5*	6.2	6.3	5.7	2.4*
Day 4 Postculling	3.7	3.7	4.0	3.3	4.1	4.1	3.7	2.8*
Day 7	3.7	3.7	3.9	3.2	4.1	4.1	3.5	2.8*
Day 14	3.7	3.7	3.8	3.2	4.1	4.1	3.5	2.7*
Day 21	3.7	3.7	3.8	3.2	4.1	4.1	3.5	2.6*
	Mean number of female pups/litter				**Mean number of female pups/litter**			
Born	7.2	7.0	7.0	5.7*	6.7	7.5	6.2	6.3
Born Alive	7.0	6.7	6.9	5.3*	6.3	7.5	6.2	5.9
Day 4 Preculling	7.0	6.5	6.7	4.1*	6.0	7.2	5.4	2.9*
Day 4 Postculling	4.0	4.1	3.9	4.0	3.6	3.9	3.8	3.3
Day 7	4.0	4.1	3.9	3.9	3.6	3.9	3.5	3.3
Day 14	4.0	4.1	3.9	3.9	3.6	3.9	3.4	3.1
Day 21	4.0	4.1	3.9	3.9	3.6	3.9	3.4	3.1

* Statistically significant difference from control at $p < 0.05$

Table 633: Summary of litter data

Indices	F_1 Pups				F_2 Pups			
	0 ppm	25 ppm	75 ppm	150 ppm	0 ppm	25 ppm	75 ppm	150 ppm
Sex ratio (males)[a]	0.46	0.47	0.51	0.47	0.51	0.47	0.49	0.47
Gestation index[b]	100	100	100	100	100	100	100	100
Mean % born alive	95.8	98.4	98.7	95.3	94.6	97.9	100*	90.3
0-4 day viability	99.8	93.5	97.6	78.3*	95.4	95.7	91.1	54.2*
Lactation index[c]	100	100	98.6	98.4	100	100	92.8	89.3
Litter survival[d]	100	95.7	100	88.5	100	100	100	65.0*

[a] Ratio of males born to total number of sexable pups born.
[b] Percent litters delivered having at least one live pup.
[c] Mean percent survival from day 4 postculling to day 21.
[d] Percent viable litters born with at least one pup alive on day 21.
* Statistically significant from control ($p \leq 0.05$).

Table 644: Summary of mean pup weights

Indices	F_1 generation				F_2 generation			
	0 ppm	**25 ppm**	75 ppm	**150 ppm**	**0 ppm**	**25 ppm**	**75 ppm**	**150 ppm**
	Mean pup weights (g)				**Mean pup weights (g)**			
Day 0	6.9	6.9	6.4*	6.0*	7.1	6.9	6.6	5.7*
Day 4 Preculling	11.8	11.7	10.1*	9.6*	12.4	11.6	10.2	8.4*
Day 4 Postculling	11.7	11.6	10.2*	9.6*	12.5	11.7	10.3	8.5*
Day 7	19.0	18.4	16.2*	14.4*	19.9	18.9	16.2*	13.1*
Day 14	38.2	36.8	33.4*	29.5*	38.6	37.8	32.3*	28.1*
Day 21	59.9	58.6	52.9*	44.9*	63.2	61.8	51.5*	43.8*
	Mean male pup weights (g)				**Mean male pup weights (g)**			
Day 0	7.0	7.1	6.6*	6.2*	7.3	7.0	6.8	5.9*
Day 4 Preculling	12.1	12.0	10.4*	9.9*	12.6	11.8	10.3*	8.6*
Day 4 Postculling	12.0	12.0	10.3*	9.9*	12.7	11.8	10.3*	8.6*
Day 7	19.5	19.0	16.7*	15.0*	20.1	19.3	17.0	13.8*
Day 14	39.1	37.7	33.9*	30.5*	38.4	38.3	33.1*	28.0*
Day 21	61.2	60.5	54.1*	46.3*	64.4	63.0	53.2*	44.1*
	Mean female pup weights (g)				**Mean female pup weights (g)**			
Day 0	6.7	6.7	6.3*	5.8*	6.9	6.7	6.4	5.5*
Day 4 Preculling	11.5	11.3	9.9*	9.4*	12.2	11.4	10.0	8.3*
Day 4 Postculling	11.5	11.2	10.0*	9.4*	12.4	11.5	10.2	8.5*
Day 7	18.5	17.8	15.7*	14.1*	19.6	18.6	16.3	12.5*
Day 14	37.4	35.9	32.8*	28.8*	38.8	37.3	32.8*	26.7*
Day 21	58.6	56.7	51.5*	43.8*	61.7	60.6	51.4*	41.9*

* Statistically significant difference from control at $p < 0.05$

III. CONCLUSIONS

The generational study HLR 423-90, originally submitted under EU Rev8 Point IIA 5.6.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 83-4 (1982). A review of this study indicates that it partially meets the current guideline B.35, deviations include:

- Food consumption was not measured during lactation.
- Organs other than reproductive were not weighted (brain, liver, kidneys, spleen, pituitary, thyroid, and adrenals).

- Histopathology was not conducted on cervix. Detailed histopathology was not conducted on the testes or on the ovary.
- Sperm analysis was not carried out.
- Estrus cycle length and normality was not evaluated.
- Individual pups were not weighed at birth (but were weighed on Day 4 and Day 21 postpartum).
- Only litters were weighed at birth and on Days 7 and 14.
- Physical development of pups (*e.g.*, ear and eye opening, tooth eruption and hair growth, sexual maturation parameters and functional investigations) were not assessed.

Parental NOEL: 25 ppm (equivalent to 1.43 mg/kg bw/day). **This was based on adverse treatment-related effects on body weight and weight gains at ≥ 75 ppm.**

Reproductive NOEL: 25 ppm (equivalent to 1.43 mg/kg bw/day). This was based on body weight effects ≥75 ppm and decreased pup survival at 150 ppm. A reduction in the mean number of pups (males and females combined) born per litter and pups born alive per litter was noted in both generations. This may reflect an adverse effect on *in utero* development or a fertility related effect.

However, reconduct is unlikely to yield a significantly different result because no reproductive effects were observed in this study, and the NOAEL is based on a slight decrease in body weight, which is consistently observed in other feeding studies. Moreover, the most sensitive effect of oxamyl is neurotoxicity (acetylcholinesterase inhibition). It is therefore unlikely that new or more reproductive toxicity information will impact the departure points for the risk assessments.

RMS comments and conclusion for the renewal

Despite the deviations with respect to the current guideline, it was not consider appropriate to ask for the an additional study for this end-point which is unlikely to yield a significantly different result. This consideration is based on the fact that no reproductive effects were observed in all the available studies and no endocrine activity has been demonstrated for oxamyl as part of the U.S. EPA Endocrine Disruptor Screening Program Tier 1 (see below Section B 6.8.3 for summaries of these studies). Moreover, the NOAEL in this study is based on a slight decrease in body weight, which is consistently observed in other feeding studies; the most sensitive effect of oxamyl is neurotoxicity (acetylcholinesterase inhibition). It is therefore unlikely that new or more reproductive toxicity information will impact the departure points for the risk assessments.

Therefore the study is considered valid.

A 1-generation study rat study was submitted in the EU Dossier in 2001 and included in the first EU approval review ([redacted] [redacted] **1969:** Ninety-day feeding study in rats with 1-(dimethylcarbamoyl)-N-(methylcarbamoyloxy)-thioformimidic acid, methyl ester [IND-1410]: Reproductive and fertility effects with Oxamyl (IN D1410) Multigeneration reproduction study in rats). However the study is dated and present several deviations with respect to the current standard for this kind of studies and was considered as supplementary to the above described study also in the previous evaluation. It was therefore not described in details here and only the agreed conclusions reported in the first EU approval review are reported.

Conclusion:

This study is considered supplementary to B.6.6.1.1 above. Very limited pup/litter parameters were measured allowing little insight into the effect on pup weight, e.g., pups/litters were not weighed at birth or on day 4 but only at weaning. The study gives some information on the effect of feeding of the test substance on fertility and reproductive performance over one generation. As pup body weights at weaning were significantly reduced at all dose levels, no NOEL can be proposed for offspring. The reproductive NOEL was 50 ppm based on an apparent decrease in litter size and the number of live pups at 100 and 150 ppm.

Separate male and female studies

The two-generation reproduction study in the rat reported above (HLR 432-90) provided sufficient information to fully interpret the effects of oxamyl on reproduction, and no further studies were deemed necessary.

Three segment designs

The two-generation reproduction study in the rat reported above (HLR 432-90) provided sufficient information to fully interpret the effects of oxamyl on reproduction, and no further studies were deemed necessary. A 3-generation study was submitted in the EU Dossier in 2001 and included in the first EU approval review ([redacted] **1972:** Long-term feeding study in rats and dogs with 1-(dimethylcarbamoyl)-N-(methylcarbamoyloxy)-thioformimidic acid, methyl ester (IND-1410)). However the study is dated and present several deviations with respect to the current standard for this kind of studies and was considered as supplementary to the above described study also in the previous evaluation. It was therefore not described in details here and only the agreed conclusions reported in the first EU approval review are reported.

Conclusion:

This study is considered supplementary to B.6.6.1.1 above. Very limited pup/litter parameters were measured allowing little insight into the effect on pup weight, e.g., pups/litters were not weighed at birth or on day 4 but only at weaning. The study gives some information on the effect of prolonged feeding of the test substance on fertility and reproductive performance.

The parental NOEL is taken from the feeding phase of this study where mean body weights were reduced at ≥ 100 ppm (50 ppm (equivalent to 2.3 mg/kg bw/day in males and 2.8 mg/kg bw/day in females). The offspring NOEL is 50 ppm (2.5 mg/kg bw/day) – based on pup body weight effects at ≥100 ppm. The reproductive NOEL is 50 ppm, based on a possibly treatment-related reduction in litter size at higher doses.

Dominant lethal assay for male fertility

The two-generation reproduction study in the rat reported above (HLR 432-90) provided sufficient information to fully interpret the effects of oxamyl on reproduction, and no further studies were deemed necessary.

Cross matings of treated males with untreated females and *vice versa*

The two-generation reproduction study in the rat reported above (HLR 432-90) provided sufficient information to fully interpret the effects of oxamyl on reproduction, and no further studies were deemed necessary.

Effects on spermatogenesis

Sufficient information to fully interpret the effects of oxamyl on reproduction has been provided, and no further studies were deemed necessary.

Effects on oogenesis

Sufficient information to fully interpret the effects of oxamyl on reproduction has been provided, and no further studies were deemed necessary.

Sperm motility, mobility, and morphology

Sufficient information to fully interpret the effects of oxamyl on reproduction has been provided, and no further studies were deemed necessary.

Investigation of hormonal activity

The two-generation reproduction study in the rat reported above (HLR 432-90) provided sufficient information to fully interpret the effects of oxamyl on reproduction, and there were no effects in the study that could be potentially attributed to effects on hormone activity. In addition, a full battery of endocrine studies has been completed for oxamyl as part of the U.S. EPA Endocrine Disruptor Screening Program (EDSP Tier 1). Please see Section B 6.8.3 for summaries of these studies. None of the studies, including the reproduction, developmental, and specific endocrine studies provided any evidence that oxamyl has the potential to perturb endocrine systems. Therefore, it can be concluded that oxamyl is not an endocrine active substance.

B.6.6.2 Developmental toxicity studies

Teratogenicity test by the oral route in the rat

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.6.2/01

Reference: --	**Report:**	[redacted] (1988); Teratogenicity study of IN D1410-196 in the rat **DuPont Report No.:** HLR 473-88 **Guidelines:** U.S. EPA 83-3 (1982) GLP: Yes

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.2%

Deviations: Based on OECD test guideline 414 the following deviations were identified in the study protocol.

- Dosing was from days 7 to 16 of gestation instead of the more usual treatment period of days 5 – 16/17 in the rat
- It was reported that gravid uterine weight was recorded but the data were not included in the report

I. MATERIALS AND METHODS:

Groups of 25 female Sprague-Dawley rats each received daily oral doses of Oxamyl (DPX- D1410 technical; batch IN-D1410-196; purity 97.2%) in distilled water at concentrations of 0, 0.2, 0.5, 0.8, and 1.5 mg/kg bw/day by gavage from days 7 to 16 of gestation. The dosing volume was 10 mL/kg bw.

The day copulation was confirmed (by the presence of a vaginal plug) was designated as day 1 of gestation. The animals were observed for clinical signs of toxicity in the morning on days 1-22 of gestation and at approximately 2 hours post dosing on days 7-16 of gestation. Body weights were recorded on days 1, 7-17, and 22 of gestation. Food consumption was recorded on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, and 22 of gestation. Females were sacrificed on day 22 of gestation and were examined for gross lesions. The liver, gravid uterus and non-gravid uterus were weighed. Uteri were examined and types of nidations (live and dead foetuses and resorptions) were recorded. Resorptions were classified. Corpora lutea were counted. Foetuses were weighed and examined externally, viscerally, and skeletally.

II. RESULTS

Maternal effects:
General observations:

There was no test substance-related mortality. Clinical signs of toxicity were significantly increased in the high dose group during treatment including tremors, salivation, diarrhoea, eye discharge and wet areas of the body. Tremors were observed in 4/25 animals of the 0.8 mg/kg bw/day rats during the dosing period. Significant dose-related decreases in body weight changes were noted at 0.8 and 1.5 mg/kg bw/day during the dosing period (reduced by ~21% and 30%, respectively, compared with controls) and specifically on days 7-9 and 9-11 (Table 65). A slight (not statistically significant) decrease in body weight gain was noted in 0.5 mg/kg bw/day rats during the dosing period (reduced by ~9% compared to controls). Significant dose-related effects on food consumption were noted in 0.8 and 1.5 mg/kg bw/day rats during the dosing period.

Table 655: Maternal body weight gain

Group (mg/kg bw/day)	Mean maternal weight gain (g)	% relative to control
0	57.3	-
0.2	57.1	99.7
0.5	52.4	91.4
0.8	45.2*	78.9
1.5	40.0*	69.8

* Statistically significant difference from control at $p < 0.05$

Reproductive outcomes:

No treatment-related effects on reproductive parameters (sex ratio, pregnancy rate, incidence of total resorptions, incidence of early deliveries, corpora lutea, or the number of stunted foetuses) were observed.

Gross pathology, organ weights:

No test substance-related gross pathological lesions were observed at necropsy. An upward trend in increased relative liver weights was observed (no statistical significance was noted in any group).

Foetal effects:

Significant reductions in mean foetal body weights were noted at 0.8 and 1.5 mg/kg bw/day (Table a). A slight, statistically significant decrease in mean foetal body weight was also noted at 0.5 mg/kg bw/day. A number of malformations were seen in treated groups including one hydrocephalic foetus at 0.20 mg/kg bw/day, one foetus with fused vertebral arches at 0.05 mg/kg bw/day, one foetus with fused ribs at 0.80 mg/kg bw/day and one foetus with gastroschisis at 1.5 mg/kg bw/day. There was no apparent relationship with treatment.

The spectrum of variations (distended ureters, small renal papillae, retarded ossification) seen in treated groups was not apparently different from controls. There was a higher incidence of rudimentary cervical rib in the 1.5 mg/kg bw/day ((1(4), 2(1), 0, 0, 14(7)) and also a genesis of the vertebra ((0, 0, 0, 0, 9(4) at 0, 0.2, 0.5, 0.8, and 1.5 mg/kg bw/day respectively), which were not statistically significant.

Table 666a: Summary of reproductive parameters

Parameter		Dosage (mg/kg bw/day)				
		0	**0.2**	**0.5**	**0.8**	**1.5**
Number copulated		25	25	25	25	25
Number pregnant		24	25	24	23	24
Number of deaths		0	0	0	0	0
Number with total resorptions		0	0	0	0	0
Number of early deliveries		0	0	2[a]	1[b]	0
Number of litters		24	25	24[a]	22[b]	24
MEANS PER LITTER:						
Live foetuses	Total	15.4	14.6	14.7	16.1	15.4
	Males	7.4	7.5	7.3	8.5	7.0
	Females	8.0	7.1	7.4	7.6	8.4
Resorptions	Total	0.6	0.8	0.3	0.5	0.8
	Early	0.6	0.8	0.3	0.5	0.8
	Late	0.0	0.0	0.0	0.0	0.0
Nidations		16.0	15.4	15.0	16.7	16.1
Mean corpora lutea		17.4	16.8	16.4	17.5	16.8
Percent resorptions per litter	Total	4.0	6.3	2.4	3.3	4.8
	Early	4.0	6.3	2.4	3.0	4.8
	Late	0.0	0.0	0.0	0.3	0.0
Mean foetal weight (g)[c]	Total	5.33	5.24	5.12*	4.97*	4.96*
	Males	5.47	5.38	5.25*	5.09*	5.09*
	Females	5.21	5.11	5.01*	4.83*	4.84*
Number of stunted foetuses		0	0	2	1	0

[a] One dam delivered 5 pups and another dam delivered 2 pups just prior to sacrifice; reproductive data are included in calculations.

[b] One dam delivered entire litter early on day 22 of gestation. The litter is not included in number of litters reported and reproductive data are not included in calculations.
[c] Significant trend (Jonckheere's test) at $p \leq 0.05$
*Significantly different from controls at $p < 0.05$

Table 676b: Summary of fetal malformations

INCIDENCE OF FETAL MALFORMATIONS[a,b]

GROUP:	I	II	III	IV	V
DOSE (MG/KG/DAY):	0	0.2	0.5	0.8	1.5
EXTERNAL					
No. examined[c]	370[24]	365[25]	352[24]	355[22]	369[24]
Umbilicus - Gastroschisis	...[d]	...	...	...	1(1)
No. affected[c]	0[0]	0[0]	0[0]	0[0]	1[1]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.3
(S.D.)					(1.28)
(S.E.)					(0.26)
VISCERAL					
No. examined	190[24]*	189[25]	183[24]	186[22]	193[24]
No. affected	0[0]	0[0]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.0	0.0	0.0	0.0
HEAD					
No. examined	191[24]	189[25]	182[24][f]	185[22][g]	192[24][h]
Brain - Hydrocephaly	...	1(1)	...	...	...
No. affected	0[0]	1[1]	0[0]	0[0]	0[0]
Mean percent affected per litter	0.0	0.5	0.0	0.0	0.0
(S.D.)		(2.50)			
(S.E.)		(0.50)			
SKELETAL					
No. examined	370[24]	365[25]	352[24]	355[22]	369[24]
Rib - Fused	...	...	...	1(1)	...
Vertebra - Fused	...	...	1(1)	...	...
No. affected	0[0]	0[0]	1[1]	1[1]	0[0]
Mean percent affected per litter	0.0	0.0	0.2	0.3	0.0
(S.D.)			(1.20)	(1.25)	
(S.E.)			(0.25)	(0.27)	
TOTAL NUMBER AFFECTED	0(0)	1(1)	1(1)	1(1)	1(1)
MEAN PERCENT AFFECTED	0.0	0.3	0.2	0.3	0.3
PER LITTER (S.D.)		(1.33)	(1.20)	(1.25)	(1.28)
(S.E.)		(0.27)	(0.25)	(0.27)	(0.26)

FOOTNOTES:

[a] Individual fetal alterations are presented in Appendix J.

[b] The incidence of malformations was not signfficantly different ($p \leq 0.05$) between test and control groups.

[c] Numbers examined and affected, including numbers affected with the listed malformations, are expressed as Fetuses [Litters] or Fetuses (Litters).

[d] For ease of reading, zeros have been replaced with ellipses for specific malformations.

[e] No visceral exam was conducted on Fetus #11 from Dam #437726, due to technical error.

[f] One stunted fetus (#6) from Dam #437624 did not have a head exam.

[g] One stunted fetus (#2) from Dam #437785 did not have a head exam.

[h] One malformed fetus (#6) from Dam #437721 did not have a head exam.

III. CONCLUSIONS

The developmental toxicity study HLR 473-88, originally submitted under EU Rev8 Point IIA 5.6.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 83-3 (1982). A review of this study indicates that it partially meets the current guideline B.31 with the following deviations:

- Dosing was from Days 7 to 16 of gestation instead of the more usual treatment period of Days 5 - 16/17 in the rat
- It was reported that gravid uterine weight was recorded but the data were not included in the report

It was considered that the deviations from the current standard would not impact significantly on the results of the study.

There was a dose-related adverse effect on maternal body weight gain, which was statistically significant at 0.8 and 1.5 mg/kg bw/day and a decrease at 0.5 mg/kg bw/day which was probably also related to treatment. Clinical signs of toxicity, including signs of cholinesterase inhibition, were increased at 1.5 mg/kg bw/day with some tremors also at 0.8 mg/kg bw/day. There was a treatment and dose-related reduction in foetal body weights from 0.5 mg/kg bw/day.

The **developmental NOAEL was 0.2 mg/kg bw/day** based on the slight but significant reduction in foetal weight from 0.5 mg/kg bw/day. The **maternal NOAEL was 0.5 mg/kg bw/day** based on slight reduction in mean weight gain at this dose level and significant reduction at 0.8 and1.5 mg/kg bw/day.

RMS comments and conclusion for the renewal
This study is considered valid.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.6.2/02

Reference: --	**Report:**	Munley, S.M. (1998); DuPont's position on foetal weight changes in rats following developmental toxicity testing with oxamyl **DuPont Report No.:** DuPont-1954 **Guidelines:** Not given

1. Test material: Not applicable
   Lot/Batch #: Not applicable
   Purity: Not applicable

The developmental toxicity position paper DuPont-1954 was originally submitted under EU Rev8 Point IIA 5.6.2. Guidelines were not given. This study is a supplemental study accompanying the DuPont report above (HLR 473-88).

As reported in the first EU approval review, the notifier supplied this position paper in which they 'review and re-evaluate compound-related effects reported for the rat developmental toxicity study with Oxamyl as evaluated in Section B.6.6.2.1 above. In the analysis of the study data and historical control data on mean foetal weight, they stressed that the foetal weight effect seen at 0.5 mg/kg bw/day was marginal and occurred in the presence of a slight, statistically insignificant, non-adverse effect on maternal weight gain seen at the same dose. They conclude that the conceptus was found to be sensitive only at dose levels at or near those that were also toxic to the dam. This conclusion was considered reasonable in the 2003 evaluation.

An additional developmetal toxicity study in rat was submitted in the EU Dossier in 2001 and included in the first EU approval review ([redacted]**, 1971:** Teratogenic study in rats with S-methyl-1-dimethylcarbamoyl-N- [(Methylcarbamoyl)oxy] thioformimidate (IND-1410)). However the study is dated and present several deviations with respect to the current standard for this kind of studies; those tested are higher and a NOAEL for maternal toxicity could not be established, since body weight was affected in all treated group. Despite this no developmental effects were reported. It can therefore be considered only as supplementary to

the above described study. It was not described in details here and only the agreed conclusions reported in the first EU approval review are reported.

Conclusion:

The test substance was administered in the diet to pregnant dams from days 6 to 15 of gestation at dose levels up to 300 ppm (equivalent to 20.5 mg/kg bw/day). Maternal body weight gain was adversely affected in all treated groups. Therefore, a NOEL for maternal toxicity could not be established. Developmental parameters were unaffected by treatment. Therefore, the developmental NOEL is 20.5 mg/kg bw/day.

Teratogenicity test by the oral route in the rabbit

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.6.2/03

Reference:	**Report:**	[redacted] (1980); Teratology study in rabbits - Oxamyl **DuPont Report No.:** HLO 801-80 **Guidelines:** Not given
--		

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.1%

Deviations: Based on OECD test guideline 414 the following deviations were identified in the study protocol.

- Relative humidity (up to 92%) exceeded the maximum recommended 70%.
- Only 17 impregnated does were assigned to each treatment group. The guideline recommends 20 does with implantation site at necropsy with 17 as the minimum requirement. In this study the number of pregnant does was less than 17 in all treated groups.
- Animals were first weighed on day 6 of treatment rather than day 3.
- The heads of one third of the foetuses were examined whereas the guideline recommends examining 50% of the foetuses.

I. MATERIALS AND METHODS

Groups of 17 pregnant female New Zealand White rabbits each received Oxamyl (DPX- D1410 technical; batch D1410-196; purity 97.1%) in distilled water at concentrations of 0, 1, 2, and 4 mg/kg bw/day orally by gavage from days 6 to 19 of gestation in a dosing volume of 1 mL/kg bw/day. Females were artificially inseminated and the day after artificial insemination was designated as day 0 of gestation. Animals were observed for clinical signs of toxicity daily. Individual body weights were recorded on days 6, 11, 15, 19, and 29 of gestation. Individual food consumption was recorded on days 7 through 29 of gestation. On day 29 of gestation, female rabbits were sacrificed, the foetuses were taken by caesarean section and the does were examined for visceral gross pathology. The ovaries and uterus of each rabbit were weighed and the number of corpora lutea and implantations determined. Each foetus was examined externally, body weight and crown-rump distance determined and a visceral examination conducted. The heads of one-third of the foetuses were removed and sectioned by Wilson's technique for examination. All foetuses were evaluated for skeletal anomalies following fixation and staining.

II. RESULTS

Maternal effects

General Observations:

No treatment-related mortality was observed. One low dose pregnant rabbit and one high dose non-pregnant rabbit died during the study (deaths were considered likely to be due to intubation error). No treatment-related clinical signs of toxicity were noted. Sporadic incidences of eye discharge and red stains in the tray under the cage were noted in the control and mid-dose groups. Statistically significant decreases in body weight gain were

noted in the 2 and 4 mg/kg bw/day groups during gestation days 6-19. No treatment-related effects on food consumption were noted.

Table 687: Maternal body weight gain

Gestation days	Mean body weight change (g)			
	Control	1 mg/kg bw	2 mg/kg bw	4 mg/kg bw
0-6	58	66	59.7	113.1
6-19	169.5	154.4	65.2*	55.6*
19-29	138.4	144.1	157.1	218.6
0-29	365.9	368.7	282	387

* Level of significance not given.

Gross pathology, organ weight:

No test substance-related gross pathological lesions were observed at necropsy.

Reproductive outcomes:

No treatment-related effects were noted on pregnancy rate, numbers of corpora lutea and implantations or implantation efficiencies. No treatment-related effects were noted on foetal body weights and lengths. In the 2 and 4 mg/kg bw/day groups, a slightly increased incidence of resorption was noted (statistically insignificant; 2 high-dose does had 100% resorption; 1 dead foetus in the mid-dose group). Foetal viability was subsequently slightly lower at 4 mg/kg bw/day.

Table 698: Summary of pregnancy status, litter data and ovarian and uterine weights in maternal rabbits

Parameter	Dosage (mg/kg bw/day)			
	0	1	2	4
Number of dam	17	17	17	17
Number of pregnant dams	17	15	15	13
Pregnancy rate (%)	100	88	88	76
Number surviving to day 29	17	16	17	16
Survival rats (%)	100	94	100	94
Mean number of:				
Corpora lutea	11.1	9.9	11.1	9.8
Implantations	7.4	7.0	7.0	7.0
Resorptions	0.8	0.5	1.0	1.2
Foetuses live	6.6	6.6	5.9	5.8
dead	0.0	0.0	0.1	0.0
Indices calculated per litter basis				
Mean implantation efficiency (%)	68.9	72.9	65.2	74.2
Mean incidence of resorption (%)	10.4	8.7	15.9	24.8
Mean incidence of foetal mortality (%)	0.0	0.0	1.4	0.0
Mean incidence of foetal viability (%)	89.7	90.8	82.7	75.2
Live foetuses				
Mean male body weight (g)	44.49	45.08	45.04	43.08
Mean female body weight (g)	43.35	43.83	43.26	44.76
Mean male crown-rump distance (cm)	9.24	9.33	9.37	9.28
Mean female crown-rump distance (cm)	9.31	9.27	9.32	9.45
Percent males	63.2	50.7	60.3	54.5
Mean ovarian and uterine weights with foetuses (g)	418.7	416.6	384.9	413.5
Mean ovarian and uterine weights without foetuses (g)	62.4	62.3	59.7	53.5

Foetal effects:

Some visceral variations and malformations were observed and occurred in both controls and the low dose group and were not apparently treatment-related. Skeletal variations occurred in controls and the low and mid

dose group equally and were not seen in the high dose group. A single skeletal malformation was found in the low dose group (incomplete vertebral column).

Table 709: Summary of visceral findings in foetuses

Findings	Dosage (mg/kg bw/day)			
	0	1	2	4
Number of foetuses examined	113	90	89 (1)	75
Number that appeared normal	110	79	86 (1)	74
Number of heads examined	38	30	30 (1)	23
Number that appeared normal	38	28	30 (1)	23
Number with variants	2	10	3	1
Number with anomalies	1	3	0	0

Table 80: Summary of skeletal findings in foetuses

Number of foetuses examined	113	90	89 (1)	75
Number that appeared normal	108	82	86 (1)	75
Number with variants	5	8	3	0
Number with anomalies	0	1	0	0

Values in parenthesis are the number of dead foetuses examined or foetuses with a particular finding

Table 71: Mean incidence for visceral and skeletal findings in foetuses per litter (values are means calculated on a per litter basis)

Findings	Dosage (mg/kg bw/day)			
	0	1	2	4
VISCERAL				
Number with variants	0.1	0.7	0.1	0.1
Number with anomalies	0.2	0.2	0.0	0.0
Incidence of variants (%)	2.0	10.4	7.5	0.9
Incidence of anomalies (%)	0.6	4.1	0.0	0.0
SKELETAL				
Number with variants	0.3	0.6	0.2	0.0
Number with anomalies	0.0	0.1	0.0	0.0
Incidence of variants (%)	6.4	10.5	8.1	0.0
Incidence of anomalies (%)	0.0	1.4	0.0	0.0

III. CONCLUSIONS

The developmental toxicity study HLO 801-80 was originally submitted under EU Rev8 Point IIA 5.6.2 and conducted with test material pure oxamyl (PAI). Guidelines were not given. A review of this study indicates that it partially meets the current guideline B.31 with the following deviations:

- Relative humidity (up to 92%) exceeded the maximum recommended 70%.
- Only 17 impregnated does were assigned to each treatment group. The guideline recommends 20 does with implantation site at necropsy with 17 as the minimum requirement. In this study the number of pregnant does was less than 17 in all treated groups.
- Animals were first weighed on Day 6 of treatment rather than Day 3.

- The heads of one third of the fetuses were examined whereas the guideline recommends examining 50% of the fetuses.

It was considered that the deviations from the current standard would not impact significantly on the results of the study and reconducting such a kind of study is unlikely to yield a different result because the most sensitive effect of oxamyl is neurotoxicity (acetylcholinesterase inhibition) and effects on the foetus were only observed in the presence of toxicity to the dam. It is therefore unlikely that new or more developmental toxicity information will impact the departure points for the risk assessments.

The NOEL for maternal toxicity was 1 mg/kg bw/day and was based on statistically significant decreased body weight gain at 2 and 4 mg/kg bw/day. The NOEL for developmental toxicity was 2 mg/kg bw/day, based on slightly increased percent resorptions at 4 mg/kg bw/day.

RMS comments and conclusion for the renewal

This study is considered valid.

B.6.6.3 Summary of reproduction and developmental studies

Results of the reproduction and developmental studies with oxamyl are summarised in the following table.

Table 82 Summary of reproduction and developmental studies for oxamyl

Type of study	Species	Doses/ concentra-tions tested	NOAEL	LOAEL	Target organ(s) and effects	Reference[a]
Two-generation reproduction	Rat	0, 25, 75, 150 ppm	**Parental**: 25 ppm (**1.43 mg/kg bw/day**) Pup: 25 ppm **Reproductive**: ≥150 ppm (HDT)[b] (**≥12.2 mg/kg bw/day**)	Parental: 75 ppm (4.22 mg/kg bw/day) Pup: 75 ppm Reproductive: >150 ppm (HDT)[b] (>12.2 mg/kg bw/day)	↓ parental body weight/body weight gain, food consumption & efficiency ↓ pup body weight No effects on reproduction	HLR 423–90
Three-generation reproduction	Rat	0, 50, 100, 150 ppm	Parental: 50 ppm (2.5 mg/kg bw/day[c]) Pup: 50 ppm Reproductive: ≥150 ppm (HDT) (≥7.5 mg/kg bw/day[c])	Parental: 100 ppm (4.9 mg/kg bw/day[c]) Pup: 100 ppm Reproductive: >150 ppm (HDT) (>7.5 mg/kg bw/day[c])	↓ parental body weight ↓ pup body weight ↓ litter size No effects on reproduction	HLR 37–72[d]
One-generation reproduction	Rat	0, 50, 100, 150 ppm	Parental: 50 ppm (3.9 mg/kg bw/day) Pup: 50 ppm Reproductive: >150 ppm (14.0 mg/kg bw/day)	Parental: <50 ppm (3.9 mg/kg bw/day) Pup: <50 ppm Reproductive: >150 ppm (14.0 mg/kg bw/day)	↓ parental body weight gain, blood in urine, ↓ absolute organ weight ↓ litter size & live pups No effects on reproduction	HLR 308–69[d]
Teratology —gavage	Rabbit	0, 1, 2, 4 mg/kg bw/day	**Maternal: 1 mg/kg bw/day Foetal: 2 mg/kg bw/day Developmental: ≥4 mg/kg bw/day** (HDT)	Maternal: 2 mg/kg bw/day Foetal: 4 mg/kg bw/day Developmental: >4 mg/kg bw/day (HDT)	↓ maternal body weight gain ↑ resorptions No effects on development	HLO 801–80

Table 82 Summary of reproduction and developmental studies for oxamyl (continued)

Type of study	Species	Doses/ concentra-tions tested	NOAEL	LOAEL	Target organ(s) and effects	Reference[a]
Teratology —gavage	Rat	0, 0.2, 0.5, 0.8, 1.5 mg/kg bw/day	**Maternal: 0.5 mg/kg bw/day Foetal: 0.2 mg/kg bw/day Developmental: ≥1.5 mg/kg bw/day (HDT)**	Maternal: 0.8 mg/kg bw/day Foetal: 0.5 mg/kg bw/day Developmental: >1.5 mg/kg bw/day (HDT)	↓ maternal body weight gain; clinical signs ↓ foetal body weight No effects on development	HLR 473–88
Teratology —diet	Rat	0, 50, 100, 150, 300 ppm (0, 4.5, 8.2, 11.6, 20.5 mg/kg bw/day)	Maternal: 50 ppm (4.5 mg/kg bw/day) Foetal and Developmental: ≥300 ppm (≥20.5 mg/kg bw/day) (HDT)	Maternal: 100 ppm (8.2 mg/kg bw/day) Foetal and Developmental: >300 ppm (>20.5 mg/kg bw/day) (HDT)	↓ maternal body weight gain No effects on development	HLR 5-71[d]

[a] Summarised in Point CA 5.6.2 in this document, except where noted.
[b] HDT=Highest dose tested.
[c] Calculated using standard conversion method (ppm x 0.05 mg/kg bw/day).
[d] Study submitted in the EU Dossier in 2001 and included in the first EU approval review. Now cited in Reference Lists "Documents Not Submitted and Not Relied Upon." Included for comparison purposes.

A two-generation rat reproduction study conducted with oxamyl did not reveal evidence of reproductive toxicity. Parental toxicity consisted of decreased body weight, body weight gain, and food consumption in males and females of both generations at the mid- and high-dose levels (75 and 150 ppm, respectively). A statistically significant increase in the incidence of alopecia was noted in F1 females at the mid- and high-dose during the premating and gestation periods, and an increased incidence of hyperactivity was noted in F1 males and females (statistically significant in males) at the high dose. The NOEL for parental toxicity was 25 ppm (equivalent to 1.43 mg/kg bw/day). No treatment-related reproductive effects were noted; however, pup toxicity (decreased viability and decreased body weight) occurred in the 150 ppm group at the same dose levels causing parental toxicity. A statistically significant decrease in the mean number of pups (males and females combined) born per litter, born alive per litter and alive on day 4 preculling in the F_1 and F_2 generations was also recorded at 150 ppm. A significant treatment-related reduction in F_1 and F_2 male and female pup mean body weights also occurred at ≥ 75 ppm throughout most of the lactation period. Adverse foetal effects were seen at dose levels (≥ 75 ppm) which also caused significant effects on parental weight gain giving a developmental NOEL of 25 ppm (equivalent to 1.43 mg/kg bw/day).

A three-generation rat reproductive study with oxamyl was conducted as part of a chronic toxicity study, which due to some limitations can only be considered as a supporting study. No treatment-related reproductive effects were noted. Parental toxicity consisted of decreased body weight in males and females at the mid- and high-dose levels of 100 and 150 ppm, respectively, and decreased food consumption and blood cholinesterase activity in males and females at the high dose. Slightly decreased weanling weight was observed at 100 and 150 ppm but was reversible when pups were transferred to control diet. The offspring NOEL was 50 ppm (2.5 mg/kg bw/day) and was based on the reduction in pup body weight at ≥100 ppm. The reproductive NOEL was 50 ppm (2.5 mg/kg bw/day) based on a possibly treatment-related reduction in litter size at ≥100 ppm, higher than the value derived from the key study, described above.

The potential to cause developmental toxicity was investigated in rat and rabbit developmental studies. Oxamyl showed no evidence of developmental toxicity in the rat and rabbit developmental studies. In the rabbit developmental study, maternal toxicity consisted of decreased body weight gain during the treatment period at

the mid- and high-dose levels (2 and 4 mg/kg bw/day, respectively). Slightly decreased foetal viability was noted at the high dose, but this finding was not statistically significant.

In the rat developmental study in which oxamyl was administered as a bolus dose by gavage, maternal toxicity consisted of transient tremors and decreased body weight changes and food consumption at the two highest dose levels (0.8 and 1.5 mg/kg bw/day, respectively). Slight but statistically significant decreases in foetal body weight were noted at dose levels of ≥0.5 mg/kg bw/day; the slightly reduced foetal body weight for the 0.5 mg/kg bw/day group was within the laboratory's historical control range, and corresponds to a equally slight but not statistically significant decrease body weight in the dams. It is considered that the appropriate foetal NOAEL for this study is 0.5 mg/kg bw/day, which is the same as the NOAEL for maternal toxicity, also in view of results obtained in another a rat developmental study (only supporting) in which oxamyl was administered by diet. Significantly less maternal toxicity was observed, again primarily consisting of effects on body weight and nutritional parameters at 100, 150, and 300 ppm (8.2, 11.6, and 20.5 mg/kg bw/day, respectively). No effects were observed on the reproductive outcome or in the foetuses. The NOEL in this study is 50 ppm (4.5 mg/kg bw/day), approximately 10-fold greater than the NOEL obtained in the rat developmental study when oxamyl was administered by gavage.

Oxamyl is therefore, not considered to be uniquely toxic to the conceptus and is not considered to have any unique toxicity to the reproductive system.

B.6.7 Neurotoxicity

Oxamyl has been evaluated in a number of studies to check for the neurotoxic potential in mammals. These studies are summarised below. Oxamyl is a carbamate insecticide that, unlike some organophosphates, does not pose a risk for delayed neurotoxicity. It is now well understood textbook information that carbamates do not bind to neurotoxic esterase (NTE) (Casarett and Doull's Toxicology, 1996 The Basic Science of Poisons, Fifth Edition, Ed. C. Klaasen, McGraw-Hill, New York) and, therefore, do not cause organophosphate-induced neuropathy. Regardless, a test for delayed neurotoxicity in hens was conducted some years ago (summarised below). Although this study does not meet the current testing guidelines for the 28-day delayed neurotoxicity study in hens, it does provide scientific evidence that oxamyl does not cause delayed neurotoxicity at dose levels up to 40 mg/kg bw.

B.6.7.1 Neurotoxicity studies in rodents

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and some were found not to be relevant to the risk assessment in the context of this assessment. A reference for those articles can be found in Appendix 1. Neurotoxicity studies that were found to be relevant have been summarized below.

Acute neurotoxicity - rat

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.1/01

Reference:	**Report:**	[redacted] (1997); Acute oral neurotoxicity study of oxamyl technical in rats **DuPont Report No.:** HLR 1118-96 **Guidelines:** U.S. EPA 81-8 (1982) GLP: YES
--		

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.3%

I.MATERIALS & METHODS

Crl:CD®BR rats were quarantined and observed for body weight, food consumption and signs of disease or injury. After 6 –7 days, healthy animals were chosen for the study.

In a pilot study, 6 male and 6 female Crl:CD®BR rats received Oxamyl (DPX-D1410 technical; batch D1410-196; purity 98.3%) in aqueous solution by oral gavage (adjusted for purity) at doses of 0, 0.1, 0.75, 1.5 and 2.0 mg/kg bw (males) or 0, 0.1, 0.25, 0.75 and 1.5 mg/kg bw (females). No compound-related clinical signs or effects on motor activity were recorded in either sex at 0.1 mg/kg bw or in females at 0.25 mg/kg bw. A dose-related increase in clinical signs was recorded at the higher dose levels which peaked between 30 and 60 minutes after dosing. Based on these results and previously-reported LD50 values of 3.1 mg/kg bw (males) and 2.5 mg/kg bw (females) the doses selected for the main study were 0, 0.1, 1.0 and 2.0 mg/kg bw (males) and 0, 0.1, 0.75 and 1.5 mg/kg bw (females).

In the main study, single doses of aqueous Oxamyl solution were administered by gavage at 10 mL/kg to groups of 42 male and female rats that had been fasted overnight. Aliquots of dosing solutions were analysed for concentration verification. Purina® Certified Rodent Chow® #5002 and water were available ad libitum except for prior to dose administration (water only available) and during neurobehavioural assessments. Food and water were periodically analysed for contamination. All animals were weighed immediately before dosing.

Twelve rats/sex/group were used in neurotoxicity studies. These animals were weighed on days 2, 8 and 15. Food consumption was determined for the neurotoxicity subgroup only for the 1-2, 2-8 and 8-15 day intervals. Clinical signs were recorded once each day until day 15 with the exception of days on which a neurobehavioural evaluation was conducted. Six of the neurotoxicity subgroup rats/sex/group were euthanized on day 16 by pentobarbital anaesthesia followed by exsanguination and whole body perfusion fixation. These animals were subjected to a gross examination prior to perfusion and nervous tissue extracted. A histopathological examination was performed on tissue samples from control and high-dose groups.

Neurobehavioural evaluations (Functional Observation Battery (FOB) and Motor Activity (MA)) were performed on the neurotoxicity subgroup prior to dosing, at 30 – 60 minutes on test day 1 and on test days 8 and 15. A pre-test neurobehavioural test battery (FOB and MA) was performed to establish baseline measurements. The FOB assessment was conducted prior to the MA test and involved evaluations inside the home cage, outside the home cage while being handled and in a standard 'open field' arena. MA was assessed using automated activity monitors (Coulbourn® Instruments) over a 1-hour period.

Thirty rats/sex/group were used for clinical pathology. Clinical signs were recorded for the clinical pathology subgroup before dosing and 30 – 60 minutes after dosing on day 1 and also on either day 2 or 15. Plasma and erythrocyte acetylcholinesterase activity was assayed in blood sampled from the orbital sinus of each rat while under light CO_2 anaesthesia. Samples were collected prior to dosing and 30 – 60 minutes after dosing on day 1 from 10 rats/group, on day 2 from another 10 rats/group and on day 15 from the remaining 10 rats/group. Shortly after sampling, the rats were sacrificed and the brains removed and frozen at –70°C for subsequent cholinesterase analysis.

Positive control neurobehavioural and neuropathological data were provided from in-house studies on acrylamide, carbaryl, 1,1'-[2,2,2-trichloroethylidine]-bis [4-chloro-benzene] (DDT), d-amphetamine and trimethyl tin.

II. RESULTS

Oxamyl concentration in the dose samples ranged from 91.3 – 106% of nominal values. It was not detected in control samples.

Mortality:

One male rat in the clinical pathology subgroup dosed at 2 mg/kg bw died on test day 1. Low posture, tremors and salivation were recorded in this animal and the mortality was considered to be treatment-related. Brain acetylcholinesterase determination and gross necropsy were not conducted on this animal.

Clinical signs:

Clinical signs of toxicity were recorded from the outset in both the neurotoxicity and clinical pathology subgroups. However, statistical significance was reported in the latter subgroup only. These signs were indicative of cholinesterase inhibition and were recorded in both sexes at the mid and high doses. They were recorded on day 1 of the study and were considered to be treatment-related (Table 72). Similar clinical signs

were noted in the neurobehavioural study rats on day 1. By day 2, the rats had recovered and no further clinical signs of toxicity were recorded for the remainder of the study.

Table 723: Statistically significant clinical observations in male and female rats in the clinical pathology subgroup on day 1 of the study (N = 30 / group).

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
Low Posture	0	0	21*	28*	0	0	13*	30*
Salivation	0	0	22*	30*	0	0	4*	24*
Tremors	0	0	30*	30*	0	0	29*	30*
Wet Perineum	0	0	0	0	0	0	4*	7*

* Statistically significant difference from control at $p < 0.05$ by Cochran-Armitage Test for Trend

Body weight / body weight gain:

No significant differences in absolute body weight were recorded. Body weight gain in the 2 mg/kg bw male rats of the clinical pathology subgroup was significantly reduced on days 1 and 2 of the study to 51% of the control value. A reduction of 16% in body weight gain was also recorded in 1 mg/kg bw males although statistical significance was not achieved. These reductions were corroborated by a decrease in food consumption and were considered to be treatment-related. Recovery in body weight gain was recorded in both groups over days 2 – 8. High-dose female rats of the clinical pathology subgroup experienced a decrement in body weight gain of 12% compared to controls over test days 1 and 2. Statistical significance was not achieved and food consumption over the same period was higher than control values. However, the reduction in body weight gain was considered to be toxicologically significant. Body weight gain recovered to control levels over days 2 – 8. Female body weight gain was significantly reduced in the 0.75 mg/kg bw group over days 2 – 8. However, this was an isolated finding and not considered to be treatment-related (Table 73).

Table 734: Mean body weight gain in male and female rats (grams)

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
Test day 1-2	28.1	26.3	23.5	13.9*	18.1	18.3	17.9	15.9
2-8	42.3	41.7	43.0	46.7	20.4	20.5	15.3*	21.1
8-15	39.9	40.7	41.7	40.7	14.7	16.2	15.5	15.8
1-15	110.3	108.7	108.2	101.3	53.2	55.0	48.7	52.8

* Statistically significant difference from control at $p < 0.05$ by Cochran-Armitage Test for Trend

Food consumption / efficiency:

Mean daily food consumption was significantly reduced in mid and high-dose males over days 1 – 2. These reductions were corroborated by decreases in body weight gain at these dose levels and were considered treatment-related. Recovery in food consumption was recorded in both groups over days 2 – 8. No effects on food consumption were recorded in females (Table 74).

Table 745: Mean daily food consumption in male and female rats (g)

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
Test day 1-2	29.8	28.4	25.9*	17.7*	19.1	20.0	19.6	22.4
2-8	26.6	26.6	25.2	24.8	18.2	18.3	17.1	18.1
8-15	24.8	25.6	24.8	25.0	16.5	17.0	16.2	16.6
1-15	25.9	26.2	25.1	24.4	17.4	17.8	16.8	17.6

* Statistically significant difference from control at $p < 0.05$

Functional observation battery:

Forelimb and hind limb grip strength was compromised in high-dose males and females on day 1 reaching statistical significance in males only (compared to control). This finding was considered to be biologically significant in both sexes. No effects on forelimb and hind limb grip strength were recorded on days 8 and 15. The incidence of hind limb foot splay was slightly higher in high dose males compared to control or baseline values on day 1 and was considered to be treatment related. Effects on this parameter were not recorded in females or in males on days 8 and 15.

Significant deviations in other FOB endpoints were effectively confined to the day 1 evaluation (Table 75). A reciprocal relationship between curled-up posture and palpebral closure in 1 and 2 mg/kg bw males and 0.75 and 1.5 mg/kg bw females indicated that this posture was not due to sleeping. The statistically significant increase in the incidence of dilated pupils in 1 and 2 mg/kg bw males and 1.5 mg/kg bw females is a paradox as cholinesterase inhibition is associated with 'pin-point' pupils. The incidence of defecation and urination in males in the open field evaluation was the opposite to that recorded by the motor activity monitor. Perturbations in FOB parameters in 1 and 2 mg/kg bw males and 0.75 and 1.5 mg/kg bw females are generally consistent with the effects of cholinesterase inhibition.

Table 756: Functional observation battery – selected findings in male and female rats on day 1 of the study

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
HOME CAGE								
Posture - Curled-up / asleep / head hung	7	3	10	11*	2	2	8*	9*
Palpebral closure - Appeared to be sleeping	4	2	0*	0*	1	1	1	0
OUTSIDE THE HOME CAGE								
Ease of removal - No resistance	0	0	1	5*	0	0	0	3*
Ease of handling - Too easy	0	0	1	4*	0	0	0	2
Fur appearance - Slightly soiled	0	0	7*	11*	0	0	2	7*
Lacrimation - Slight	0	0	4*	8*	0	0	0	5*
Salivation – Severe (drooling)	0	0	3*	10*	0	0	0	4*
Exophthalmus – Dilated pupils facing upward	0	0	4*	6*	0	0	1	4*
OPEN FIELD								
Righting reflex - Slow	0	0	3*	5*	0	0	5*	3*
Laboured breathing - Present	0	0	1	7*	0	0	0	2
Convulsions – Slight tremors	0	0	11*	12*	0	0	8*	12*
Coordination – Unbalanced / swaying	0	0	2	7*	0	0	1	5*
Gait - Abnormal (dragging limbs / hopping)	0	0	3*	9*	0	0	2	8*
Locomotion – Somewhat impaired	0	0	3*	9*	0	0	0	5*
Arousal – Very low (stupor)	0	0	3*	3*	0	0	0	1
Vocalisation – Only when handled	1	1	0	0	3	3	0	0*
Defecation - Present	3	2	0	0*	0	0	1	0
Urination - Present	4	1	0*	0*	0	0	1	0
Splayed limbs - Present	0	0	6*	11*	0	0	4*	9*
Approach & touch – No reaction	1	0	1	2	0	0	1	3*
Tail pinch – No response	0	1	6*	9*	0	0	1	9*
MOTOR ACTIVITY MONITOR								
Urination - Present	6	9	12*	11*	3	7	11*	8*
Pupillary response - Absent	0	1	2	6*	0	1	0	5*

* Statistically significant difference from control by Cochran-Armitage test for trend at $p < 0.05$

Motor activity:

A number of statistically significant findings were recorded in males on day 1 only. The mean duration of movement and the mean number of movements were decreased compared to the control value in 1.0 and 2.0 mg/kg bw males during the first and second 10-minute intervals. The mean number of movements was also decreased in 2.0 mg/kg bw males during the fifth 10-minute interval. Total decrease in both parameters was significant in 2.0 mg/kg bw males and in 1.0 mg/kg bw males for the mean number of movements. The occurrence of statistically significant decreases in mean duration of movement in females was the same as that

in males with the addition of a decrease in 1.5 mg/kg bw females during the third 10- minute interval. The mean number of movements was significantly lower in 1.5 mg/kg bw females during the first, second and third 10-minute intervals and over the hour evaluation period. Increases in the mean number of movements in females on days 8 and 15 were sporadic and not considered to be treatment-related. The decreases recorded in both parameters in the higher dose groups of both sexes on day 1 correlate with the perturbations recorded in neurobehavioural parameters in the same groups. Therefore, the decreases are considered to be treatment-related.

Cholinesterase inhibition:

Plasma, erythrocyte and half brain cholinesterase activities were significantly decreased compared to controls in both sexes in the mid and high dose groups on day 1 (Table 76). These decreases were part of a dose-response trend and the magnitude of the deceases was approximately 40%. All animals had recovered by day 2. Other deviations in cholinesterase activity compared to control values were recorded on days 2 and 15. These were sporadic and of no toxicological concern.

Table 767: Mean plasma and erythrocyte cholinesterase activity (units / litre) in male and female rats prior to treatment (Day 0) and 30-60 minutes after treatment (Day 1)

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
Erythrocyte acetylcholinesterase activity								
Day 0	1634	1510 (-7.6)	1676 (+2.6)	**2102*** (+28.6)	2070	2298 (+11)	2020 (-2.4)	2054 (-0.8)
Day 1	2138	2000 (-6.5)	**914*** (-57.2)	**800*** (-62.6)	2010	2270 (+12.9)	**914*** (-54.5)	**606*** (-69.9)
Plasma cholinesterase activity								
Day 0	481	396* (-17.7)	408* (-15.2)	406* (-15.6)	958	1015 (+5.9)	1099 (+14.7)	1059 (+10.5)
Day 1	428	387 (-9.6)	**170**[#] (-60.3)	**99**[#] (-76.9)	994	1082 (+8.9)	**613*** (-38.3)	**279*** (-71.9)

Values in parentheses are the % change in cholinesterase activity compared to the control value
* Statistically significant difference from control by Dunnett's test at $p < 0.05$
[#] Statistically significant difference from control by Dunn's Multiple Comparisons at $p < 0.05$
Biologically significant (≥ 20%) reductions in cholinesterase activity compared to controls

Cholinesterase activity in the 0.1 mg/kg bw female cerebellum was significantly reduced on day 1 in a dose-dependent manner. While this reduction was also biologically significant (- 24.6% compared to control activity), its toxicological significance was contested. It occurred in females only. Decreases in activity in the other brain regions were neither statistically nor biologically significant. The decrease was not corroborated by clinical signs of cholinesterase inhibition and plasma and erythrocyte cholinesterase activity were increased compared to controls on day 1. Doubts were expressed regarding the reproducibility of the sampling technique and the reliability and value of the data obtained from each brain region due to the high coefficient of variation associated with the cholinesterase activity and weight measurements (Table 77).

While statistical or biological significance was not achieved regarding reductions in cholinesterase activity in the various brain regions in either sex at 0.1 mg/kg bw on day 1, the reductions that were recorded were part of a dose-response trend, especially in females. A similar trend was recorded for plasma and erythrocyte cholinesterase activity on day 1 in male rats only. These reductions were not corroborated by clinical signs of toxicity.

Table 778: Brain acetylcholinesterase activity (units / gram tissue) in male and female rats shortly after treatment (Day 1)

Dose (mg/kg bw):-	Male				Female			
	0	**0.1**	**1.0**	**2.0**	**0**	**0.1**	**0.75**	**1.5**
Cortex	10.19	8.23 (-19.2)	**4.53*** (-55.5)	**2.98*** (-70.8)	10.03	8.56 (-14.7)	**4.16**[#] (-58.5)	**3.18**[#] (-68.3)
Hippocampus	13.03	11.69 (-10.3)	**8.06** (-38.1)	**3.40**[#] (-73.9)	10.15	8.71 (-14.2)	**6.09*** (-40.0)	**2.97*** (-70.7)
Midbrain	11.63	11.96 (+2.8)	**6.95*** (-40.2)	**4.64*** (-60.1)	13.60	12.48 (-8.2)	**6.82*** (-49.9)	**4.09*** (-69.9)
Cerebellum	4.83	5.04 (+4.3)	**3.59** (-25.7)	**1.43**[#] (-70.4)	5.61	**4.23*** (-24.6)	**2.77*** (-50.6)	**1.45*** (-74.2)
Half brain	11.13	10.96 (-1.5)	**5.89**[#] (-47.1)	**3.76**[#] (-66.2)	11.97	11.89 (-0.7)	**6.43*** (-46.3)	**3.94*** (-67.1)

Values in parentheses are the % change in cholinesterase activity compared to the control value
* Statistically significant difference from control by Dunnett's test at $p < 0.05$
[#] Statistically significant difference from control by Dunn's Multiple Comparisons at $p < 0.05$
Biologically significant (≥ 20%) reductions in cholinesterase activity compared to controls

Gross pathology / neuropathology:

No treatment-related gross pathological or microscopic lesions were recorded in the neurotoxicity subgroup rats. Axonal / myelin degeneration was recorded in individual nerve fibres only and in terms of their frequency and severity, there was no difference between control and test groups. They were not considered to be treatment-related.

III. CONCLUSIONS

The neurotoxicity in rodents study HLR 1118-96, originally submitted under EU Rev8 Point IIA 5.8.2.1 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 81-8 (1982). A review of this study indicates that it fully meets the current guideline B.43 and is relied upon.

One treatment-related mortality was recorded in high-dose males. Treatment-related decreases in body weight gain were recorded in high dose males and females and mid dose males over days 1- 2 and a decrease in food consumption was recorded in these male groups over the same period. Clinical signs of cholinesterase inhibition, perturbations in FOB parameters and motor activity parameters on day 1 and plasma, erythrocyte and brain cholinesterase inhibition on day 1 were recorded in mid and high dose males and females. **The NOAEL for acute oral neurotoxicity is 0.1 mg/kg bw for males and females.**

RMS comments and conclusion for the renewal

The study is acceptable as a key study

Subchronic neurotoxicity in rats – 90 day

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.1/02

Reference:	**Report:**	
--		[redacted] (1998); Oxamyl technical: subchronic oral neurotoxicity study in rats. **DuPont Report No**.: HL-1998-00708 **Guidelines:** U.S. EPA 81-8 (1982)

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.3%

Deviations: Based on OECD test guideline 424 the following deviations were identified in the study protocol:-

- No ophthalmological investigation was performed on any group during this study.

I. MATERIALS & METHODS

Crl:CD®(SD)BR strain rats were released from quarantine after 6 days on the basis of body weight and clinical signs of disease.

The dose levels were selected on the basis of a range-finding study that was conducted in preparation for a 2-year study (B.6.5.1). The initial dose levels selected were 0, 10, 100 and 300 ppm (adjusted for purity). However, due to severe body weight loss and clinical signs of toxicity at 300 ppm the intermediate and high doses were reduced to 30 and 250 ppm on test day 7. The mean daily Oxamyl intake over the entire 90-day study period was 0, 0.55, 1.69 and 15.3 mg/kg bw/day (males) and 0, 0.67, 2.03 and 20.3 mg/kg bw/day (females).

Oxamyl (DPX-D1410 technical; batch D1410-196; purity 98.3%) was administered by dietary admixture in Certified Rodent DietTM #5002 chow. Aliquots of diet mixture were analysed periodically for concentration, homogeneity and stability verification. Food and water were available ad libitum and were periodically analysed for contamination.

All animals were weighed immediately before dosing and weekly during the exposure period. The neurotoxicity subgroup animals were also weighed on days of FOB and MA assessment. Food consumption and food efficiency were also determined weekly.

During the first 2 weeks, all rats were handled daily and clinical signs recorded. This was subsequently reduced to once per week for the 0, 10 and 30 ppm groups. Daily cage-side observations continued to be conducted on these groups. Daily clinical observations were continued in high-dose rats until week 4. Thereafter, such observations were conducted weekly but cage-side observations continued daily. Clinical signs were not recorded on days of FOB and MA unless warranted by cage-side observations.

Twelve rats/sex/group were designated as neurotoxicity subgroup animals and were used in neurobehavioural evaluations (FOB and MA assessment conducted on the same day) during weeks 4, 8 and 13. A pre-test neurobehavioural test battery (FOB and MA) was performed to establish baseline measurements. The FOB assessment was conducted prior to the MA test and involved evaluations inside the home cage, outside the home cage while being handled and in a standard ‘open field’ arena. MA was assessed using automated activity monitors (Coulbourn® Instruments) over a 1-hour period.

Six neurotoxicity subgroup rats/sex/group and 6 unexposed control animals of each sex were euthanized at week 13 by pentobarbital anaesthesia followed by exsanguination and whole body perfusion fixation. A gross pathological examination was performed. Nervous tissue and muscle samples were extracted from control and high-dose groups for histopathological examination.

Thirty rats/sex/group were designated as the clinical pathology subgroup. Plasma and erythrocyte cholinesterase activity was assayed in blood samples collected prior to dosing and during week 4 from 10 rats/group, during week 8 from another 10 rats/group and during week 13 from the remaining 10 rats/group. Shortly after sampling, the rats were sacrificed and the brains removed and frozen at –70°C for subsequent acetylcholinesterase analysis.

Positive control neurobehavioural and neuropathological data were provided from in-house studies on acrylamide, carbaryl, DDT, d-amphetamine and trimethyl tin.

II. RESULTS

Oxamyl concentration in the dose samples ranged from 86 – 106% of nominal values. It was not detected in control samples.

Mortality:

No mortalities were recorded during the study.

Clinical signs:

Clinical signs indicative of cholinesterase inhibition were recorded in both sexes at ≥100 ppm. The increased incidence of several of these signs achieved statistical significance at the high dose over at least one of the four recording intervals. Other signs that did not achieve statistical significance but are nonetheless indicative of cholinesterase inhibition include an increased dose-related incidence of lacrimation, wet chin and stained/wet fur of the inguen and/or perineum in males and piloerection and stained perineum in females. These findings are considered to be biologically significant. The incidence of certain clinical signs seemed to decrease after approximately 1 month including tremors, ptosis, hyperreactivity, exophthalmus, lacrimation and piloerection. Alopecia is not normally associated with cholinesterase inhibition and the incidence recorded in both sexes is possibly attributable to stress secondary to systemic toxicity. The incidence of tremors in 100 ppm females during week 1 was significantly higher than in controls (Table 78).

Table 789: Frequency of statistically significant clinical observations in male and female rats (% total number of rats affected per group) in the clinical pathology subgroup at the four evaluation times.

	Male				Female			
Dose (ppm):-	**0**	**10**	**100**	**300**	**0**	**10**	**100**	**300**
	Days 0 – 7				**Days 0 – 7**			
Abnormal gait / mobility	0	0	0	14*	0	0	0	31*
Alopecia	10	12	7	10	0	0	5	10*
Hunched over	0	0	0	2	0	0	0	50*
Tremors	0	0	0	62*	0	0	29*	88*
	Male				**Female**			
Dose (ppm)	**0**	**10**	**30**	**250**	**0**	**10**	**30**	**250**
	Days 8 – 27				**Days 8 – 28**			
Abnormal gait / mobility	0	0	0	62*	2	0	0	95*
Coloured eye discharge	5	10	2	26*	2	0	0	0
Exophthalmus	0	0	0	40*	0	0	5	88*
Hunched over	0	0	0	10*	0	0	2	60*
Hyperactive	Not recorded				0	0	0	10*
Hyperreactive	2	0	2	40*	0	0	0	45*
Lacrimation	0	0	0	2	0	0	0	7*
Piloerection	0	0	0	10*	0	0	0	5
Ptosis	0	0	0	76*	0	0	0	71*
Tremors	0	0	0	86*	0	0	0	98*
	Days 28 – 55				**Days 29 – 56**			
Alopecia	6	16	16	31*	19	6	19	44*
Exophthalmus	0	0	0	3	0	0	9	31*
Hyperreactive	0	0	0	19*	0	0	0	9*
Ptosis	0	0	0	19*	0	0	0	47*
Stained fur	Not recorded				0	0	0	9*
Tremors	0	0	0	6	0	0	0	22*
	Days 56 – 97				**Days 57 – 97**			
Alopecia	9	14	14	36*	23	5	23	36
Exophthalmus	0	0	0	14*	5	0	14	23*
Hunched over	0	0	0	14*	Not recorded			
Ptosis	0	0	0	14*	0	0	0	45*
Stained fur	Not recorded				0	0	0	23*
Stained perineum	0	0	0	9	0	5	5	18*
Tremors	0	0	0	27*	0	0	0	36*

* Statistically significant difference from control at $p < 0.05$ by Cochran-Armitage Test for Trend

Body weight / body weight gain:

Significant reduction in group mean body weights were recorded in both sexes at the high dose each week from week 1 – 13. Significant reductions were also recorded in the intermediate dose groups at weeks 1, 2, 3 and 5 (males) and weeks 1 and 3 (females). Body weight gain was significantly lower in high-dose males at the end of weeks 1, 3, 5 and 11 and in high-dose females at the end of weeks 1, 7 and 11. Body weight gain in the latter group was significantly increased at the end of weeks 2, 4, 8 and 12. Body weight loss in high-dose animals during week 1 was substantial compared to the control body weight gain, especially in males. This may have contributed to the significant decrement in body weights throughout the exposure period. A significant decrement in body weight gain compared to controls was recorded in intermediate-dose animals during week 1. However, a recovery was recorded during week 2 with body weight gain in exposed animals being greater than in controls. Mean body weight gain in 10 ppm females was significantly lower than controls during week 7. This was considered to be a sporadic finding and not biologically significant (Table).

Table 90: Mean body weight gain in male and female rats (grams)

	Male				Female			
Dose (ppm):-	**0**	**10**	**100/30**	**300/250**	**0**	**10**	**100/30**	**300/250**
Test day 0-7	50.0	45.5	20.0*	-45.7*	14.6	17.0	-4.7*	-34.6*
7-90	231.6	235.4	252.1	195.7*	84.5	91.8	101.6*	109.4*
0-90	281.1	284.2	272.2	148.8*	98.8	109.0	96.5	76.1*

* Statistically significant difference from control by one-way ANOVA and Dunnett's test at $p < 0.05$

Food consumption / food efficiency:

Mean daily food consumption was significantly reduced in all male exposed groups and in intermediate and high dose females compared to controls during week 1. A significant reduction was recorded in high dose males for the duration of the exposure period and in high dose females during week 2. Significant increases were recorded in this group during weeks 4, 9, 10, 12 and 13. The reduction in food consumption in 10 ppm males was not corroborated with a decrease in food efficiency or body weight gain and was therefore not considered to be biologically significant. Food efficiency was significantly reduced in high dose males and in intermediate and high dose females during week 1. It was also reduced in intermediate dose males during week 1 but the decrease was insignificant. The recovery in food consumption and efficiency in intermediate dose males after week 1 corroborates the recovery in body weight gain. Significant increases in food efficiency were recorded in intermediate and high dose males during weeks 2, 4 and 6. However, these were exceptional and the decreases in food efficiency recorded during the other weeks corroborates the decreases in food consumption and body weight gain. Significant increases and decreases in food efficiency were recorded in all female groups throughout the study (Table).

Table 91: Mean daily food consumption (grams) and food efficiency (mg wt gain/gram food consumed) in male and female rats

	Male				Female			
Dose (ppm):-	**0**	**10**	**100/30**	**300/250**	**0**	**10**	**100/30**	**300/250**
Test day 0-7	24.1 295	22.2* 306	19.0* 146	7.1* -1072*	16.4 125	16.9 142	12.1* -73*	7.1* -833*
7-90	25.2 110	24.9 113	24.6 123*	21.4* 114	17.1 59	17.5 63	17.3 70*	18.3 73*
0-90	25.2 124	24.8 127	24.2 124	20.3* 85*	17.1 64	17.5 69	16.9 63	17.5 49*

* Statistically significant difference from control by one-way ANOVA and Dunnett's test at $p < 0.05$

Functional observation battery:

Forelimb grip strength was not affected by treatment. Hindlimb grip strength was lower in high dose males and females during the 4th week evaluation achieving statistical significance in males and in high dose females during the 8th and 13th week evaluations as well. Hindlimb foot splay was significantly lower in high dose males and females during the 13th week evaluation and in high dose females during the 8th week evaluation. These findings were considered to be biologically significant.

A number of treatment-related and biologically significant findings were recorded in high dose males for various FOB parameters. The incidence of ptosis during the 4th, 8th and 13th week homecage, outside the homecage and open field evaluations was considered to be biologically significant although statistical significance was not always achieved. A significantly higher incidence of piloerection was recorded on removal from the home cage. Since this was also recorded during the clinical observations made between days 8 and 27 it was considered to be biologically significant. Abnormal gait (statistically insignificant) measured during the 8th and 13th week evaluations and absent pupillary response during the 4th, 8th and 13th week evaluations were also considered to be biologically significant. The incidence of low arousal was significantly lower than controls during the 4th week evaluation in the open arena. However, this finding was not part of a dose-response relationship and did not correlate with the reduction in motor activity recorded in the same group. The statistically significant decrease in the incidence of defecation during the 4th week open field evaluation was not reproduced in the motor activity monitor and was therefore not considered to be biologically significant. Statistically significant differences in the incidence of urination in 10 ppm and 30 ppm males during the 8th and 4th week evaluations, respectively, were considered spurious. Unlike the findings of the clinical observations made over the 28 – 97 day period, no tremors were recorded in males during the FOB evaluation. This may be due to the development of tolerance, the variability in the onset and duration of tremors or the low number of rats used in the FOB which may have excluded susceptible animals (Table).

A number of treatment-related and biologically significant findings were recorded in high dose females for various FOB parameters. No treatment-related or statistically significant findings were made during the homecage evaluations. Ptosis recorded on removal from the homecage during the 4th, 8th and 13th week evaluations, although statistically significant only during the 8th week evaluation, was considered treatment-related. A significantly higher incidence of vocalisation on removal from the homecage during week 4 was recorded against a background of considerable variability in the incidence of vocalisation over the course of the study and was therefore not considered to be biologically significant. In the open field arena, abnormal gait was recorded during the 4th and 8th week evaluation and, although not statistically significant, this finding was considered to be treatment-related. The incidence of absent pupillary response was significantly higher during the 4th, 8th and 13th week evaluations. This finding was considered to be treatment-related as was the incidence of twitching back muscles during the 13th week evaluation. Again, no tremors were recorded in females during the FOB evaluation unlike the findings of the clinical observations made over the 29 – 97 day period (Table).

Table 92: FOB-selected biologically significant findings in male and female rats

Dose (ppm):-	Baseline				Week 4				Week 8				Week 13			
	0	**10**	**30**	**250**	**0**	**10**	**30**	**250**	**0**	**10**	**30**	**250**	**0**	**10**	**30**	**250**
MALES																
Home cage																
Palpebral closure - ptosis	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	1
Outside the home cage																
Palpebral closure - ptosis	0	0	0	0	0	0	0	5*	0	0	0	2	0	0	0	1
Piloerection - present	0	0	0	0	0	0	0	5*	0	0	0	0	0	0	0	0
Open field																
Palpebral closure - ptosis	0	0	0	0	1	1	1	11*	0	1	0	9*	0	2	1	5*
Gait - abnormal	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	2
Motor activity monitor																
Pupilary response - absent	0	0	1	0	0	0	0	9*	1	0	0	10*	1	0	0	10*
FEMALES																
Outside the home cage																
Palpebral closure - ptosis	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
Open field																
Palpebral closure - ptosis	0	0	0	0	0	0	0	1	0	0	0	4*	0	0	0	1
Gait - abnormal	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0
Twitching back muscles	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Motor activity monitor																
Pupilary response - absent	0	0	1	0	0	0	0	10*	0	1	1	10*	0	1	0	7*

* Statistically significant difference from control by Cochran-Armitage test for trend at $p < 0.05$

Motor activity:

The mean number of movements during the 5th 10-minute interval of the motor activity session performed during the 13th week evaluation was significantly reduced in 250 ppm male rats compared to controls. Although no other significant decreases in the duration or number of movements were recorded in either sex, the decreases recorded in the 250 ppm groups were considered to be treatment- related and therefore biologically significant (Table 79).

Table 79: Total 1 hour (6 x 10-minute readings) motor activity assessment in male and female rats (% increase / decrease compared to the control value)

Dose (ppm)	Males			Females		
	10	**30**	**250**	**10**	**30**	**250**
Mean duration of movement in seconds						
Baseline	9	8	11	10	-7	-8
Week 4	3	15	-28	13	0	-1
Week 8	10	11	-13	-1	-17	-5
Week 13	0	9	-14	-8	-20	-24
Mean number of movements						
Baseline	7	13	10	10	-4	1
Week 4	-6	14	-27	24	9	15
Week 8	3	7	-11	11	-7	-6
Week 13	-4	9	-19	9	-8	-21

Cholinesterase inhibition:

Plasma, erythrocyte and brain cholinesterase activities (all regions) were reduced in 250 ppm males and females on the 27-, 55- and 94-day evaluations. Some of these reductions reached statistical significance when compared to controls while all the reductions were biologically significant (□□20%). These reductions correlated with clinical signs of toxicity and perturbations in FOB parameters and MA at this dose level. The magnitude of the reduction in cholinesterase activity did not increase with time. Statistically or biologically significant treatment-related effects were not recorded at 10 or 30 ppm (Table 80).

Table 804: Cholinesterase activity in male and female rats dosed at 250 ppm (% of control value)

	Males				Females			
	Day -8	**Day 27**	**Day 55**	**Day 94**	**Day -8**	**Day 28**	**Day 56**	**Day 95**
Plasma	95	66*	72	76	95	33*	44*	40*
Erythrocyte	122	60*	74	52*	118*	35*	59*	45#
Cortex		54*	65*	60*		45#	64*	49*
Hippocampus		69*	66*	73*		40*	60*	58*
Mid-brain		70	64*	69*		60#	80	49#
Cerebellum		69	76	68*		63	68	61#
Half brain		62#	65	58#		44#	59	51*

* Statistically significant difference from control at the 5% level by Dunnett's criteria
# Statistically significant difference from control at the 5% level by Dunn's Multiple Comparisons criteria

Gross pathology / neuropathology:

Sporadic incidences of axon / myelin degradation were recorded in neuropathology rats. However, the incidences recorded at 250 ppm were similar in character and severity to controls and were known to occur spontaneously in the rat.

III. CONCLUSIONS

The neurotoxicity in rodents study HL-1998-00708, originally submitted under EU Rev8 Point IIA 5.8.2.2 and conducted with test material pure oxamyl (PAI), was conducted under guideline U.S. EPA 81-8 (1982). A review of this study indicates that it essentially meets the current guideline B.43 and the deviation from the guideline is considered insignificant for the outcome of the study.

Treatment-related effects recorded in the 250 ppm dose groups include clinical signs indicative of cholinesterase inhibition, significant reductions in body weight, food consumption and food efficiency, perturbations in FOB and motor activity parameters and significant reductions in plasma, erythrocyte and brain cholinesterase activity. Treatment-related effects recorded in the 100 ppm dose groups during the first week of the study include tremors in females and significant reductions in body weight, body weight gain, food consumption and food efficiency. **The NOAEL for subchronic oral neurotoxicity is 30 ppm (1.69 mg/kg bw/day for males and 2.03 mg/kg bw/day for females)**, even higher than the acute NOAEL for the same end-point.

RMS comments and conclusion for renewal

The study is considered acceptable as a key study

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.1/03

Reference: --	**Report:**	[redacted] (1999); A randomised double blind ascending oral dose study with oxamyl **DuPont Report No.:** HLO-1998-01505 **Guidelines:** Declaration of Helsinki (1964) as amended by the 29th Medical World Assembly in Tokyo (1975), 35th Medical World Assembly in Venice (1983), 41st Medical World Assembly in Hong Kong (1989), 48th General Assembly, Somerset West, Republic of South Africa (1996) GLP: It has been declared as a GLP study and effectively conducted according to the GLP principle, althugh outside their applicability domain (human studies are not included in the pre-clinical evaluations) Additional to this study is Green, J.W. (2003); Statistical analysis of HLO-1998-01505: Assessment of study design and power **DuPont Report No.:** DuPont-12251

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 97.6%

I. MATERIALS & METHODS

Pre-screened healthy non-smoking human male volunteers aged 19 – 39 years (mean 30.1 years) weighing between 50 and 100 kg (mean 76.56 kg) were used in a double blind comparative study of the effects of Oxamyl (DPX-D1410 technical; batch D1410-196; purity 97.6%) and a placebo (lactose). The initial study design involved the administration of 0.005, 0.015, 0.03, 0.06 and 0.09 mg/kg bw Oxamyl (blended with lactose at 1% w/w and administered orally in gelatin capsules) in an escalatory manner to 33 subjects over 7 sessions. Each subject received a single dose of placebo or Oxamyl. On completion of the 7th session, a protocol amendment

was issued detailing the dosing regimen in two further sessions involving the administration of 0.15 mg/kg bw Oxamyl and a further 7 subjects (Table 81).

Table 815: Dosing schedule (number of subjects per dose per session)

Dose (mg/kg bw)	Placebo	0.005	0.015	0.030	0.060	0.090	0.150
Session 1	2						
Session 2	1	1					
Session 3	1	4	1				
Session 4	1		4	1			
Session 5	1			4	1		
Session 6	1				4	1	
Session 7	1					4	
Session 8	1						1
Session 9	1						4

The criteria for halting the dose escalation procedure included the occurrence of severe or clinically significant signs of carbamate toxicity requiring atropine sulphate administration, ≥40% inhibition of erythrocyte acetylcholinesterase in a subject at a single time point or ≥25% inhibition at two consecutive time points. If such inhibition was associated with clinical signs of carbamate toxicity, no further administration at that dose level occurred.

All subjects remained under close medical and nursing supervision throughout the study. Plasma and erythrocyte cholinesterase activity was determined 2 days and 16 hours prior to dosing. Saliva collection and pupillometry were performed at the latter time point.

Subjects were fasted from 23:00 h on day –1. On day 1 of the study the subjects were given a standard breakfast at 08:00 h. Approximately 5 minutes later Oxamyl or placebo was swallowed with 150 mL water. The subjects remained inactive and were fasted for 3 hours post-dosing after which time they could be ambulant and were allowed decaffeinated fluids. A light lunch was provided at +4 hours and the subjects were discharged at +24 hours. All subjects returned at 7 ±2 days for a check-up and for blood sampling for plasma and erythrocyte cholinesterase determination.

Standing and supine heart rate was determined by palpation while supine systolic and diastolic arterial pressure was determined by sphygmomanometry. Readings were taken at screening, 16 h and 30 min pre-dosing and at 1, 2, 3, 4, 8 and 24 hours post-dosing. Oral temperature was recorded at screening and 30 min pre-dosing and at 1, 2 and 24 hours post-dosing. A 12- lead electrocardiogram (ECG) was obtained at screening and 30 min pre-dosing and at +30 min, 1, 2 and 24 hours post-dosing. A single-channel ECG was performed from 30 min pre-dosing to 3 hours post-dosing. Subjects exhibiting bradycardia or tachycardia for >30 seconds were subjected to a clinical investigation. Haematology and clinical chemistry were performed at screening and 30 min pre-dosing and at 24 hours post-dosing. Urinalysis was performed at screening and 24 h post-dosing. Pupillometry was performed using a pupilscan hand-held electronic pupillometer and saliva was collected (using 2 dental rolls for 5 minutes) and weighed at 16 h and 30 min pre-dosing and at 1, 2, 3, 4, 8 and 24 hours post-dosing. Blood for cholinesterase activity determination (Ellman method) was collected and frozen at screening, 48 h, 16 h and 30 min pre-dosing and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12 and 24 hours and 7 ±2 days post-dosing.

II. RESULTS

There were no major differences in mean age, height or weight between the dose groups. A number of deviations from protocol occurred that did not impact on the interpretation of the results or the outcome of the study.

Adverse events:

No serious adverse events requiring treatment with atropine were documented. Six adverse events occurred in 3 subjects administered the placebo. These included bleeding gums (subject 002), headache (subject 026; 19 hour duration), fever, tremor, muscular pains and right sided groin pain (subject 035). Three adverse events were documented in two 0.015 mg/kg bw subjects including headache (subject 009; commenced pre-dose and lasted

over 22 hours) and nausea (subject 009; duration approximately 1 hour which was not correlated by cholinesterase inhibition or effects on pupil size or salivation) and abdominal pain (subject 011; 30 minute duration). None of these effects was considered treatment-related. Only 1 adverse event was documented in a 0.03 mg/kg bw group subject (018) namely an earache lasting 12 hours which was not considered treatment-related. Three adverse events occurred in one 0.15 mg/kg bw subject (037) including headache lasting over 15 hours, increased sweating lasting 4 hours and a nose bleed. The time course of these events did not coincide with cholinesterase inhibition. It is therefore doubtful if they were treatment-related. All adverse events had resolved by the post-study visit.

Vital signs:

Changes in mean supine systolic or diastolic pressure and changes in mean supine pulse rate indicative of a treatment-related effect were not recorded. Dose-related increases in the number of mean supine or erect pulse rate decreases were not recorded. Subject 035 (placebo group) contracted a viral illness after dosing and had a temperature of 38.1°C at approximately +6 hours accompanied by an elevated pulse rate (100 b.p.m) and blood pressure (150/100 mmHg). His temperature was normal at other time points. This was not considered to be treatment-related.

Electrocardiogram (ECG):

Subject 008 (0.005 mg/kg bw dose group) had a prolonged PR interval (>210 ms) at 1, 2 and 24 hours post-dosing. However, this was also recorded at –30 min pre-dosing and therefore this was not considered to be treatment-related. No other treatment-related changes in 12-lead or single channel ECG were recorded.

Haematology / Clinical chemistry:

No treatment-related changes in haematological or clinical chemistry indices (including indices of liver and kidney function) were recorded at either analysis time.

Urinalysis:

No treatment-related changes in urinalysis measurements were recorded at either analysis time.

Pupillometry:

Statistically significant deviations in initial, minimum or recovery pupil size (% change from baseline at –30 min) when compared to the placebo were elucidated by statistical analyses of the total data and/or the data excluding outlying values. A decrease in initial pupil size compared to the placebo was recorded in the 0.09 and 0.15 mg/kg bw groups at +1 hour while increases were recorded at 2, 3, 4, 8 and 24 hours in the 0.005 mg/kg bw group and at 8 hours in the 0.15 mg/kg bw group. Deviations in minimum pupil size did not occur at any time point. Increases in recovery pupil size were recorded at 2, 4, 8 and 24 hours in the 0.005 mg/kg bw group. The mean difference between the initial and minimum pupil size at each time point for each dose group was reported. However, no statistical analysis was performed on this data. The increases in pupil size were not considered to be biologically relevant or related to treatment as cholinesterase inhibition is associated with pupillary constriction. The decrease in initial pupil size (excluding outlying values) in the 0.09 (-17.7%) and 0.15 mg/kg bw (-19.6%) groups at +1 hour was not considered to be treatment-related as it was not associated with any significant decrease in minimum or recovery pupil size.

Saliva collections:

A statistically significant increase in the quantity of saliva collected was documented for the 0.15 mg/kg bw group at +1 hour (160.67 ±176.43 (mean % change from baseline at –30 min ±SD)).

Cholinesterase activity:

Statistically significant perturbations in mean plasma cholinesterase activity (% reduction compared to the average of the screening, -48 h, -16 h and –30 min cholinesterase values) when compared to the placebo were elucidated by statistical analyses of the total data and/or the data excluding outlying values. Decreases of 8.0 - 43.4% were recorded at 15 through 180 minutes post dosing in the 0.15 mg/kg bw dose group. The maximum

decrease was achieved between 45 and 60 minutes and by 4 hours post dosing activity levels had returned to baseline values. Mean decreases of 11.6, 10.1 and 10.2% were recorded in the 0.09 mg/kg bw dose group at 75, 90 and 120 minutes, respectively. Although these values were significantly greater than the corresponding mean perturbations in placebo plasma cholinesterase activities, they were not considered to be biologically significant as the individual decreases were within the range covered by individual placebo values. A decrease of 13% at 12 hour in the 0.005 mg/kg bw dose group was considered to be sporadic and unrelated to treatment. A number of statistically significant increases were documented in the 0.015 and 0.03 mg/kg bw dose groups. Such increases were considered to be biologically irrelevant.

Statistically significant perturbations in mean erythrocyte acetylcholinesterase activity were similarly elucidated using statistical analyses of the total data. Decreases of 9.2 to 27.9% were recorded at 30 through 105 minutes post dosing in the 0.15 mg/kg bw dose group. Maximum decrease was achieved between 30 and 60 minutes and by 3 hours post dosing activity levels had returned to baseline values. A mean decrease of 7.3% recorded in the 0.09 mg/kg bw dose group at 30 minutes was not considered to be biologically significant as mean decreases of a similar magnitude were recorded in the placebo group throughout the study. An increase of 21.3% documented in the 0.09 mg/kg bw dose group at 6 hours post dosing was considered to be biologically irrelevant.

III. CONCLUSIONS

The supplementary neurotoxicity on human volunteers study HLO-1998-01505, originally submitted under EU Rev8 Point IIA 5.8.2.3 and conducted with test material pure oxamyl (PAI), was conducted under Declaration of Helsinki (1964) as amended by the 29th Medical World Assembly in Tokyo (1975), 35th Medical World Assembly in Venice (1983), 41st Medical World Assembly in Hong Kong (1989), 48th General Assembly, Somerset West, Republic of South Africa (1996). There is no guideline applicable to this study.

The adverse events recorded in the placebo group were severe for lactose. Subject 035 experienced fever, tremors and muscular and groin pain which are symptoms associated with the CNS and muscarinic effects of cholinesterase perturbation. However, this subject did not register a lower than normal plasma cholinesterase reading at any evaluation time and registered a higher than normal erythrocyte acetylcholinesterase reading at +24 hours.

No treatment-related effects on vital signs, ECG, haematology / clinical chemistry or urinalysis were recorded. Although the adverse events documented in the treatment groups involved muscarinic effects similar to those that are consequential to cholinesterase inhibition, they mimicked those documented in the placebo group and/or did not coincide with the time course of cholinesterase inhibition. All adverse events had resolved by the post-study visit. At +1 hour, a decrease in the initial pupil size in the 0.09 and 0.15 mg/kg bw dose groups was recorded that, although not corroborated by effects on minimum or recovery pupil size, did corroborate with a statistically significant increase in the quantity of saliva collected (0.15 mg/kg bw group only).

RMS comments and conclusion for renewal

This study is relied upon as supplemental information regarding the threshold for neurological effects in humans.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.1/04

Reference:	**Report:**	[redacted] (1997); Reversibility study with carbamate insecticides in rats **DuPont Report No.:** HL-1997-00641 **Guidelines**: Not given GLP: YES
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1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.3%

I. MATERIALS & METHODS:

The objective of this investigation was to determine the time for cholinesterase activity levels to return to baseline values after Oxamyl-induced inhibition. A pilot study showed that the approximate time-to-peak inhibition of cholinesterase activity was 30 minutes post-dosing with reductions of 65% and 57% in male and female plasma cholinesterase activity, respectively, and reductions of 70% and 62% in male and female erythrocyte acetylcholinesterase activity, respectively. These reductions were accompanied by clinical signs which, together with cholinesterase inhibition, had resolved by 3 hours post-dosing. Since the magnitude of inhibition at 1 mg/kg bw was > 50%, this dose was chosen for the main study.

A single dose of Oxamyl (DPX-D1410 technical; batch D1410-196; purity 98.3%), adjusted for purity, was administered in deionised water by gavage in a dosage volume of 10 mL/kg to 40 male and 40 female Crl:CD®BR rats giving a final dose of 1 mg/kg bw. The same number of rats was used in a control group. The animals were 48 – 49 days old. Stock solutions of Oxamyl were freshly prepared before dosing. The animals were fed Purina Certified Rodent Chow ® #5002 and water ad libitum. Both food and water were periodically analysed for the presence of contaminants. Test solution and vehicle were analysed for concentration verification by HPLC.

Body weight was determined prior to treatment to calculate the dosing volume. However, body weight data was not reported. Clinical signs were reported prior to dosing and at approximately 0.5, 2, 3 and 4 hours post-dosing. Blood was collected from the orbital sinus and the rats were subsequently sacrificed by CO2 asphyxiation and the brains removed and frozen. Plasma, erythrocyte and whole-brain cholinesterase activity was determined in 10 rats per group at approximately 0.5, 2, 3 and 4 hours post-dosing. Individual rats were sampled once only. Cholinesterase activity was determined using a modification of the Ellman photometric assay.

II. RESULTS

The measured concentration of Oxamyl in the test solution was approximately 97% of the nominal value. Oxamyl was not detected in the vehicle control.

Clinical signs:

There were no mortalities during the course of the study. Most animals (38 males and 36 females) experienced tremors while 1 male had a wet chin indicative of salivation. These effects occurred at the 30-minute evaluation and were considered treatment-related. No clinical signs of toxicity were recorded at the 2 hour evaluation.

Cholinesterase activity:

Moderately severe inhibition of plasma, erythrocyte and whole-brain cholinesterase activities was recorded in both sexes at the 30-minute evaluation time. Although statistically significant decreases were recorded in brain acetylcholinesterase activity at 2 hours, these decreases were not considered biologically significant (<20%) and they were within the mean activity values of the 8 control groups in the study. It was therefore concluded that activity had recovered by 2 hours. No significant decreases in cholinesterase activity were recorded at 3 or 4 hours (Table 82).

Table 826: Perturbation in plasma, erythrocyte and whole-brain cholinesterase activity (% change compared to the mean control cholinesterase activity value).

Time	Male			Female		
	Erythrocyte	**Plasma**	**Brain**	**Erythrocyte**	**Plasma**	**Brain**
½ hour	-58*	-57*	-45*	-61*	-50*	-48*
2 hour	+2	-9	-4*	-6	-6	-11*
3 hour	+12	+3	-1	+3	-7	-3
4 hour	-13	+1	+5*	+8	+7	0

* Statistically significant difference from control at the 5% level by Dunnett's criteria

III. CONCLUSIONS

The neurotoxicity in rodents study HL-1997-00641 was originally submitted under EU Rev8 Point IIA 5.8.2.4 and conducted with test material pure oxamyl (PAI). Guidelines were not given, but there is no current specific guideline applicable to this study.

This study is supplemental. Inhibition of plasma, erythrocyte and brain cholinesterase activity was recorded in rats 30 minutes after dosing and this was accompanied by clinical signs of cholinesterase inhibition specifically tremors. By 2 hours post-dosing, no treatment-related effects on cholinesterase activity or associated clinical signs were evident.

RMS comments and conclusion for the renewal

This stuy is accepted and considered supplemental information regarding the mechanism of action and rapid reversibility of the acetylcholinesterase inhibition by oxamyl.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.1/05

Reference:	**Report:**	[redacted] (2001); Cholinesterase inhibition determination in rats exposed to inhalation atmospheres of oxamyl technical (96.9%) **DuPont Report No.:** DuPont-4383, Revision No. 1 **Guidelines:** Not given GLP: YES

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 96.9%

I. MATERIALS & METHODS

The objective of this study was to investigate cholinesterase activity in rats exposed by inhalation to atmospheres of Oxamyl.

Young adult male and female (nulliparous and non-pregnant) Crl:CD®(SD)IGS BR rats were quarantined for 6 days and observed for clinical signs of disease. The animals were administered Certified Rodent LabDiet® 5002 (PMI Nutrition International Inc.) and water ad libitum except during exposure. Both food and water were periodically analysed for the presence of contaminants. Test material was assumed to be stable throughout the exposure period as no evidence of instability was observed.

Two groups of 20 rats (10 animals per sex) were exposed nose-only for 4 hours to aerosol atmospheres of Oxamyl (DPX-D1410 technical; batch D1410-196; purity 96.9%). The test material was mixed with distilled water as a 1% or 5% w/w solution to give final atmospheric concentrations of 0.024 or 0.0049 mg/L, respectively. A similar sized group of rats was exposed to air only and served as a control for each exposure group. At the time of exposure the animals were approximately 6.5 weeks old and weighed 185-240 g (males) and 138-170 g (females).

The rats were observed for clinical signs of toxicity and mortality during exposure following which all rats were sacrificed by CO_2 anaesthesia and exsanguination. Blood and brain samples were collected for cholinesterase analysis. Anticoagulated blood was analysed within 15 minutes of collection and brain samples were stored at –70°C and analysed within 24 hours of collection. Cholinesterase activity was determined using a modification of the Ellman photometric assay.

Aerosol atmospheres of Oxamyl were achieved using a Spraying Systems Nebuliser. The atmospheric concentration of Oxamyl was verified periodically by gravimetric analysis.

II. RESULTS

The mass median aerodynamic diameter (MMAD) was 0.85 – 1.2 µm.

Clinical signs:

There were no mortalities or clinical signs of toxicity during the study. Immediately following exposure, rats exposed to 0.024 mg/L Oxamyl exhibited tremors and lethargy. Other clinical signs recorded immediately following exposure in the control and both groups of test rats include wet/stained fur, wet perineum, nasal / ocular discharge and diarrhoea. At 0.0049 mg/L the clinical signs were mild and similar in incidence to controls.

The mean body weight at the commencement of exposure was 206 and 153 g in 0.024 mg/L males and females, respectively, and 197 and 152 g in 0.0049 mg/L males and females, respectively.

Cholinesterase activity:

Statistically and biologically significant decreases in plasma, erythrocyte and whole-brain cholinesterase activity were recorded in males and females exposed to 0.024 mg/L Oxamyl. These findings correlated with the clinical signs recorded for both sexes at this exposure dose. Statistically significant decreases in cholinesterase activity were recorded in both sexes exposed to 0.0049 mg/L Oxamyl with the exception of female plasma cholinesterase activity. The decreases in plasma cholinesterase was not biologically significant. The decreases in erythrocyte acetylcholinesterase activity were biologically significant in both sexes. On an individual animal basis, brain acetylcholinesterase activity was less than 80% of the control mean in 3/10 treated males and 0/10 treated females. It was therefore concluded than brain acetylcholinesterase activity was marginally affected at 0.0049 mg/L (Table 837).

Table 837: Group mean plasma, erythrocyte and whole-brain cholinesterase activity in male and female rats exposed to 0.024 and 0.0049 mg/L Oxamyl

		0.024 mg/L			0.0049 mg/L		
		Control	Test	% decrease[a]	Control	Test	% decrease[a]
Males	Plasma (U/L)	470	131.8	72*	495.5	436.1	12*
	Erythrocyte (x20 U/L)	1766	500	72#	1806	1300	28*
	Whole-brain (U/g)	11.64	3.77	68#	11.9	10.09	15*
Females	Plasma (U/L)	688.3	167.9	76#	742.5	694.4	6
	Erythrocyte (x20 U/L)	1984	540	73*	1854	1312	29*
	Whole-brain (U/g)	11.61	3.78	67*	12.16	11.09	9*

[a] % decrease in test group mean cholinesterase activity compared to the control group mean
* Statistically significant difference from control at p<0.05 by parametric test (Dunnett / Tamhane-Dunnett)
# Statistically significant difference from control at p<0.05 by nonparametric test (Dunn's)

III. CONCLUSIONS

The neurotoxicity in rodents study DuPont-4383, Revision No. 1, was originally submitted under EU Rev8 Point IIA 5.8.2.6 and conducted with test material pure oxamyl (PAI). Guidelines were not given. A review of this study indicates that there is no current guideline applicable to this mechanistic study.
The highest concentration tested was approximately half the lowest concentration tested in the acute inhalation toxicity study (B.6.2.3). However, in the present test Oxamyl was administered as an aerosolised aqueous solution instead of a dry dust. Male and female rats exposed to 0.024 mg/L Oxamyl had statistically and biologically significant decreases in plasma, erythrocyte and whole-brain cholinesterase activity accompanied by clinical signs of cholinesterase inhibition. Both sexes had statistically and biologically significant decreases in erythrocyte acetylcholinesterase activity at 0.0049 mg/L as well as statistically significant inhibition of whole-brain acetylcholinesterase activity. These findings were not associated with significantly increased clinical signs of toxicity compared to controls. A NOAEL could not be determined.

RMS comments and conclusion for renewal

This study is acceptable. It is relied upon as supplemental information regarding the mechanism of action with regard to acute inhalation exposure and time to peak effect as well as reversibility of the carbamylation of acetylcholinesterase.

Study submitted to the EU for the first time in this submission.

B.6.7.1/06

Reference: CA 5.7.1/01	**Report:**	[redacted] (2005); Oxamyl (DPX-D1410) technical (98% w/w): Relative sensitivity of preweanling rat pups and adult rats to inhibition and recovery of cholinesterase activity **DuPont Report No.:** DuPont-16755 **Guidelines:** Not given (mechanistic study) **Deviations:** Not applicable **Testing Facility:** [redacted] **Testing Facility Report No.:** DuPont-16755 **GLP:** Yes. The study final report seems to comply with the GLP principles. However, the statement indicating that the document reports results coming from two 'protocols' is not clear: in case teo study plans have been combined in a single Final report, one of the basis of the GLP principle (1 study plan= 1 Final report) would have been not respected. **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

The relative sensitivity of preweanling rat pups (lactation Day 11) and young adult rats to inhibition and recovery of cholinesterase activity was studied in male and female Crl:CD®(SD)IGS BR rats administered pure oxamyl (PAI) by single-dose oral gavage in NanoPure® water. Three subsets of experiments were performed to evaluate the peak inhibition and recovery of cholinesterase activity in pups (Subset 1), determine the dose response of acetylcholinesterase activity inhibition at the time of peak inhibition in pups (Subset 2), and evaluate dose responses at peak inhibition and at recovery in young adult rats (Subset 3). For Subset 1, doses in male and female rats were 0.0 or 0.1 mg/kg body weight (bw). For Subset 2, doses in male and female rats were 0, 0.075, 0.1, 0.125, or 0.15 mg/kg bw. For Subset 3, doses in male and female rats were 0, 0.15, 0.20, or 0.25 mg/kg bw. Dose volumes were 2 mL/kg bw for males and females in all subsets. Rats were euthanised at 30, 60, 90, 120, 180, 240, or 360 minutes after dose administration (Subset 1); 30 minutes after dose administration (Subset 2); or 30 or 240 minutes after dose administration (Subset 3). For all subsets, blood and brain were collected at sacrifice for determination of cholinesterase activity.

The greatest degree of cholinesterase inhibition in preweanling male and female rat pups dosed with 0.1 mg/kg generally occurred at 30 minutes after dosing. Based on this and previous studies in adult rats, 30 minutes post-dosing was chosen as the sampling time for peak effects in subsequent dose-response studies. During dose-response studies, red blood cell (RBC) and brain cholinesterase activities were decreased in preweanling and adult rats (adults at higher doses only) at 30 minutes after dosing, and returned to baseline by 240 minutes post-dose for both sexes. For both adults and pups, the degree of RBC cholinesterase inhibition was greater than brain cholinesterase indicating that RBC cholinesterase activity is the more sensitive parameter. For RBC cholinesterase inhibition (the more affected parameter), effects were essentially the same in preweanling pups and adults based on results at the dose in common (0.15 mg/kg) and linear trends across the range of doses tested. For brain cholinesterase (the less affected parameter), preweanling pups appeared to be slightly more sensitive than adults based on results at the common dose. At lower doses (0.075, 0.1, and 0.125 mg/kg), however, the range of responses for brain cholinesterase activity in pups tended to overlap with the linear trend line in dose response for adults.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Off-white solid
	CAS #:	23135-22-0
	Stability of test compound:	The test material was stable in the vehicle when held for 5 hours at room temperature.
2.	Vehicle and/or negative control:	NanoPure® water
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD®(SD)IGS BR
	Age at dosing:	11 days (pups); 42 days (young adults)
	Weight at dosing:	Not reported
	Source:	[redacted]
	Acclimation period:	Approximately 4 days
	Diet:	PMI® Nutrition International, LLC, Certified Rodent LabDiet® (#5002), *ad libitum*
	Water:	Tap water, *ad libitum*
	Housing:	Dams and their litters were housed in shoe box cages with Bed-o'cobs® bedding. Young adult animals were housed singly in stainless steel, wire-mesh cages suspended above cage boards.
4.	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. *In-life initiated/completed*

01-February-2005 to 17-February-2005

2. *Preparation and analysis of the dosing solutions*

The test substance was dissolved in NanoPure® water on the day of dosing. Nominal concentrations reflect adjustments for purity as reported by the supplier. The dosage volumes were approximately 2 mL/kg bw, and were based on each rat's body weight on the day of dosing. Samples of each concentration of the dosing formulation were collected and analysed on the first day of dosing for the preweanling rats in Subset 2 and for the young adult rats in Subset 3 to verify the test substance concentration in the dosing formulation and to verify 5-hour stability.

3. *Animal assignment and treatment*

Following previous method development studies, doses of 0.0 and 0.1 mg/kg bw (Subset 1) or 0, 0.075, 0.1, 0.125, and 0.15 mg/kg bw (Subset 2) were selected for preweanling pups. Based on previous studies in adult rats, doses of 0, 0.15, 0.20, or 0.25 mg/kg bw were selected for Subset 3. The day after arrival, pups were cross-fostered and numbered. Pups numbered 1–10 were fostered with the first dam; pups numbered 11–20 were fostered with the second dam, and so on. Young adult rats were assigned to dose groups by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means within a sex.

Rats were given a single dose of oxamyl by gavage. The test substance was administered in NanoPure® water at a volume of 2 mL/kg bw. Animals were observed for clinical signs at least once daily prior to test substance administration. The animals were weighed on the day of dosing to determine the amount of test substance to be administered. Since these weights were recorded prior to administration of the test substance, they were not included in this report; however, they were retained in the study records. At specified timepoints (Table 84), preweanling rats were anesthetised with an intraperitoneal injection

of Beuthansia® and blood was collected by cardiac puncture using a syringe and needle. Young adult rats were euthanized by CO_2 asphyxiation and blood was collected from the vena cava using a syringe and needle. Brains were collected immediately after blood collection, weighed, snap-frozen in liquid nitrogen, and maintained frozen at approximately -80°C until analysis.

Table 848 Study design: Relative sensitivity of preweanling rat pups and adult rats to inhibition and recovery of cholinesterase activity following administration of oxamyl

Experiment	Dose level (mg/kg bw)	Age (days)	Number of animals		Time of sacrifice (minutes post-dose)	Samples
			Male	Female		
Subset 1	0.0	11	5	5	60	Blood, brain
		11	5	5	120	
		11	5	5	240	
	0.1	11	5	5	30	
		11	5	5	60	
		11	5	5	90	
		11	5	5	120	
		11	5	5	180	
		11	5	5	240	
		11	5	5	360	
Subset 2	0.0	11	10	10	30	Blood, brain
	0.075	11	10	10		
	0.1	11	10	10		
	0.125	11	10	10		
	0.15	11	10	10		
Subset 3	0.0	42	10	10	30	Blood, brain
	0.15	42	10	10		
	0.20	42	10	10		
	0.25	42	10	10		
	0.0	42	10	10	240	
	0.15	42	10	10		
	0.20	42	10	10		
	0.25	42	10	10		

4. *Measurement of red blood cell cholinesterase activity (RCHE)*

Red blood cells (RBCs) were separated from plasma by centrifugation in a high-speed refrigerated centrifuge (approximately 13000 rpm for 4 minutes at 2–8°C). After the plasma supernatant and buffy coat (containing white blood cells and platelets) was discarded, a 50 µL sample of the vortexed RBCs was pipetted into phosphate buffer containing 0.5% Triton X-100 to prepare a 1:20 RBC hemolysate. The resulting hemolysate was vortexed for 5–10 seconds. The RBC hemolysates were placed in sample cups and assayed using the manufacturer's modified reagents on a Roche Behringer-Mannheim Hitachi 717 chemistry analyser. Sample blanks were also run to correct for any interference. Except where indicated, blood samples were processed and analysed within 15 minutes of collection.

5. *Measurement of brain cholinesterase activity (BCHE)*

Phosphate buffer (0.1 M) was added to thawed brain tissue, and the buffered tissue sample was homogenised using an automated homogeniser for approximately 10–15 seconds until a unifory smooth homogenate was achieved. A 3-fold dilution was prepared for the homogenate using phosphate buffer with 0.5% Triton X-100, and the dilution was thoroughly mixed by vortexing. The diluted homogenate samples were placed in sample cups and assayed using the manufacturer's modified reagents on a Roche Behringer-Mannheim Hitachi 717 chemistry analyser. Sample blanks were run to correct for any interference. Except where indicated, tissue analysis was completed within 30 minutes from the end of preparation of the tissue homogenate dilution. Samples were maintained on ice during the preparation procedures.

6. *Statistics*

Group mean and standard deviation were calculated for RBC and brain cholinesterase activity. Coefficients of variation and mean or individual percent inhibition values were also calculated for RBC and brain cholinesterase activities. For this study, the determination of compound-related effects was based on assessment of means, standard deviations, individual values of dosed animals, and comparisons of data from treated animals to those of control and recovery animals from the current study.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was uniformly mixed in the vehicle at all levels, was at the targeted concentration in the samples, and was stable in the vehicle when held for 5 hours at room temperature.

B. CLINICAL PATHOLOGY EVALUATION: SUBSET 1

The time to peak inhibition and time to recovery for RBC and brain cholinesterase activity was determined in preweanling (11-day old) rat pups dosed with 0.0 (control) or 0.1 mg/kg of the test substance.

Cholinesterase (both RBC and brain) activities were determined in control pups (0 mg/kg) at 60, 120, and 240 minutes after dosing. There were no sex differences in cholinesterase activities for male and female pups. There were no consistent time-related changes in RBC cholinesterase for control pups between 60 minutes and 240 minutes post-dosing. However, for both male and female control pups, brain cholinesterase activity increased between 60 and 120 minutes, and then remained relatively constant between 120 and 240 minutes after dosing. These results are likely due to the normal variability in cholinesterase parameters.

RBC and brain cholinesterase activities were determined in pups dosed with 0.1 mg/kg of the test substance at 30, 60, 90, 120, 180, 240, and 360 minutes after dosing. The lowest RBC and brain cholinesterase activities occurred at the first time point (30 minutes after dosing) for male RBC and brain cholinesterase and female brain cholinesterase. Female RBC cholinesterase activities were lowest at 90 minutes after dosing. However, due to the variability in cholinesterase values at 30, 60, 90, 120, and 180 minutes, the biological relevance of the apparent depression in female RBC cholinesterase at 90 minutes is questionable. For pups, brain cholinesterase activities were similar to controls by 180 minutes after dosing, and RBC cholinesterase activities were similar to controls at 240 minutes or earlier after dosing. These data are reflected in the calculated values for percent inhibition (Table).

Table 99 Summary of cholinesterase values for male and female 11-day old pups—Subset 1

Dose	**0 mg/kg bw**			**0.1 mg/kg bw**						
Time post-dose (min.)	**60**	**120**	**240**	**30**	**60**	**90**	**120**	**180**	**240**	**360**
Male										
RCHE[a] % Inhibition	0	0	0	25[b]	19[b]	14[c]	16[d]	13[e]	6[f]	1[f]
BCHE[g] % Inhibition	0	0	0	26[b]	21[b]	12[c]	15[d]	1[e]	-6[f]	-6[f]
Female										
RCHE % Inhibition	0	0	0	6[b]	18[b]	36[c]	16[d]	11[e]	-21[f]	-17[f]
BCHE % Inhibition	0	0	0	23[b]	19[b]	6[c]	-2[d]	3[e]	10[f]	-4[f]

[a] RCHE = red blood cell cholinesterase activity
[b] Compared to the 60-minute control group.
[c] Compared to the average of the 60- and 120-minute control groups.
[d] Compared to the 120-minute control group.
[e] Compared to the average of the 120- and 240-minute control groups.
[f] Compared to the 240-minute control group.
[g] BCHE = brain cholinesterase activity

C. CLINICAL PATHOLOGY EVALUATION: SUBSET 2

The dose response of RBC and brain cholinesterase activity in preweanling rat pups was determined at 30 minutes after dosing pups with 0.0 (control), 0.075, 0.1, 0.125, or 0.15 mg/kg of the test substance. Activities were similar between males and females at a given dose for both RBC cholinesterase and brain cholinesterase. Activities for each parameter were slightly lower than control rats at doses of 0.075, 0.1, and 0.125 mg/kg (RBC cholinesterase was inhibited 15 to 20%, while brain cholinesterase was inhibited 7 to 12%), but showed no dose-response relationship across these three doses (Table). However, activities for RBC cholinesterase and brain cholinesterase in both males and females at the highest dose tested (0.15 mg/kg) were lower compared to activities in pups dosed with 0.075–0.125 mg/kg. At 0.15 mg/kg, RBC cholinesterase was inhibited 36–38% and brain cholinesterase was inhibited 23% for both sexes. For a given dose and sex, inhibition of brain cholinesterase activities was slightly less than inhibition of RBC cholinesterase activities.

Table 100 Summary of cholinesterase values for male and female 11-day old pups—Subset 2

Dose (mg/kg bw)	0.0	0.075	0.1	0.125	0.15
Time post-dose (min.)	**30**	**30**	**30**	**30**	**30**
Male					
RCHE[a] % Inhibition	0	18[b]	17[b]	15[b]	38[b]
BCHE[c] % Inhibition	0	11[b]	12[b]	12[b]	23[b]
Female					
RCHE % Inhibition	0	16[b]	20[b]	15[b]	36[b]
BCHE % Inhibition	0	7[b]	10[b]	9[b]	23[b]

[a] RCHE = red blood cell cholinesterase activity
[b] Compared to control group.
[c] BCHE = brain cholinesterase activity

D. CLINICAL PATHOLOGY EVALUATION: SUBSET 3

Dose response and recovery of RBC and brain cholinesterase activity in young adult rats were determined at 30 and 240 minutes after dosing young adult rats with 0.0 (control), 0.15, 0.20, or 0.25 mg/kg of the test substance.

At 30 minutes after dosing, RBC cholinesterase activity decreased in a dose-related pattern in males and females. RBC cholinesterase activities were similar between sexes administered the same dose. At this same time point, brain cholinesterase activity was also decreased in a dose-related pattern in males and females at all doses. However, brain cholinesterase activity at 0.15 mg/kg in adult females was similar to control and recovery values. In both males and females, the inhibition of brain cholinesterase activity was less than inhibition of RBC cholinesterase activity (Table).

At 240 minutes after dosing, RBC and brain cholinesterase activities of treated male and female rats were similar to the cholinesterase activity of concurrent control animals. A previous study had shown complete reversibility of oxamyl-induced RBC and brain cholinesterase activities at 180 minutes (3 hours) after a dose of 1.0 mg/kg, a dose that is 4-fold greater than the highest dose for adults in the current study. Therefore, any apparent changes in this study at 240 minutes after dosing were considered to represent normal variability in RBC and brain cholinesterase measurements, and these animals were considered to be unaffected.

Table 101 Summary of cholinesterase values for adult male and female rats—Subset 3

Dose (mg/kg bw)	0.0	0.15	0.20	0.25	0.0	0.15	0.20	0.25
Time post-dose (min.)	**30**				**240**			
Male								
RCHE[a] % Inhibition	0	25[b]	49[b]	46[b]	0	-1[c]	15[c]	14[c]
BCHE[d] % Inhibition	0	10[b]	13[b]	22[b]	0	3[c]	2[c]	1[c]
Female								
RCHE % Inhibition	0	22[b]	37[b]	53[b]	0	-8[c]	-4[c]	-7[c]
BCHE % Inhibition	0	3[b]	15[b]	19[b]	0	-1[c]	-5[c]	0[c]

[a] RCHE = red blood cell cholinesterase activity
[b] Compared to the 30-minute control group.
[c] Compared to the 240-minute control group.
[d] BCHE = brain cholinesterase activity

E. COMPARISON OF CHOLINESTERASE ACTIVITIES BETWEEN PUPS AND ADULTS

Cholinesterase activity data collected 30 minutes after dosing were evaluated for differences between pup and adult rats with respect to absolute activities in control pups and adults, variability of the data, and magnitude of cholinesterase activity inhibition in treated groups. All pup and adult dose groups, including those with no treatment-related effects, were used for this comparison.

1. *Comparison of baseline (control) cholinesterase activities in pups and adults*

Baseline cholinesterase activities are age-dependent. RBC and brain cholinesterase activities of control (0 mg/kg) rat pups were compared to those of control adult rats. RBC cholinesterase activities for control pups were higher than those of adults (pup means ranged from 2590–3072 U/L, and adult means ranged from 1982–2297 U/L). Brain cholinesterase activities for control pups were lower than those of adults (pup means ranged from 5.2 to 6.8 U/g, and adult means ranged from 9.1 to 10.2 U/g). There was no sex difference in these measurements for either adults or pups.

2. *Variability of cholinesterase activity measurements in pups and adults*

The variability of the data was determined by calculating the coefficient of variation for each group of similarly treated pup and adult rats (*e.g.,* male pups and adults dosed with 0.15 mg/kg at 240 minutes post-dosing). The coefficient of variation for RBC cholinesterase activity ranged from 4%–31% for pups and 17%–30% for adults. For brain cholinesterase activity, the coefficient of variation ranged from 1%–18% for pups and 5%–18% for adults. The variability in RBC and brain cholinesterase activities in both pups and adults was consistent between sexes and was not affected by the dose of test substance administered. The variability of brain cholinesterase activity was slightly lower than that of RBC cholinesterase activity. Because cholinesterase activities are quite variable, the data must be interpreted in light of the coefficients of variation.

3. *Percent inhibition at time of peak inhibition (30 minutes after dosing)*

One dose of the test substance (0.15 mg/kg) was common to both pups and adults and thus comparisons at this dose were primarily used to evaluate relative sensitivity. In addition, where appropriate, linear trends in dose response were used to evaluate the relative sensitivity in groups of rats of different ages.

Percent inhibition for RBC and brain cholinesterase activity for each sex was compared between pups and adults. Because the standard calculation of percent inhibition does not consider the variability among individual animals, inhibition was also calculated using each individual rat's cholinesterase activity value compared to the appropriate mean control value.

Based on linear trend lines as well as results for the common dose (0.15 mg/kg), inhibition of male and female pup RBC cholinesterase activity was essentially the same as inhibition of adult RBC cholinesterase activity over the range of doses tested. The trend line for male and female brain cholinesterase indicated slightly more inhibition in pups compared to adults; however, the difference

between the trend lines was generally less than the standard deviations at each dose. In addition, the greater slopes of both male and female pup trend lines for brain cholinesterase inhibition were due primarily to slightly greater inhibition (compared to adults) at the highest dose tested in pups (0.15 mg/kg), rather than the inhibitions at the three lower doses (0.075, 0.10, and 0.125 mg/kg) tested in pups. These findings indicate that for brain cholinesterase, pups are slightly more sensitive than adults at the dose in common.

III. CONCLUSIONS

The purpose of this study was to determine the relative sensitivity of male and female preweanling rat pups and adult rats to inhibition and recovery of cholinesterase activity following administration of a single oral dose of Oxamyl Technical. The study is acceptable.

In untreated preweanling pups, RBC cholinesterase activities (U/L) were higher and brain cholinesterase activities (U/g) were lower compared to the corresponding activities in untreated adults. Within an age group, there was no consistent sex difference in the degree of RBC or brain cholinesterase inhibition over the range of doses tested.

The greatest degree of cholinesterase inhibition in preweanling male and female rat pups dosed with 0.1 mg/kg generally occurred at 30 minutes after dosing. Based on this and previous studies in adult rats, 30 minutes post-dosing was chosen as the sampling time for peak effects in subsequent dose-response studies. During dose-response studies, RBC and brain cholinesterase activities were decreased in preweanling and adult rats (adults at higher doses only) at 30 minutes after dosing, and returned to baseline by 240 minutes post-dose for both sexes. For both adults and pups, the degree of RBC cholinesterase inhibition was greater than brain cholinesterase indicating that RBC cholinesterase activity is the more sensitive parameter.

For **RBC cholinesterase inhibition (the more affected parameter), effects were essentially the same in preweanling pups and adults based on results at the dose in common (0.15 mg/kg)** and linear trends across the range of doses tested. For **brain cholinesterase** (the less affected parameter), **preweanling pups appeared to be slightly more sensitive than adults based on results at the common dose.** At lower doses (0.075, 0.1, and 0.125 mg/kg), however, the range of responses for brain cholinesterase activity in pups tended to overlap with the linear trend line in dose response for adults.

Study submitted to the EU for the first time in this submission.

B.6.7.1/07

Reference: **CA 5.7.1/03**	**Report:**	Moser, V.C., McDaniel, K.L., Phillips, P.M, Lowitt, A.B. (2007); Time-course, dose-response, and age comparative sensitivity of N-methyl carbamates in rats **Source:** Toxicological Sciences 114(1), 113–123 (2010) This is a paper published in the open literature, with no indication about GLP, or applied guidelines. Some parameters usually requested in the reporting format of the GLP-compliant final report are not include in the publication.

Executive summary:

To study potential age–related differences, seven carbamates (carbaryl, carbofuran, formetanate, methiocarb, methomyl, **oxamyl**, and propoxur) were evaluated in preweanling (17 days old or postnatal day [PND] 17) male rats. Motor activity was monitored, and ChE inhibition was measured in brain and red blood cells (RBCs) using a radiometric assay that minimized reactivation of ChE.

First, time-course studies in PND17 Long–Evans male rats, using a single oral dose of each carbamate, were conducted. Almost all carbamates showed maximal ChE inhibition at a 45-min time point; only methomyl showed an earlier peak effect (15 minutes). At 24 hours, most inhibition had recovered.

Next, dose-response data were collected for each carbamate, using four doses and control, with motor activity testing beginning 15 minutes after dosing and tissue collection at 40–45 minutes. RBC ChE was generally inhibited to a greater degree than brain. Motor activity was not as sensitive a measure for some of the carbamates, with some differences across carbamates in the shapes of the dose-response curves. Additional studies documented age-related differences by comparing ChE inhibition in PND11, PND17, and adult rats following administration of carbaryl or carbofuran. Only the youngest (PND11) rats were more sensitive than

adults to carbaryl, but both younger ages showed more effects than adults with carbofuran. Comparisons of the other carbamates (including oxamyl) to previous studies in adult rats confirm avalialble data, showing the time-course and dose-response characteristics for each carbamate and documenting greater sensitivity of the young for carbofuran and carbaryl only.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	Not given
	Purity:	99.0%
	Description:	Not given
	CAS #:	23135-22-0
	Stability of test compound:	Not given
2.	Vehicle and/or negative control:	Deionized water
3.	Test animals	
	Species:	Rat
	Strain:	Long-Evans hooded timed-pregnant or adult male rats (Charles River Laboratories, Raleigh, NC)
	Age at dosing:	Pregnant rats were allowed to deliver naturally; day of birth is considered PND0. On PND4 (time-course), PND3 (PND11 studies), or PND2 (dose-response), all pups were grouped by sex and redistributed to the dams, assuring that littermates were spread across litters. All litters were culled to eight pups, with six males in each. Only males were used in these studies. Pups in each litter were dosed in a split-litter design, *i.e.*, with no more than one pup within a litter receiving the same treatment. For the time-course studies, all six males in a litter were dosed, and for the dose-response studies, five males in a litter were dosed to assure that no more than one pup in a litter received the same dose. General observations of the litters indicated that dams did not treat the pups differently based on their dosing conditions. For the adult studies, male rats were received at 90 days of age and tested within 1 week.
	Weight at dosing:	Not given
	Source:	Charles River Laboratories, Inc., Raleigh, North Carolina
	Acclimation period:	Not given
	Diet:	Purina Formulab Diet #5008 for dams and Purina Rodent Chow #5001 for adult males
	Water:	Filtered tap
	Housing:	Singly housed on hardwood chip bedding (Beta-Chip) in temperature and humidity controlled facilities. Pregnant rats also had either a cotton pad (Nestlet) (time-course studies) or Enviro-Dri (dose-response studies) in each cage to serve as nesting material.
4.	Environmental conditions	
	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	Not reported

B. STUDY DESIGN AND METHODS

In-life testing: Body weight on the day of dosing was used to calculate dosing volume at 2 mL/kg. Within each litter, pups were randomly assigned to treatment groups. Adult rats were also weighed a few days after arrival and assigned to dose group using a stratification process by weight. All chemicals were administered *via* oral gavage at 2 mL/kg to provide greater accuracy in the dosing volume.

Range-finding studies were conducted with each carbamate, in which one to three dose levels were administered to separate animals (two to four per dose) to determine doses which produce moderate but not extreme signs of toxicity. Doses for the subsequent studies were based on these results. Time-course and dose-response studies were conducted for each carbamate in PND17 rats; additional ages (adult, PND11) were included for carbaryl and carbofuran. Dosing was spaced so that sacrifice and tissue collection can take place at the same approximate time after dosing for all pups within the treatment group. To the extent possible, treatments were counterbalanced across the days of testing (note, the treatment groups for the longer time points were dosed earlier in the day). The time-course and dose-response studies for each carbamate were conducted separately.

For the time-course, rats (n = 6 per dose at each time) were dosed with either vehicle or a single dose of one of the carbamates (carbaryl 30 mg/kg, carbofuran 1 mg/kg, formetanate 3 mg/kg, methiocarb 12 mg/kg, methomyl 2.5 mg/kg, **oxamyl 0.5 mg/kg**, and propoxur 10 mg/kg). Nominal time points for the time-course study were 15, 45, 90, 180, or 1440 minutes (24 hours); in practice, precise times were 15–20, 45–55, 90–95, 180–190, and 1440–1450 minutes after dosing. Vehicle-treated control rats were included only at 45, 180, and 1440 minutes.

For the dose-response study, rats (n = 10 per dose for PND17, n = 8 per dose for PND11, and n = 6 per dose for adult) were dosed with one of five doses of each carbamate. For PND17 rats, motor activity measurements were included in the dose-response study. Fifteen minutes after dosing, rats were placed in activity chambers shaped like a figure eight (Reiter, 1983). Photobeams spaced around the chamber detected movement as counts for a total of 20 minutes. Immediately after the activity session, rats were euthanized for tissue collection (40–45 minutes after dosing). For the PND11 and adult studies, rats remained in the home cage until euthanasia in the same time frame.

Cholinesterase assay: At the appropriate time, rats were decapitated quickly under CO_2-induced anesthesia. Trunk blood was collected in heparinized tubes. The whole brain was removed from the skull, split sagitally (including cerebellum), and placed in dry ice. Whole blood was spun at 1000 × g for 10 minutes, and RBCs were collected and diluted with chilled 0.1 M $NaPO_4$, pH 8.0/1.0% Triton buffer at a 1:2 dilution (one to two parts), and then placed in dry ice. Tissues were stored at –80°C until the day of assay.

A radiometric assay was used to determine brain and blood ChE activity (Johnson and Russell, 1975). On the day of assay, brain tissues and RBC were thawed on ice (about 20 minutes). Brains were diluted in two volumes of chilled 0.1 M sodium phosphate buffer (pH 8.0) with 1% Triton X-100 and homogenized for 20–30 seconds (Polytron homogenizer, Kinematica Model PT3100, Littau, Switzerland). RBC samples were used directly as prepared on the day of collection. The assay was conducted with a small reaction volume (20 µL sample plus 80 µL substrate) to minimize tissue dilution. The final ACh iodide concentration was 1.2 mM. Reactions took place in a water bath at 26°C; incubation times were 30 seconds for brain and 2 minutes for RBC (lower ChE activity in RBC required longer times to produce reliably measured hydrolysis product). The reaction was then stopped using acid buffer. A toluene-based scintillant was added, the vials were shaken to allow extraction of the labeled [^{3}H]acetate, and [^{3}H] activity was counted within a few hours in a Beckman scintillation counter (model LS6000LL; Fullerton, CA). All samples were run in duplicate; duplicates >20% apart were not used (this only occurred in 0.5% of the total samples assayed). Negative values after blank subtraction were set to zero.

II. RESULTS AND DISCUSSION

There was internal consistency across the studies in that the degree of ChE inhibition in the time-course studies was similar to the inhibition produced when the same dose was used in the dose-response studies.

Time-Course

ChE activity for the control groups in the time-course studies was very similar within each study as well as between studies (range, brain 4.98–5.803 μmol ACh hydrolyzed/min/mg tissue and RBC 0.678–0.896 μmol/min/mL) with the exception of the carbaryl study that had somewhat higher brain ChE activity (mean, 6.651 μmol/min/mg). There were no marked differences between control ChE activities on the day of dosing compared to the 24-hour time point.

For all carbamates, brain and RBC ChE showed considerable inhibition (brain, 30–70% inhibition and RBC, 60–90% inhibition) on the day of dosing. Treated groups were not significantly different from each other from 15 to 180 minutes for carbaryl-induced inhibition of brain and RBC ChE and for carbofuran inhibition of RBC. In contrast, methomyl showed the most rapid recovery during that time frame. The time of peak effect of each carbamate ranged from 15 to 90 minutes for both compartments. Based on statistically significant differences across the time points, the peak inhibitions were obtained at: carbaryl brain and RBC, 15–180 minutes; carbofuran brain, 15–45 minutes, and RBC, 15–180 minutes; formetanate brain, 45–90 minutes, and RBC, 15–90 minutes; methiocarb brain, 15–90 minutes, and RBC, 15–45 minutes; methomyl brain and RBC, 15 minutes; **oxamyl brain and RBC, 45–90 minutes**; and propoxur brain, 15–90 minutes, and RBC, 15–45 minutes. However, it should be noted that while the use of Tukey's test does provide alpha protection in multiple comparisons, small but significant differences at certain times may not be reproducible. Our choice of 40–45 minutes for the dose-response was based on these data as well as our need to have a common test time for all carbamates. Recovery of ChE activity to control levels was evident at 24 h for all except carbaryl RBC ChE (13% inhibition, $p = 0.0173$) and methomyl brain ChE (6% inhibition, $p = 0.0385$). In both cases, the degree of inhibition at 24 hours was marginal.

Dose-Response

PND17 control ChE values obtained across the dose response studies were similar and compared well with those data from the time-course studies (range, brain 4.98–5.79 μmol ACh hydrolyzed/min/mg tissue and RBC 0.686–1.11 μmol/min/mL). Brain ChE activity in controls was lowest in PND11 pups (3.38–3.70 μmol/min/mg tissue) and highest in adults (6.38–6.69 μmol/min/mg tissue), whereas there was little difference across ages for RBC ChE in untreated animals (PND11 0.635–0.871 and adults 0.606–0.599 μmol/min/mL). In contrast to the ChE data, there were clear study-to-study differences in control motor activity levels. Controls in the formetanate study were the highest, being significantly different than all the other control groups. The values of the carbofuran and methomyl controls were the lowest, but these differences were not statistically significant different from the other groups (except formetanate). All carbamates produced monotonic dose-related decreases. **For oxamyl, RBC ChE inhibition was significantly different from control in all dose groups, whereas the lowest dose did not produce significant inhibition of brain ChE. Only the highest dose decreased motor activity levels**. There was very little concordance between motor activity depression and the degree of ChE inhibition ($r^2 = 0.112$ and 0.059 for brain and RBC, respectively).

III. CONCLUSIONS

The study reveals good concordance between brain and RBC ChE inhibition, with RBC being generally more sensitive, following a single dose of N-methyl carbamates in PND17 rats. Onset and peak effect were rapid, and recovery began within hours after a single dose. ChE was inhibited in a dose-dependent manner, and for several carbamates, even the lowest dose tested produced significant inhibition. In contrast, motor activity dose–response curves were more variable and did not always show clear dose-related decreases.

The time-course of ChE inhibition was generally similar across most of the carbamates with the exceptions of methomyl, which showed significant recovery at the 45-minute time point and almost complete recovery at 180 minutes, and oxamyl, which showed greater inhibition at 45 minutes compared to 15 minutes. The choice of 40–45 minutes for the dose-response data was optimal for all except methomyl, for which the test time may have been slightly later than the peak effect; however, this would only serve to underestimate the degree of toxicity. Decarbamylation of the ChE enzyme occurs at the same rate regardless of which carbamate is involved (Reiner, 1971), so differences in these time-course patterns are likely due to differing rates at which the chemical is removed from the target organ through metabolism and elimination.

Regarding the age related toxicity, the youngest rats tested, PND11, were the most sensitive to brain ChE inhibition produced by both carbaryl and carbofuran and to carbofuran-induced RBC ChE inhibition: PND11

rats were more sensitive than PND17, which were more sensitive than adults to carbofuran, whereas the PND17 and adult rats were equally sensitive to carbaryl.

For the other five carbamates (formetanate, methiocarb, methomyl, **oxamyl**, and propoxur), only adults were tested, therefore the direct comparison could not be carried out. It is only possible to compare these dose-response data to those collected in adult rats previously (McDaniel *et al.*, 2007, summarised below) or with other literature data. There was greater inhibition in pups compared to adults at similar doses for formetanate, methiocarb, oxamyl, and propoxur but not methomyl. While these comparisons suggest age-related differences in sensitivity for at least some of the carbamates, such observations should be interpreted cautiously, as age-related differences in dose-response curves are best described using systematic comparisons in the same laboratory.

These data do not directly impact the risk assessment as no direct age-related comparison was possible in rats treated with oxamyl provided within this study for adult vs. PND11 or PND17 rats.

Study submitted to the EU for the first time in this submission.

B.6.7.1/08

Reference: **CA 5.7.1/02**	**Report:**	McDaniel, K.L., Padilla, S., Marshall, R.S., Phillips, P.M., Podhorniak, L., Qian, Y., Moser, V.C. (2007); Comparison of acute neurobehavioral and cholinesterase inhibitory effects of N-methylcarbamates in rat **Source:** Toxicological Sciences 98(2), 552–560 (2007) This is a paper published in the open literature, with no indication about GLP, or applied guidelines. Some parameters usually requested in the reporting format of the GLP-compliant final report are not include in the publication.

Executive summary:

In the present study about the acute toxicity of seven N-methyl carbamate pesticides, the dose-response profiles of cholinesterase (ChE) inhibition in brain and erythrocytes (RBCs) as well as motor activity (both horizontally and vertically directed) and clinical signs of overt toxicity were evaluated. The chemicals tested were carbaryl, carbofuran, formetanate, methiocarb, methomyl, **oxamyl**, and propoxur. All were administered orally, and rats were tested in 20-minute activity sessions beginning 15 minutes after dosing; tissues were collected immediately after activity sessions. In general, motor activity was a sensitive measure of ChE inhibition for all these carbamate pesticides, and vertical activity showed the greatest magnitude of effect at the highest doses compared to either horizontal activity or ChE inhibition. Brain and RBC ChE activities were generally affected similarly. Pearson correlation coefficients of within-subject data showed good correlation between the behavioral and biochemical end points, with brain ChE inhibition and horizontal activity showing the highest correlation values. Determination of benchmark dose levels for 10% change in each end point also revealed that these two measures produced the lowest estimates. Thus, motor activity decreases are highly predictive of ChE inhibition for N-methyl carbamates, and *vice versa*.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	Not given
	Purity:	99.0%
	Description:	Not given
	CAS #:	23135-22-0
	Stability of test compound:	Not given
2.	Vehicle and/or negative control:	Deionized water
3.	Test animals	
	Species:	Rat
	Strain:	Male Long-Evans rats (Charles River Laboratories, Raleigh, NC)
	Age at dosing:	Adult (~97 days old)
	Weight at dosing:	Not reported
	Source:	Charles River Laboratories, Inc., Raleigh, North Carolina
	Acclimation period:	Not given
	Diet:	Not given
	Water:	Not given
	Housing:	Singly housed on heat-treated pine shavings in temperature and humidity controlled facilities
4.	Environmental conditions	
	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	Not reported

B. STUDY DESIGN AND METHODS

Rats were weighed the day prior to dosing and assigned to a dose group using stratification of weights. On the morning of testing, each rat (n = 10/dose group) received the treatment in a single oral gavage dose (given at 1 mL/kg). Approximately 10–12 minutes later, each animal was visually examined and received a score, termed ‘‘Tox Score”. The Tox Score was a ranked, global description of degree of overt cholinergic signs, including, but not limited to, lacrimation, miosis, fasciculations, smacking, tremors, polyuria, and diarrhea (any of these alone or in combination). Rats were scored as 1, normal; 2, some effects that were not very obvious; and 3, severe and obvious effects. The examiners had no knowledge of the treatment of the animals.

Motor activity assessment began 15 minutes after dosing, and the session length was 20 minutes. Activity was monitored in a photocell-based chamber shaped like a figure eight (Reiter, 1983). A set of eight photocells spread throughout the chamber measured horizontal activity, and a bank of photocells placed 14 cm above the flooring measured vertical activity.

At the end of the activity session, half of the rats (n = 5/dose group) were removed and immediately decapitated under CO_2 anesthesia for blood and brain collection. The other half of the rats were returned to their home cages and were not used further. Thus, tissues were collected within 35–40 minutes after dosing. Whole brain was collected and immediately placed on dry ice. Trunk blood was collected in a heparinized tube and centrifuged at 1000 × g for 10 minutes to separate plasma and RBC. The RBC fraction was diluted 1:3 (1 part RBC plus two parts 0.1 M sodium phosphate buffer, pH 8.0 containing 1% Triton). Brain and diluted RBC were stored at −80°C until assayed.

ChE Assay

The brain tissue was thawed on ice and prepared on the day of analysis. The brain was weighed and diluted (weight/volume) with 0.1 M sodium phosphate buffer, pH 8.0 containing 1% Triton. The final dilution was 1:3. The brain tissue was homogenized on ice using a Polytron (model PT3100, probe 3012/2TM, 20000 rpm, Brinkman Industries, Westbury, NY) for 20 seconds. Special care was taken to limit reactivation of

the brain and RBC carbamylated ChE. Tissue dilution was kept to a minimum, and tissues were not further diluted until the exact moment of adding the substrate at the beginning of the assay. Tissues were kept on ice until the exact moment of the assay, and the time between homogenization (brain) and assay was minimized (preliminary experiments indicated that brain homogenate diluted 1:3 kept on ice for 90 minutes did not show any significant reactivation).

The radiometric assay was essentially as described by Johnson and Russell, with a total reaction volume of 100 μL with a final substrate concentration of 1.2 mM acetylcholine iodide spiked with 0.1 μCi of [^{3}H]acetylcholine iodide (76.0 mCi/mmol, Perkin Elmer Life Sciences, Boston, MA). The assay was conducted at 26°C using an incubation of 1 minute for brain homogenate and 3 minutes for RBC. After the reaction was stopped and scintillant was added, activity was counted within 24 hours of the assay in a Beckman scintillation counter (model LS6000LL, Fullerton, CA). Counting efficiency, as determined by an external quench standard, was approximately 62%. On the day of each assay, reference standards (serial dilutions of control rat brain homogenate kept frozen at −80°C) were analyzed immediately before the experimental tissues to ascertain that the assay was performing correctly. These reference values varied no more than 10% over the course of the experiments.

II. RESULTS AND DISCUSSION

There were no significant changes in any end point observed at the two lower doses of oxamyl (see figure below). At the higher doses, RBC ChE was inhibited to a greater degree than brain, and in fact, the magnitude of change was more similar to the motor activity decreases. Only one and two rats in the 1 and 1.5 mg/kg dose groups, respectively, showed overt toxicity (Tox Scores of “2”). Correlation coefficients were high, with brain ChE being significantly more predictive than RBC ChE for horizontal but not vertical activity. BMD10 values were very similar (0.25–0.51 mg/kg).

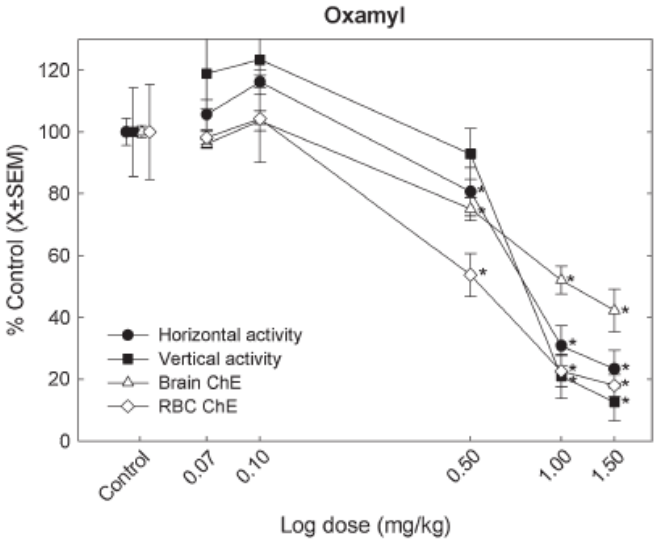

FIG. 6. Effects of oxamyl on motor activity (horizontal and vertical) and ChE activity (brain and RBC), expressed as percent control. * indicates doses significantly different from control; $n = 5$/dose for ChE measurements, $n = 10$/dose for motor activity.

III. CONCLUSIONS

Motor activity depression was generally greater than the ChE inhibition, and vertical activity was depressed to a greater extent than horizontal activity at the high end of the dose-response curves. This greater sensitivity of vertical activity could be due to other influences on the behavior (*e.g.*, vestibular effects) or the configuration of the chambers (rearing is only measured in the central arena). When focusing on the low end of the dose response, however, significant motor activity decreases were not observed in the absence of significant ChE inhibition. Oxamyl produced significantly more inhibition in RBCs than in brain across most doses—this same pattern of inhibition was also noted in the time-course assessment (Padilla *et al.* 2007), which is summarised below.

In the present study, doses of 1 and 1.5 mg/kg produced 80–90% blood inhibition and almost complete suppression of motor activity. These results do not impact the risk assessment and confirm the results of other acute studies including HLR 1118-96 (summarised in Point CA 5.7.1).

Study submitted to the EU for the first time in this submission.

B.6.7.1/09

Reference: **CA 5.7.1/04**	**Report:**	Padilla, S., Marshall, R.S., Hunter, D.L., Lowit, A. (2007); Time course of cholinesterase inhibition in adult rats treated acutely with carbaryl, carbofuran, formetanate, methomyl, methiocarb, oxamyl or propoxur **Source:** Toxicology and Applied Pharmacology 219, 202–209 (2007)

Executive summary:

To compare the toxicity of seven N-methyl carbamates, time course profiles for brain and red blood cell (RBC) cholinesterase (ChE) inhibition were established for each. Adult, male, Long Evans rats (n = 4–5 dose group) were dosed orally with either carbaryl (30 mg/kg in corn oil); carbofuran (0.5 mg/kg in corn oil); formetanate HCl (10 mg/kg in water); methomyl (3 mg/kg in water); methiocarb (25 mg/kg in corn oil); oxamyl (1 mg/kg in water); or propoxur (20 mg/kg in corn oil). This level of dosing produced at least 40% brain ChE inhibition. Brain and blood were taken from 0.5 to 24 hours after dosing for analysis of ChE activity using two different methods: (1) a radiometric method which limits the amount of reactivation of ChE activity, and (2) a spectrophotometric method (Ellman method using traditional, unmodified conditions) which may encourage

reactivation. The time of peak ChE inhibition was similar for all seven N-methyl carbamate pesticides: 0.5–1.0 hour after dosing. By 24 hours, brain and RBC ChE activity in all animals returned to normal. The spectrophotometric method underestimated ChE inhibition. Moreover, there was a strong, direct correlation between brain and RBC ChE activity (radiometric assay) for all seven compounds combined (r2 = 0.73, slope 1.1), while the spectrophotometric analysis of the same samples showed a poor correlation (r2 = 0.09). For formetanate, propoxur, methomyl, and methiocarb, brain and RBC ChE inhibitions were not different over time, but for carbaryl, carbofuran, and oxamyl, the RBC ChE was slightly more inhibited than brain ChE. These data indicate (1) the radiometric method is superior for analyses of ChE activity in tissues from carbamate-treated animals; (2) that animals treated with these N-methyl carbamate pesticides are affected rapidly, and recover rapidly; and (3) generally, assessment of RBC ChE is an accurate predictor of brain ChE inhibition for these seven pesticides.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	Not given
	Purity:	99.0%
	Description:	Not given
	CAS #:	23135-22-0
	Stability of test compound:	Not given
2.	Vehicle and/or negative control:	Deionized water
3.	Test animals	
	Species:	Rat
	Strain:	Male Long-Evans rats (Charles River Laboratories, Raleigh, NC)
	Age at dosing:	Adult (~90 days old)
	Weight at dosing:	Not reported
	Source:	Charles River Laboratories, Inc., Raleigh, North Carolina
	Acclimation period:	1 week
	Diet:	Not given
	Water:	Not given
	Housing:	Singly housed on heat-treated pine shavings in temperature and humidity controlled facilities
4.	Environmental conditions	
	Temperature:	Not reported
	Humidity:	Not reported
	Air changes:	Not reported
	Photoperiod:	Not reported

B. STUDY DESIGN AND METHODS

The animals were dosed by gavage with the N-methyl carbamate and then at specific times after dosing; each animal was anesthetized with CO_2 and killed by decapitation between 0.5 and 25 hours after dosing. The time points were chosen based on preliminary experiments. Usually there were five treated animals per time point with one control animal assigned to each time point. Whole brain was collected and immediately placed on dry ice. Trunk blood was collected in a heparinized tube. The blood was separated by centrifugation at 1000 × g for 10 minutes. After removal of the plasma, an aliquot was taken of the red blood cell fraction and diluted 1:3, *i.e.,* 1 part red blood cells to 2 parts 0.1 M sodium phosphate buffer, pH 8.0 containing 1% Triton X-100. The brain and diluted red blood cells were stored at −80°C until analysis.

The brain tissue was thawed on ice and prepared on the day of analysis. The brain was weighed and diluted (weight/volume) with 0.1 M sodium phosphate buffer, pH 8.0 containing 1% Triton. The final dilution was 1:3. The brain tissue was homogenized on ice using a Polytron (Polytron PT3100, probe 3012/2TM, 20000 rpm, Brinkman Industries, Westbury, NY) for 20 seconds.

Further dilution of both red blood cells and brain homogenate was necessary for analysis on the automated analyzer. The previously diluted (1:3) red blood cells were diluted (on ice) 1:8 for a final dilution of 1:24. The 1:3 brain homogenate was further diluted (on ice) 1:5 for a final dilution of 1:15.

Cholinesterase analyses: Each sample was analyzed on the same day using both the spectrophotometric and radiometric cholinesterase assays (described below). Both of the assays measured total cholinesterase activity (acetyl- and butyrylcholinesterase activity).

Spectrophotometric method: Total cholinesterase activity was determined for brain and red blood cells using a Hitachi 911 Automatic Analyzer (Roche Diagnostics, Indianapolis, IN) according to a method described by Hunter and coworkers (Hunter *et al.*, 1997). This variation of the Ellman method has a total assay volume of 355 µL, consisting of 5 µL of tissue, 300 µL of chromogen/buffer (0.3 mM 5,5′-dithio-bis (2-nitrobenzoic acid); final DTNB concentration in assay 0.25 mM), and 50 µL of substrate (8.45 mM acetylthiocholine iodide; final concentration in assay 1.2 mM). There is a 5-minute preincubation period and the reaction is conducted at 37°C.

Radiometric method: The radiometric assay was essentially as described by Johnson and Russell (1975), with a total reaction volume of 100 µL consisting of up to 80 µL of tissue and 20 µL of substrate (6.0 mM acetylcholine iodide and 0.1 µCi of [^{3}H]acetylcholine iodide [76.0 mCi/mmol, Perkin Elmer Life Sciences, Boston, MA] per 20 µL; final substrate concentration was 1.2 mM). The assay was conducted at 26°C using 20 µL of 1:3 brain homogenate incubated for 1 minute or 80 µL of 1:3 red blood cells incubated for 3 minutes. After the reaction was stopped and scintillant was added, activity was counted within 24 hours of the assay in a Beckman scintillation counter (model LS6000LL, Fullerton, CA). Counting efficiency, as determined by an external quench standard, was approximately 62%.

II. RESULTS AND DISCUSSION

For oxamyl, there was a significant interaction between tissue and time, so the data were further analyzed to determine which tissue was different from control at which time. The dose of 1 mg/kg produced approximately 80% inhibition in red blood cell cholinesterase and 50% inhibition of brain cholinesterase activity by 0.5 hours after dosing. By 4 hours after dosing, both the brain and red blood cell cholinesterase recovered to control levels

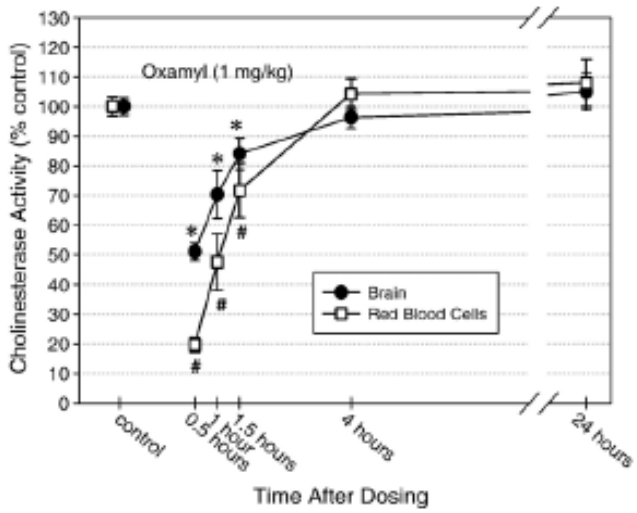

Fig. 9. Time course of brain and red blood cell cholinesterase inhibition in adult male rats dosed orally with oxamyl in water. A repeated measures ANOVA indicated an interaction between tissue (brain or red blood cell) and time (p <.0001). A post-hoc test (Fisher's PSLD) was conducted to determine which time points for brain or red blood cells were different (p<.05) from control. *Brain is different from control; #red blood cell is different from control. n=5 per time point, except n=4 at 24 h. Brain control activity=5237±154 nmol acetylcholine hydrolyzed per min/g tissue. Red blood cell control activity=441±14 nmol acetylcholine hydrolyzed per min/ml red blood cells. All data are presented as means±SE.

III. CONCLUSIONS

In the present group of studies, cholinesterase levels measured using a spectrophotometric assay (using the conditions of the traditional, unmodified Ellman assay), which requires extensive tissue dilution, were compared to a radiometric assay that can be conducted with highly concentrated tissues for very short incubation times. In this case, the red blood cells and brain tissue were treated identically until minutes before the actual assay, and yet, the spectrophotometric assay tended to underestimate the degree of cholinesterase inhibition. Interestingly, a comparison between red blood cell and brain cholinesterase inhibition showed excellent correlation if the radiometric assay was used for analysis, but that correlation became non-existent if the spectrophotometric assay under the conditions performed here was used for analysis. This most likely reflects the fact that if one does not have control of the key variables that affect reactivation, the data may become more variable and would tend, therefore, to exhibit less of a correlation between two measures.

It should be noted that the testing laboratory has shown that if the traditional Ellman assay is modified to limit reactivation by using more concentrated tissue and conducting the assay more rapidly at a lower temperature, the results may be comparable to those collected using the radiometric assay. However, because conditions of a modified Ellman assay are not yet standardized across different laboratories, ChE data generated from modified Ellman assays for carbamate pesticides can be difficult to compare across laboratories and across chemicals. The ChE data collected in this study using traditional, unmodified Ellman and radiometric assays provide a tool for evaluating ChE data generated from modified Ellman assay from other laboratories. The U.S. Environmental Protection Agency (EPA) in its cumulative risk assessment for the N-methyl carbamate pesticides (U.S. EPA, 2005) developed a critical analysis of the ChE data submitted to support pesticide registration. The EPA's analysis compared the results of the ChE data presented here for the unmodified Ellman and radiometric assays with ChE data generated primarily using modified Ellman techniques.

As can be seen in the present study, with all of the tested compounds (including oxamyl), initiation of cholinesterase inhibition was rapid (within an hour) and short acting. Within 24 hours, both red blood cell and brain cholinesterase inhibition had returned to control levels or above. This rapid and brief time course of cholinesterase inhibition after an acute dose is similar to many previously published studies on mammalian toxicity of some of the N-methyl carbamates studied here.

There is even less information in the published literature regarding formal analyses of the correlation between brain and blood (either red blood cells or whole blood) cholinesterase inhibition in N-methyl carbamate treated animals. In the present group of studies, there was, in general, excellent correlation between blood (red blood cells) and brain cholinesterase inhibition within the 24 hours after intoxication with these N-methyl carbamate pesticides. Considering the pattern of brain and red blood cell cholinesterase recovery by compound reveals some interesting patterns. The majority of the N-methyl carbamate pesticides presented a similar time course in recovery of red blood cell and brain cholinesterase inhibition.

RMS comments and conclusion for the renewal

These results coming from papers published after the first oxamyl evaluation corroborate the results of previous studies, also described in the RAR:

- **they further demonstrate rapid recovery, after oxamyl intoxication**
- **they indicate that doses of 1 and 1.5 mg/kg produced 80–90% blood inhibition and almost complete suppression of motor activity. These results do not impact the risk assessment and confirm the results of other acute studies including HLR 1118-96 (summarised in Point CA 5.7.1).**

B.6.7.2 Delayed polyneuropathy studies

Oxamyl is a carbamate insecticide that, unlike some organophosphates, does not pose a risk for delayed neurotoxicity. The majority of the literature indicates that carbamates do not bind to neuraltoxic esterase (NTE) and, therefore, do not cause organophosphate-induced neuropathy. Regardless, a test for delayed neurotoxicity in hens was conducted some years ago (summarised below). Although this study does not meet the current testing guidelines for the 28-day delayed neurotoxicity study in hens, it does provide scientific evidence that oxamyl does not cause delayed neurotoxicity at dose levels up to 40 mg/kg bw.

Delayed neurotoxicity following acute exposure

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.7.2/01

Reference: --	**Report:**	[redacted] (1970); Oral ALD and delayed paralysis test (white leghorn chickens) **DuPont Report No.:** HLR 234-70 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: Not given
   Purity: 95.0%

Deviations: Based on OECD test guideline 418 the following deviations were identified in the study protocol:-

- A preliminary study should be performed to determine the dose that should be used in the main study.
- No information on the preparation and living conditions of the animals was given.
- Neuropathy target esterase (NTE) activity was not determined at any stage.
- At least 6 hens should be used for interim NTE analyses (preferably at 2 sampling times) and at least 6 hens should be available for terminal sacrifice and for histopathological examination.
- The positive control should incorporate at least 3 hens for biochemistry and 3 for pathology.
- There was no indication that the hens destined for pathology were subjected to forced motor activity at least twice per week to evaluate minimal toxic effects.
- Negative control groups were not employed.
- Data on each animal was not provided.
- Histopathology was limited to the sciatic nerve. Evaluations are also recommended on the cerebellum, medulla oblongata, spinal chord (upper cervical, mid-thoracic and lumbo-sacral) and peripheral nerves (distal regions of the tibial nerves plus branches to the gastocnemial muscle).

I. MATERIALS & METHODS:

In an oral approximate lethal dose study (ALD), a single dose of Oxamyl (DPX-D1410 technical; batch not provided; purity 95.0%), as a 1% aqueous suspension, was administered directly into the crop of 1-year old white leghorn chickens at doses of 4.3, 12, 15, 17, 26, 30, 40, and 60 mg/kg bw per chicken.

In a delayed paralysis study, two groups of 5 chickens received intramuscular injections of 0.5 mg/kg bw atropine followed by a single oral dose of 20 or 40 mg/kg bw Oxamyl. The birds were monitored for 28 days for delayed neurotoxic effects.

As a positive control, 1200 mg/kg bw triorthocresyl phosphate was administered to 5 chickens after intramuscular injection of 0.5 mg/kg bw atropine.

A gross pathological and histopathological examination of sciatic nerve tissue was performed on all chickens.

II. RESULTS

The chickens administered 40 and 60 mg/kg bw Oxamyl died within 5 minutes of dosing. The estimated ALD was 40 mg/kg bw. Clinical signs of toxicity included sudden depression, respiratory difficulty, salivation, ataxia, in-coordination, tremors, drooping wings, 'star gazing posture', recumbency, opisthotonos and convulsions. Sudden depression, ruffled feathers, slight respiratory difficulty and ataxia were documented immediately after dosing at 12, 17 and 30 mg/kg bw. The respiratory difficulty was not evident after 30 minutes. The other symptoms persisted for several hours. No adverse clinical effects were recorded after administration of 4.3, 15 and 26 mg/kg bw. Gross and histopathological changes in the sciatic nerve were not recorded.

In the delayed paralysis study, sudden depression, lethargy, ruffled feathers, slight respiratory difficulty, ataxia and in-coordination were recorded at both dose levels. The respiratory difficulty was not evident after 30 minutes. The other symptoms persisted for 12 hours after which no clinical signs of toxicity were evident. Egg production was unaffected. There were no mortalities. No gross or histopathological changes were recorded in the sciatic nerve.

After triorthocresyl phosphate administration, transitory depression, respiratory signs and nervous signs were recorded which persisted for 12 hours. Two weeks after dosing all chickens developed in-coordination, ataxia, 'star gazing posture' and recumbency and egg production ceased. Gross changes in the sciatic nerve were not recorded. Histopathological analysis recorded myelin fragmentation, axonal disintegration, Schwann cell proliferation and round cell infiltration.

III. CONCLUSIONS

The delayed polyneuropathy studies study HLR 234-70 was originally submitted under EU Rev8 Point IIA 5.7.1 and conducted with test material pure oxamyl (PAI). Guidelines were not given. A review of this study indicates that it partially meets the current guideline B.37, the following deviations were noted:

- A preliminary study was not performed to determine the dose that should be used in the main study.
- No information on the preparation and living conditions of the animals was given.
- Neuropathy target esterase (NTE) activity was not determined at any stage.
- At least six hens should be used for interim NTE analyses (preferably at 2 sampling times) and at least six hens should be available for terminal sacrifice and for histopathological examination.
- The positive control should incorporate at least three hens for biochemistry and three for pathology.
- There was no indication that the hens destined for pathology were subjected to forced motor activity at least twice per week to evaluate minimal toxic effects.
- Negative control groups were not employed.
- Data on each animal were not provided.
- Histopathology was limited to the sciatic nerve. Evaluations are also recommended on the cerebellum, medulla oblongata, spinal chord (upper cervical, mid-thoracic and lumbo-sacral), and peripheral nerves (distal regions of the tibial nerves plus branches to the gastocnemial muscle).

Despite the deviation, it is clear that Nno clinical signs of toxicity were evident after 12 hours and no histopathological changes in the sciatic nerve were recorded. This is to confirm tst book information about the inability of carbamates to induce delayed neurotoxicity . Oxamyl does not induce delayed neurotoxicity in hens.

RMS comments and conclusion

This study is acceptable and relied upon despite the many deviations, since it provides further evidence that oxamyl does not cause delayed neurotoxicity. It is indeed well known that carbamate insecticides do not bind to NTE, and therefore do not have the potential to cause delayed neuropathy. There is no nedd for further studies.

28-Day delayed neurotoxicity

This is not a data requirement according to Regulation (EC) 1107/2009.

A delayed neurotoxicity study was not required for oxamyl. None of the subchronic and chronic studies conducted with oxamyl in rats, mice, or dogs demonstrated any effects suggestive of delayed neurotoxicity.

B.6.7.3 Summary of neurotoxicity

The results from the acute and subchronic neurotoxicity studies conducted with oxamyl are summarised below.

Table 102 Summary of neurotoxicity studies for oxamyl

Type of study	Dose range tested	NOAEL		LOAEL		Target organ(s) and effects	Reference[a]
		ppm	mg/kg/d	ppm	mg/kg/d		
Oral, acute Human	0, 0.005, 0.015, 0.03, 0.06, 0.09, 0.15 mg/kg bw (males)	—	**0.09**	--	0.15	Acetylcholinesterase inhibition and increased saliva	HLO-1998-01505
Acute neurotoxicity (gavage) Rat	0, 0.1, 1.0, 2.0 mg/kg bw (males) 0, 0.1, 0.75, 1.5 mg/kg bw (females)	—	**m: 0.1** f: 0.1	--	m: 1.0 f: 0.75	Acetylcholinesterase inhibition, decreased body weight and food consumption	HLR 1118-96
Subchronic neurotoxicity (Feeding), 90-d Rat	0, 10, 100/30, 300/250 ppm (equivalent to 0, 0.55, 1.69, 15.3 mg/kg bw/day for males and 0, 0.67, 2.03, 20.3 mg/kg bw/day for females)[b]	30	**m: 1.69** f: 2.03	100	m: 15.3 f: 20.3	Acetylcholinesterase inhibition and clinical signs	HL-1998-00708
Oral, reversibility study (gavage) Rat	0 and 1 mg/kg bw	Not applicable				Acetylcholinesterase inhibition and tremors, recovery complete within 2 hrs post-dosing	HL-1997-00641
Oral, reversibility study (gavage) Rat	1 mg/kg bw	Not applicable				Acetylcholinesterase inhibition and tremors, recovery complete by 4 hrs post-dosing	Padilla *et al.*, 2007
Acute neurotoxicity (gavage) Rat	1 and 1.5 mg/kg bw	Not applicable				Acetylcholinesterase inhibition and clinical signs, BMD10 for brain ChE 0.25 mg/kg bw and RBC ChE 0.51 mg/kg bw	McDaniel *et al.*, 2007
Acute inhalation neurotoxicity Rat	0, 0.0049, and 0.024 mg/L	**<0.0049 mg/L**		0.0049 mg/L		Acetylcholinesterase inhibition	DuPont-4383, Revision No. 1

[a] Summarised in Point CA 5.7.1 in this document.

[b] After 7 days of exposure, the 2 highest dose levels were reduced to 30 and 250 ppm due to severe toxicity at the high dose level, the food intake was calculated as an overall mean.

A single oral dose study in human volunteers was conducted with oxamyl. In this study, a statistically significant and biologically relevant increase in saliva secretion was noted at the highest dose level of 0.15 mg/kg bw one hour following dose administration. In addition, statistically significant and biologically relevant decreases in plasma and RBC cholinesterase activities were observed at 0.15 mg/kg bw with the period

of maximum depression occurring at 45 and 60 minutes for plasma cholinesterase activity and from 30 to 60 minutes for RBC cholinesterase activity. Plasma and RBC cholinesterase activities returned to baseline values within 4 and 3 hours, respectively, following administration of oxamyl. No adverse treatment-related effects were noted on ECG, vital signs, haematology and clinical chemistry parameters (including plasma and RBC cholinesterase), urinalysis, or clinical signs at dose levels of **0.09 mg/kg bw (NOAEL in this study for acute neurotoxicity in human after oral administration)** and lower.

Acute and subchronic neurotoxicity studies in rats were conducted with oxamyl. In the acute neurotoxicity study, treatment-related clinical signs related to cholinesterase inhibition were noted in male and females at the mid- and high-dose levels (1 and 2 mg/kg bw for males and 0.75 and 1.5 mg/kg bw for females, respectively). Decreased body weight gain was noted in males at the mid- and high-dose and in females at the high dose. Plasma, RBC, and brain cholinesterase inhibition was noted in males and females at the mid- and high-dose levels on Day 1. No treatment-related neuropathological findings were observed. The acute neurotoxicity NOAEL in rats was derived as **0.1 mg/kg bw, indicating no significant species difference when compared to the NOAEL obtained in humans.**

In the subchronic neurotoxicity rat study, treatment-related clinical signs of toxicity related to cholinesterase inhibition were noted in males and females at ≥100 ppm. Decreased body weight, body weight gain, food consumption, and food efficiency were also noted at doses ≥100 ppm. Plasma, RBC, and brain cholinesterase inhibition was noted in males and females at 250 ppm. No treatment-related neuropathological findings were noted. The subchronic neurotoxicity NOAEL in rats was **derived as 1.6 mg/kg bw, indicating that the most relevant effect is associated with acute exposure with no cumulative effects regarding neurotoxicity.**

A reversibility study was conducted with oxamyl. The objective of this study was to determine the length of time needed for recovery from inhibition of cholinesterase activity following an acute oral exposure to oxamyl at a concentration of 1.0 mg/kg bw. Clinical signs (predominantly tremors) were noted, and plasma, RBC, and brain cholinesterase activities were decreased within 30 minutes of dosing with **recovery occurring within 2 hours post dosing**. In a subacute study in which rats were administered oxamyl at a concentration of 2.4 mg/kg bw/day five times per week for two weeks, mild fasciculations, slight pallor, salivation, and body weight loss were noted.

A 4-hour inhalation study was conducted in which marginal effects on RBC and brain cholinesterase activities were induced at 0.0049 mg/L, the lowest concentration evaluated.

Finally, a **comparison of the sensitivity to acetylcholinesterase inhibition in adult rats and pre-weanling rats** was conducted for oxamyl. For RBC cholinesterase inhibition (the more affected parameter), effects were essentially the same in preweanling pups and adults based on results at the dose in common (0.15 mg/kg) and linear trends across the range of doses tested. For brain cholinesterase (the less affected parameter), preweanling pups appeared to be slightly more sensitive than adults based on results at the common dose. At lower doses (0.075, 0.1, and 0.125 mg/kg), however, **the range of responses for brain cholinesterase activity in pups tended to overlap with the linear trend line in dose response for adults**. Therefore, the sensitivity of the pre-weanling rats was not enhanced compared to the adult rats.

In conclusion, acute acetylcholinesterase inhibition is the most sensitive effect, but is rapidly reversible as it has been observed across species that metabolism is quite rapid and complete within a few hours. Moreover, a bolus acute dose represents the most serious and relevant dosing regimen for development of a risk assessment benchmark due to the fact that in rodent studies, small amounts of the test material are consumed over a 24 hr period and therefore, metabolism and recover occurs during dosing. The point of departure for the risk assessment should be the acute neurotoxicity study conducted in the rat by gavage where the NOAEL was 0.1 mg/kg.

B.6.8 Other toxicological studies

B.6.8.1 Toxicity studies of metabolites and relevant impurities

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

The principal metabolites of oxamyl found in plants, soil, water, and/or sediment are IN-A2213, IN-N0079, IN-D2708, IN-T2921, and IN-L2953. These metabolites, with the exception of IN-T2921, have been observed

in their free or conjugated forms in metabolism studies performed in rats and mice (AMR 1226-88; O/ME 33; summarised in Point CA 5.1.1). Below levels found in urine and faeces are reported (see Table 4). IN-N0079 was degraded and eliminated mainly as conjugates of IN-D2708 and IN-KP532.

Conjugates of metabolites	% Total radioactivity	
	Urine	Faeces
IN-A2213 (I)	14.5	12.6
IN-L2953 (II)	23.9	22.2
IN-D2708 (III)	18.0	16.7
IN-KP532 (IV)	22.6	20.7
Total	**79.0**	**72.0**

IN-T2921 was not detected in either the rat or the mouse; however, this metabolite is a proposed intermediate in the formation of IN-D2708 from IN-N0079 and was identified in goat rumen fluid (DuPont Report O/ME 38 [AMR 09-80], in the Oxamyl EU Renewal Dossier, Document M-CA, Section 6, DuPont-40933 EU).

A number of non-guideline, non-GLP acute toxicity tests were carried out with metabolites IN-A2213, IN-L2953, IN-D2708, and IN-N0079 and submitted in the form of one-page data sheets. Some subacute, one subchronic and a mutagenicity test were also provided. However, these studies are considered supplementary at best, since their reliability could not be checked. By the way they are reported in the following since they can give indication about the toxicity of some oxamyl metabolites, although some of them being identified as metabolites in rodents. They indicate that the metabolites of oxamyl are much less toxic than the parent compound.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/01

Reference:	**Report:**	[redacted] (1968); Acute oral test (CHR-CD rats) **DuPont Report No.:** HLR 300-68 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)
--		

1. Test material: IN-A2213 technical metabolite
   Lot/Batch #: Not given
   Purity: Not given

Materials and methods:

Single oral doses of INA-2213 (IN-A2213 technical metabolite; batch A2213; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), evaluation 5.8.1.1, INA-2213 has a purity of 100%) were administered in peanut oil by gavage to fasted male ChR-CD rats at doses of 90, 450, 2250, 3400, 5000, 7500, and 11,000 mg/kg bw. The precise details of the dosing regimen were however unclear. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing.

Findings:

Mortality was observed at 11,000 mg/kg bw 3 days after dosing. Clinical signs of toxicity most often observed included discomfort, light-coloured faeces for 2 days after dosing, half closed eyes, salivation, and weight loss. Other clinical signs of toxicity included a stained wet area around the mouth.

Conclusions:

The toxicity of metabolites study HLR 300-68 was originally submitted under EU Rev8 Point IIA 5.8.1.1 and conducted with test material IN-A2213 technical metabolite. Guidelines were not given. The oral ALD (Acute lethal dose) for INA-2213 in male ChR-CD rats was 11,000 mg/kg bw.It does provide evidence that the primary rat metabolite IN-A2213 is much less toxic than the parent compound

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/02

Reference:	**Report:**	[redacted] (1971); Ten-dose subacute oral tests **DuPont Report No.:** HLR 228-71 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)
--		

1. Test material: IN-A2213 technical metabolite
   Lot/Batch #: A2213-1
   Purity: Not given

Materials and methods:

A ten-dose oral subacute test was performed with IN-A2213 (IN-A2213 technical metabolite; batch A2213-1; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), evaluation 5.8.1.2, INA-2213-1 has a purity of 100%). The test substance was administered by gavage to 6 young adult ChR-CD male rats as a 20% suspension in corn oil for 5 days at a dose of 2200 mg/kg bw/day. When 4 of the 6 rats died, a second test using a 10% suspension of the test substance in corn oil was performed on 6 new animals. The animals were gavaged five times a week for 2 weeks at a dose of 1000 mg/kg bw/day followed by a 14-day recovery period. Concurrent control groups were gavaged with corn oil.

A pathological exam was performed on the test animals. A number of tissues were examined histopathologically including lung, trachea, liver, kidneys, spleen, thymus, testes, epididymides, stomach, duodenum, heart, brain, adrenal, eye and bone marrow.

Findings:

Mortality:

In the first test one rat died one day after the 4^{th} dose, two rats died one day after the 5^{th} dose and one rat died two days after the 5^{th} dose. In the second test, three test and three control rats were sacrificed approximately 4 hours and 14 days after the tenth dose.

Clinical signs:

In the first study, stained perineum, chromodacryorrhea, weakness and unkemptness were recorded. In the second study, during the dosing period, clinical signs of toxicity included weakness and unkemptness.

Body weight changes:

Continuous weight loss was recorded during the first week in the first test. In the second test, continuous weight loss was recorded during dosing. During the recovery period, the weight curve was lower than but parallel to that of controls.

Pathology:

Test substance-related effects observed at the dose level of 2200 mg/kg bw/day included atrophy of spleen and thymus, hypoplasia of bone marrow, centrilobular necrosis, congestion and haemorrhage of the liver. Rats dosed with the repeated dose rate of 1000 mg/kg bw/day showed atrophy of spleen and thymus, hypoplasia of bone marrow and depletion of liver glycogen. However, these changes were reversed during the 14-day recovery period.

Conclusions:

The toxicity of metabolites study HLR 228-71 was originally submitted under EU Rev8 Point IIA 5.8.1.2 and conducted with test material IN-A2213 technical metabolite. Guidelines were not given.

INA-2213 caused mortality, body weight loss and microscopic changes in the spleen, thymus, bone marrow, and liver when administered by gavage at a dose rate of 2200 mg/kg bw/day. At 1000 mg/kg bw/day, no mortality occurred and mild, reversible histopathological changes in the lymphoid tissue and liver were recorded.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/03

Reference: --	**Report:**	[redacted] (1973); Oral LD_{50} Test **DuPont Report No.:** HLR 126-73 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-L2953 technical metabolite
   Lot/Batch #: Not given
   Purity: Not given

Materials and methods:

INL-2953 (INL-2953 technical metabolite; batch L-2953; purity not stated), as a suspension in acetone:corn oil (15:85), was administered by single-dose oral gavage to young adult male ChR-CD rats (10 animals /dose group) at doses of 4000, 4500, 5000, 6000, 6500, and 7000 mg/kg bw at a dose volume of 3.49 – 5.71 mL. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing.

Findings:

Mortalities were recorded at 4000 (10%), 6000 (20%), 6500 (30%) and 7000 (70%) mg/kg bw. Clinical signs of toxicity most often observed included lethargy, prostration, ruffled fur, polyuria, white crystals in urine, pallor, half-closed eyes and wet perineum Tremors were recorded at 6000 mg/kg bw. All surviving animals appeared normal by day 9 or earlier and throughout the remainder of the study. Body weight loss was observed for 1-5 days after dosing at the lethal doses (≥6000 mg/kg bw) and from 1-6 days after dosing at the non-lethal doses (< 6000 mg/kg bw).

Conclusions:

The toxicity of metabolites study HLR 126-73 was originally submitted under EU Rev8 Point IIA 5.8.1.3 and conducted with test material IN-L2953 technical metabolite. Guidelines were not given. The oral LD_{50} for INL-2953 in male rats was 6675 mg/kg bw.. It does provide indication that the metabolite IN-L2953 is much less toxic than the parent compound.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/04

Reference: --	**Report:**	[redacted] (1974); Acute oral test - ALD **DuPont Report No.:** HLR 585-74 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-1
   Purity: Not given

Materials and methods:

Single oral doses of INN-79-1 (IN-N0079 technical metabolite; batch N0079-1; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), evaluation 5.8.1.4, INN-79-1 has a purity of 99.57%) in corn oil were administered by gavage to male ChR-CD rats at dose concentrations of 90, 130, 200, 300, 450, 670, and 1000 mg/kg bw. Surviving animals were observed for clinical signs of toxicity, body weight effects and mortality for up to 14 days after dosing.

Findings:

Mortalities were observed at 450 mg/kg bw and above up to 6 days after dosing. The most frequent clinical signs of toxicity recorded include belly-to cage posture, salivation, hyper- responsiveness to noise and weight loss.

Conclusions:

The toxicity of metabolites study HLR 585-74 was originally submitted under EU Rev8 Point IIA 5.8.1.4 and conducted with test material IN-N0079 technical metabolite. Guidelines were not given. The oral ALD (acute lethal dose) for INN-79-1 in male ChR-CD rats was 450 mg/kg bw. It does provide indications that this metabolite is much less toxic than the parent compound.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/05

Reference: --	**Report:**	[redacted] (1976); 10 day subacute test **DuPont Report No.:** HLR 390-76

		Guidelines: Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-2
   Purity: Not given

Materials and methods:

INN-79-2 (IN-N0079 technical metabolite; batch N0079-2; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), evaluation 5.8.1.5, INN-79-2 has a purity of 99.57%) was administered by gavage as a 1.0% suspension in corn oil at a dose level of 90 mg/kg bw/day to six young adult ChR-CD male rats. The animals were gavaged five times a week for 2 weeks. Concurrent control groups were gavaged with corn oil. Three control and 3 test rats were sacrificed approximately 4 hours after the last dose. The remaining three control and three test rats were sacrificed 14 days after the last dose.

A pathological exam was performed on all test animals. Histopathology was performed on a number of tissues including liver, pancreas, skin, mammary gland, brain, eye, adrenal, kidney, testis, epididymis, stomach, esophagus, small intestine, cecum, colon, spleen, thymus, bone marrow, sternal bone, lung, trachea, thyroid, and heart.

Findings:

Mortality:

There was no test substance-related mortality observed during the course of the study.

Clinical signs:

In the first and second week of the study, limpness was the only clinical sign observed. During the recovery period animals exhibited mild lethargy.

Body weight and body weight gains:

Significantly decreased body weights and body weight gains were recorded during the treatment period.

Pathology:

Significantly decreased absolute liver and kidney weights and decreased absolute and relative spleen and thymus weights were recorded. The absolute weight of the testes was decreased but the relative weight was increased compared to controls. Three test rats sacrificed after the 10th dose showed slight atrophy of the thymus, bone marrow, and spleen. This change was not detected in rats sacrificed after a 14-day recovery period. Acute pancreatitis was observed in one test rat sacrificed after the 10th dose. Loss of cytoplasmic vacuolation of hepatocytes in the centrilobular area of the liver was observed in all treated rats sacrificed after the 10th dose. There was a partial recovery from this lesion after a 14-day recovery period.

Conclusions:

The toxicity of metabolites study HLR 390-76 was originally submitted under EU Rev8 Point IIA 5.8.1.5 and conducted with test material IN-N0079 technical metabolite. Guidelines were not given. INN-79-2 caused decreased body weight and body weight gain and organ weight alterations in the thymus, bone marrow, spleen, liver, and pancreas following 10 repeated dosages of 90 mg/kg bw/day. It does provide indication that the metabolite IN-N0079 is much less toxic than the parent compound.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/06

Reference: --	**Report:**	[redacted] (1976); Ninety day feeding study in rats with 1 cyano-N,N-dimethylformamide (INN–79) metabolite of Vydate **DuPont Report No.:** HLR 630-76 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: N0079-3
   Purity: 100.0%

Materials and methods:

In a 90-day feeding study, IN-N0079 (IN-N0079 technical metabolite; batch N0079-3; purity 100%) was administered to male and female ChR-CD rats (16 rats/sex/group) at concentrations of 0, 50, 150, and 450 ppm (approximately 0, 4.0, 11.4, and 34.3 mg/kg bw/day in males and 0, 4.2, 12.6, and 35.7 mg/kg bw/day in females, respectively). Parameters evaluated included body weight, body weight gain, food consumption, food efficiency, clinical signs, haematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. After 90 days of feeding, 10 rats/sex/group were sacrificed and examined for pathological changes. At the conclusion of the feeding portion of the study, a one-generation, one litter reproduction study was carried out with the remaining 6 male and 6 female rats per dose group. Reproductive indices and litter and pup parameters were evaluated in the reproduction portion of the study.

Findings:

Mortality:

There were no test substance-related mortalities.

Clinical signs:

Sporadic incidences of lacerations were noted in the shoulder and head region in the controls and all test groups. Exophthalmus and alopecia were observed in 2 different female rats fed 450 ppm. One female rat fed 50 ppm exhibited loss of balance and head turned to one side after 6 weeks on test which persisted throughout the remainder of the study. This clinical observation was not considered test substance-related.

Body weight/body weight gain and food consumption:

Male and female rats fed 450 ppm had significantly decreased body weight gains. Statistically insignificant reductions in body weight gain were recorded in female rats fed 150 ppm. The food consumption by male and female rats fed 450 ppm was slightly reduced compared to that of the controls.

Haematology, clinical chemistry and urinalysis:

Male rats receiving 150 and 450 ppm had significantly lower erythrocyte counts, haematocrits, and haemoglobin concentrations while female rats had significantly lower erythrocyte counts, leukocyte counts, haematocrits, and haemoglobin concentrations at these dietary levels. The urine osmolality of the female rats fed 450 ppm was

significantly lower at the 2-month examination period. Lactate dehydrogenase activity was significantly increased above controls for female rats fed 150 ppm at the 3-month examination period.

Table 853: Summary of relevant findings in male and female rats

	Males						Females					
	150 ppm			450 ppm			150 ppm			450 ppm		
Months:-	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Haematology												
RBC	-	-	-	-	↓	↓	-	-	↓	↓	↓	↓
Hb	-	-	↓	-	-	↓	-	-	-	↓	↓	↓
Ht	-	-	↓	-	↓	↓	-	-	-	-	↓	↓
WBC	-	-	-	-	-	-	-	-	↓	-	↓	↓
Urinalysis												
mOs	-	-	-	-	-	-	-	-	-	-	↓	-
Chemistry												
AP	-	-	-	-	-	-	-	-	-	-	-	-
GPT	-	-	-	-	-	-	-	-	-	-	-	↓
GOT	-	-	-	-	-	↓	-	-	-	-	-	↓
LDH	-	-	↑	-	-	-	-	-	↓	-	-	↓

↓ Significantly lower than controls at $p < 0.05$
↑ Significantly greater than controls at $p < 0.05$

Gross pathology, organ weights and histopathology:

No test substance-related gross or microscopic changes were observed during the course of this study. No test substance-related changes in mean absolute or relative organ weights were apparent at any dietary concentration.

Reproductive parameters:

There was a significant reduction in the fertility index in the F1 generation of the 150 ppm group. A corresponding decrease in fertility rate was not observed in males or females administered 450 ppm. Therefore, this effect was not considered toxicologically significant and may not have been treatment-related. Body weights of weanlings on lactation day 21 from the 450 ppm group were lower than controls. This may have been a reflection of the altered body weight status of dams at this dose level. There were no other differences in reproductive parameters among the 50, 150, and 450 ppm test groups compared to controls.

Conclusions:

The toxicity of metabolites study HLR 630-76 was originally submitted under EU Rev8 Point IIA 5.8.1.6 and conducted with test material IN-N0079 technical metabolite. Guidelines were not given.

The NOEL for IN-N0079 in the 90-day feeding study in rats was 50 ppm (4.0 and 4.2 mg/kg bw/day for males and females, respectively). This NOEL was based on reduced body weight and altered clinical chemistry parameters in males and females at > 150 ppm. NOELs for the one-generation study were the following:

Parental toxicity: 50 ppm (4.0 mg/kg bw/day for males and 4.2 mg/kg bw/day for females) based on decreased body weight in F_0 males and females at $\geq$ 150 ppm.

Reproduction and fertility: 450 ppm (34.3 mg/kg bw/day for males and 35.7 mg/kg bw/day for females) which was the highest concentration tested.

Pup growth and development: 150 ppm (11.4 mg/kg bw/day for males and 12.6 mg/kg bw/day for females) based on decreased body weight in F_1 pups during lactation at 450 ppm.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/07

Reference: --	**Report:**	Sippel, M.E. (1978); Mutagenic activity of formamide, 1-cyano-N,N-dimethyl- in the salmonella/microsome assay **DuPont Report No.:** HLR 284-78 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-N0079 technical metabolite
   Lot/Batch #: Not given
   Purity: 100.0%

Materials and methods:

IN-N0079 (IN-N0079 technical metabolite; batch N0079; purity 100%) was evaluated for mutagenicity in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 with and without an exogenous metabolic activation system (Aroclor-induced rat liver S9, 0.3 mL S9/mL S9 mix, protein concentration not given). The mutagenicity was evaluated in two trials (Trial I and II) using standard plate incorporation methods. An additional trial (Trial III) was conducted with only TA1537 for the activated test system due to the low spontaneous mutation rate observed in Trial II. Nominal concentrations of 1000, 3000, 5000, 7000, and 10,000 µg/plate were evaluated in Trial I and nominal concentrations of 250, 500, 1000, 2500, and 5000 µg/plate were evaluated in Trials II and III. Two negative controls, a non-solvent and a solvent (acetone) control, were included in both trials. In addition, a third negative control consisting of a test plate without glucose-6-phosphate and NADP was included for the activated test system only. The nominal concentrations of IN- N0079 for this control were 10,000 µg/plate in Trial I and 3000 µg/plate in Trials II and III. The highest dose level was selected based on the lack of evidence of toxicity in an initial experiment conducted only with strain TA1535 with and without S9 activation. The test substance was administered to the test system as a solution in acetone at a concentration of 50 mg/mL. Positive indicators were 2-nitrofluorene, 9-aminoacridine, N-methyl-N'-nitro-N-nitrosoguanidine, and 2-aminoanthracene. A test substance was classified as positive if the mean number of revertants in any strain at any test substance concentration was at least twice the mean of the concurrent negative control and if more than 0.02 revertants/nmole were observed.

Findings:

The number of revertants at all concentrations of the test substance was similar to concurrent controls in studies both with and without activation. There was no evidence of a test substance precipitate. Toxicity was only seen at dose levels of ≥ 1000 µg/plate in TA1537 in the activated test system.

Conclusions:

The toxicity of metabolites study HLR 284-78 was originally submitted under EU Rev8 Point IIA 5.8.1.7 and conducted with test material IN-N0079 technical metabolite. Guidelines were not given. IN-N0079 was negative for mutagenic activity in the non-activated and S9-activated test systems in the *in vitro* bacterial gene mutation assay with an independent repeat assay. It does provide indication that the metabolite IN-N0079 is not mutagenic.

RMS comments and conclusion for the renewal

The study is acceptable as supplementary information.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.6.8.1/08

Reference: --	**Report:**	[redacted] (1972); Oral LD_{50} test **DuPont Report No.:** HLR 399-72 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1. Test material: IN-D2708 technical metabolite
   Lot/Batch #: D2708-3
   Purity: Not given

Materials and methods:

Single oral doses of IN-D2708 (IN-D2708 technical metabolite; batch D2708-3; purity not specified. However, according to Annex IIA – Tier II – Document M-II summary and evaluation section 3, mammalian toxicology (DuPont-5940-EU), evaluation 5.8.1.8, IN- D2708 has a purity of 99.87%) were administered by gavage as a 20% suspension in corn oil to fasted male ChR-CD rats (5/dose) at doses of 2500 and 5000 mg/kg bw in a dose volume of 3.30 and 6.15 mL, respectively. Surviving animals were observed for clinical signs of toxicity, body weight effects, and mortality for up to 14 days after dosing.

Findings:

All rats in the 5000 mg/kg bw group were found dead by the day after dosing. No mortalities were observed in the 2500 mg/kg bw group. Clinical signs of toxicity most often observed included irregular respiration, belly-to-cage posture, half closed eyes, prostration on day of dosing, pallor, weakness, and initial weight loss.

Conclusions:

The toxicity of metabolites study HLR 399-72 was originally submitted under EU Rev8 Point IIA 5.8.1.8 and conducted with test material IN-D2708 technical metabolite. Guidelines were not given. The oral LD_{50} for IN-D2708 in male rats was 3540 mg/kg bw. It does provide indications that the metabolite IN-D2708 is much less toxic than the parent compound.

RMS comments and conclusion for the renewal **The study is acceptable as supplementary information.**

B.6.8.2 Supplementary studies on the active substance

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

Study submitted in the EU Dossier in 2001 and included in the first EU approval review.

B.8.6.2/01

Reference: --	**Report:**	[redacted] (1968); Ten dose subacute oral test **DuPont Report No.:** HLR 150-68 **Guidelines:** Not given GLP: NO (they were not in place at the time the study was carried out)

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	Not given
	Purity:	Not given

Materials & Methods:

Six young adult male Chr-CD rats were administered Oxamyl (DPX-D1410 technical; batch not provided; purity not provided) as a 0.05% solution in a 1:99 mixture of acetone and peanut oil by gavage five times per week for two weeks. The doses administered were 0 and 2.4 mg/kg bw. Three test rats and three vehicle control rats were sacrificed at 4 hours and at 14 days after the final dose.

Clinical signs of toxicity were recorded. A histopathological evaluation of certain tissues was conducted.

Findings:

There were no mortalities during the course of the study. During week 1, mild fasciculations lasting 2 to 4 hours were recorded after each dosing episode. Slight pallor, salivation and body weight loss were recorded after dosing on days 1 and 2. During week 2, mild fasciculations were recorded 1 to 2 hours after dosing with body weight loss on day 1. No clinical signs of toxicity were reported at the 4-hour or 14-day sacrifice times.

Mild inflammation of the stomach was recorded in both test and control groups at the 4-hour or 14-day sacrifice times. The severity of the finding was less in the control groups.

Conclusions:

The supplementary studies on the active substance study HLR 150-68 was originally submitted under EU Rev8 Point IIA 5.8.2.5 and conducted with test material pure oxamyl (PAI). Guidelines were not given. Oxamyl did not cause any cumulative toxicity following subacute exposure (10 doses).

RMS comments and conclusion for renewal

This study is accepted only as supplemental information

B.6.8.3 Studies on endocrine disruption

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

The potential of oxamyl to induce adverse effects on components of the endocrine system has been assessed in short-term and long-term feeding studies, in a multi-generation reproduction study, and in developmental toxicity studies in rats and rabbits. In these studies, there was no evidence to suggest that oxamyl directly interferes with the function of the oestrogen, androgen, or thyroid pathways. No effects on fertility, reproduction, development, sexual maturation, or reproductive organ toxicity were noted. There was no specific target organ toxicity indicative of interaction with the endocrine system. Further, a full battery of studies presented below designed to specifically address the effects of oxamyl on the endocrine system have been undertaken as part of the Test Order received by DuPont requiring testing of oxamyl in the Tier 1 U.S. EPA Endocrine Disruptor Screening Program (EDSP). The results of these studies are summarized below and confirm the absence of any interaction by oxamyl with the estrogen, androgen, or thyroid systems. Therefore, it can be concluded that oxamyl does exert effects on the endocrine system.

Study submitted to the EU for the first time in this submission.

B.6.8.3/01

Reference: CA 5.8.3/01	**Report:**	[redacted] (2010); Oxamyl: Additional information in support of comparative cholinesterase study DuPont-16755 (MRID 46615301) - pilot data **DuPont Report No.:** DuPont-30415 **Guidelines:** Not given **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** DuPont-30415 **GLP:** No **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

In preparation for the comparative cholinesterase (CCA) study conducted with oxamyl (DuPont-16755, Point CA 5.7.1), a series of pilot studies were performed initially for the purposes of selecting dose levels in adult rats and preweanling rat pups and subsequently for the purposes of methods development for the acetyl cholinesterase (AChE) assay. The additional cholinesterase data in red blood cells (RBCs) and in brain collected in these pilot experiments are being provided.

The first pilot study was comprised of dosing postnatal day 11 and 21 rats in which clinical signs were observed. Five subsequent pilot experiments were conducted in which cholinesterase activity was assessed in RBCs and brain in either PND 11 preweanling rats or young adult rats.

During the course of these pilot studies, a high degree of variability was observed, particularly in the RBC cholinesterase measurements. As a result, the study was eventually terminated, and further methods development work was conducted with control rats. The two primary sources of the variability in RBC cholinesterase measurements were considered to be related to either incomplete lysis of RBCs or inconsistent pipetting of blood samples for analysis. Experiments were conducted to understand the source of the variability and to subsequently control variability in RBC cholinesterase measurements. Based on the results of this work, the Standard Operating Procedure for the cholinesterase assay was updated with the modified RBC lysis procedure and the specified use of a calibrated, fixed volume microdispenser pipet, with a positive displacement piston, in advance of the subsequent main study reported in DuPont-16755 (Point CA 5.7.1).

In the early pilot work, adult animals were not fasted prior to dosing (*i.e.*, food was not withdrawn the night before treatment). However, due to the high variability in AChE values, consideration was given as to whether varying amounts of solid diet in the stomach of individual rats could have been a contributing factor. A comparison study in fasted and fed rats was conducted. The results suggested that the degree of variability in RBC AChE was somewhat reduced in fasted animals within the specific experiment. In a further pilot experiment, additional fasted adults were treated; however, the degree of variability in RBC AChE was similar to that observed in nonfasted animals. In the main CCA study, the adult animals were not fasted. Neither the nursing pups nor the lactating dams were fasted at any time during the pilot studies or the main study.

Study submitted to the EU for the first time in this submission.

B.6.8.3/02

Reference: **CA 5.8.3/08**	**Report:**	[redacted] (2012a); Pubertal development and thyroid function in intact juvenile/peripubertal male rats following oral administration of oxamyl (DPX-D1410) technical (98% w/w) **DuPont Report No.:** DuPont-33933 **Guidelines:** OPPTS 890.1500 **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** 189244 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its potential effects on the endocrine system, by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal male rat. The male pubertal assay is part of the Tier 1 battery of the United States Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirement of Federal Food, Drug, and Cosmetic Act (FFDCA) Section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that make up the full EDSP Tier 1 screening battery.

The test substance, oxamyl, in the vehicle (deionised water) was administered by oral gavage to two groups of 15 juvenile/peripubertal male Crl:CD(SD) rats, obtained from non-treated time-mated females, once daily during postnatal Day (PND) 23–53 or 54. Dosage levels were 0.25 and 0.5 mg/kg/day administered at a dosage volume of 5 mL/kg. A concurrent control group composed of 15 juvenile/peripubertal males received only the vehicle on a comparable regimen.

All males selected for study were observed twice daily for mortality and moribundity from weaning through study termination. Clinical observations were recorded daily. Animals were weighed on the day of randomisation, daily prior to test substance administration, and on the day of euthanasia. All males were observed daily (beginning on PND 30) for balanopreputial separation. A complete necropsy was conducted on all rats found dead or that survived to the scheduled euthanasia on PND 53 or 54; selected organs were weighed and preserved. Hormone (thyroxine [T4], thyroid stimulating hormone [TSH], and testosterone) and clinical pathology evaluations (creatinine and urea nitrogen) were conducted on all surviving animals on PND 53 or 54. In addition, histopathological evaluation of the thyroid, kidney, testis, and epididymis was performed.

All males survived until the scheduled sacrifice time. There were no test substance-related effects on mortality, clinical signs, body weight parameters, balanopreputial separation, thyroid hormones (T4 and TSH) or serum testosterone, serum chemistry (creatinine or urea nitrogen), gross or microscopic pathology, organ weights, at any level tested.

Based on the lack of effects on the mean age and body weight at attainment of balanopreputial separation, male reproductive organs, serum testosterone levels, or test substance-related macroscopic and microscopic findings, oxamyl did not alter the hypothalamic-pituitary-gonadal axis function when administered orally to peripubertal male rats at dosage levels of 0.25 and 0.5 mg/kg/day. Based on the absence of effects on serum thyroid hormone levels, thyroid weights, and microscopic alterations in the thyroid, oxamyl did not display the potential to disrupt hypothalamic-pituitary-thyroid axis.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.0%
   Description: White powder
   CAS #: 23135-22-0
   Stability of test compound: Considered stable while stored at room temperature
   Vehicle: Deionised water
2. Test animals
   Species: Rat
   Strain: Sprague Dawley (Crl:CD[SD])
   Age at dosing: Approximately 23 days old
   Weight at dosing: 54.6–64.5 g
   Source: [redacted]
   Acclimation period: Not applicable, since the test animals were born in the same facility that the testing was being conducted.
   Diet: Harlan Laboratories 2016*CM* Teklad Global (16% Protein Rodent Diet), *ad libitum*
   Water: Reverse osmosis purified water, *ad libitum*
   Housing: Animals were housed three animals per cage in plastic maternity cages that contained Aspen bedding. Enrichment (Gnaw Pucks®) was placed in each cage.
3. Environmental conditions
   Temperature: 20.4–22.1°C
   Humidity: 37.2–59.5%
   Air changes: 10 changes/hour
   Photoperiod: Alternating 14-hour light and 10-hour dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   07-December-2011 to 07-January-2012
2. Animal assignment and treatment

   Two groups of 15 juvenile/peripubertal male Crl:CD(SD) rats, obtained from non-treated time-mated females, were dosed by oral gavage with 0.25 and 0.5 mg/kg/day of the test substance once daily during PND 23–53 or 54. A concurrent control group composed of 15 juvenile/peripubertal males received only the vehicle on a comparable regimen. Animals were assigned to dose and were distributed by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Table 864: Study design: Oral pubertal development study in male rats

Group no.	No./ group	Daily dose (mg/kg/day)	Dose volume (mL/kg)	Treatment
I	15	0 (control)	5	Vehicle control
II	15	0.25	5	Oxamyl
III	15	0.5	5	Oxamyl

3. Dosage preparation and analysis

   The test substance formulations were prepared daily as single formulations for each dosage level and stored at room temperature. The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures. The first dosing formulations were visually

inspected by the Study Director and were found to be visibly homogeneous and acceptable for administration. Subsequent dosing formulations were not visually inspected, but were considered homogeneous based on the results of the first inspection.

4. Statistics

Histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests to compare each test substance-treated group with the control group. The tests were 1-sided at the 0.05 significance level (testing for an increase).
Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each test substance-treated group with the control group. The tests were 1-sided at the 0.05 significance level testing for increased severity. The tests were 2-sided for graded responses presented on a Grade 1–5 scale at the 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.
Data that were statistically analysed included the organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, hormone values, serum chemistries, and balanopreputial separation data. Each endpoint was tested for homogeneity of variance using Levene's test. If that test was significant at $p = 0.01$, then a log transform was applied and Levene's test conducted on the transformed data. If that test was still significant, then the square root transformation was applied to the raw data and Levene's test conducted again. If the test was still significant then a nonparametric test, as described below, was used to analyse the data.
If the variances were homogeneous then an analysis of variance (ANOVA) was conducted, on the raw or transformed data, as appropriate. The statistical model contained a factor for treatment group and a blocking factor based on the study necropsy date. A 2-sided Dunnett's test was conducted, regardless of the outcome of the ANOVA, looking for significant differences in the test substance-treated groups when compared with the control group. If the transformations were unsuccessful in making the variances homogeneous then the nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. Because these were pre-planned pairwise comparisons, Dunn's test was conducted regardless of the outcome of the Kruskal-Wallis test. The tests were 2-sided, at the 0.05 significance level, looking for significant differences from the control group.
In addition, organ weights and balanopreputial separation data were subject to the following analyses if they met the homogeneity of variance criteria: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate; 2) linear trend test using the ANOVA model; and 3) linear trend test using the ANCOVA model.

C. METHODS

1. Observations

Animals were observed twice daily for mortality and morbidity and once daily for signs of abnormal behaviour and appearance. Prior to test substance administration during the treatment period, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations.

2. Body weights

All animals were weighed on the day of randomisation, daily prior to test substance administration, and on the day of euthanasia. Body weights were also taken on the day of achievement for balanopreputial separation.

3. Balanopreputial separation

Each male was observed for balanopreputial separation beginning on PND 30 (Korenbrot *et al*., 1977[1]). In addition, the appearance of a partial and complete balanopreputial separation or a persistent thread of tissue between the glans and prepuce was recorded. The age and body weight on the day at which complete balanopreputial separation was observed was recorded for each animal. However, if any animal within any group showed incomplete separation (persistent threads) for greater than 3 days, a separate, second analysis was conducted using the age at which incomplete separation was first

[1] Korenbrot, C.C.; Huhtaniemi, I.T.; Weiner, R.I. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction* **1977**, *17*, 298-303.

observed. Examination of the males was continued daily until complete balanopreputial separation was attained or euthanasia. When an animal did not attain balanopreputial separation prior to necropsy, PND 54 or 55 (last study day + 1) and body weight at necropsy were used as the age and body weight at balanopreputial separation, respectively. This is documented on the data tables.

4. Clinical pathology (haematology and clinical chemistry)

Trunk blood samples were collected from all animals immediately upon sacrifice. Hormone analysis, serum chemistry, and testosterone analysis were all performed on the samples.

5. Sacrifice and pathology

At termination, animals were sacrificed by decapitation. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 87. Gross lesions were saved in 10% neutral-buffered formalin for possible future histopathological examination.

Table 875 Oral pubertal development study in male rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Liver	X	
Levator ani plus bulbocavernosus muscles	X	
Kidneys[a]	X	X
Adrenal glands[a]	X	
Thyroid[b]	X	X
Testes[c, d]	X	X
Epididymides[c,d]	X	X
Ventral prostate	X	
Dorsolateral prostate	X	
Seminal vesicles (with coagulating gland and fluid)[e]	X	
Pituitary gland	X	

[a] Paired organs were weighed together.
[b] Fixed with attached trachea. Trimmed and weighed after fixation.
[c] PAS and haematoxylin staining were used for the right testis and epididymis and transverse sections of 2–4 microns of the testes and longitudinal sections of the epididymides were made.
[d] Weighed individually.
[e] Weighed with and without fluid. Any loss of fluid was recorded.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no test substance-related clinical findings noted at the daily examinations in the 0.25 and 0.5 mg/kg/day groups. No clinical findings were noted at approximately 30–45 minutes following dose administration.

2. Mortality

All animals survived to the scheduled necropsy on PND 53 or 54.

B. BODY WEIGHT AND BODY WEIGHT GAIN

No test substance-related effects on mean body weights or body weight gains were noted in the 0.25 and 0.5 mg/kg/day groups. The values in the test substance-treated groups were similar to and not statistically significantly different from the control group.

C. BALANOPREPUTIAL SEPARATION

Mean ages of attainment of complete balanopreputial separation and mean body weights at the age of attainment were unaffected by test substance administration (see table below).

The % CV for body weight at age of attainment of complete balanopreputial separation in the control group (8.19%) was greater than the maximum acceptable value in the performance criteria (7.57%); however, the mean weight was within the acceptable range.

Table 1. Preputial Separation; General Growth

OXAMYL		Effect	Transform or nonparam	Pairwise test	Vehicle Control				0.25 mg/kg/day					0.5 mg/kg/day				
					MEAN	SD	CV	N	MEAN*	SD	CV	N	P-VALUE	MEAN*	SD	CV	N	P-VALUE
AGE AT PPS (PND)	U			DUNNETT	46.0	1.85	4.03	15	45.5	3.16	6.95	15	0.8386	46.1	2.69	5.83	15	0.9964
AGE AT PPS (PND)	A			DUNNETT	46.0	1.85	4.03	15	45.5	3.16	6.95	15	0.8129	46.0	2.69	5.83	15	0.9959
AGE AT PPS (INCOMPLETE) (PND)	U			DUNNETT	44.1	2.43	5.52	15	43.5	1.46	3.35	15	0.7281	43.9	1.85	4.21	15	0.9420
AGE AT PPS (INCOMPLETE) (PND)	A			DUNNETT	44.0	2.43	5.52	15	43.6	1.46	3.35	15	0.7174	43.8	1.85	4.21	15	0.9435
BODY WEIGHT AT PPS (g)	U			DUNNETT	243.3	19.92	8.19	15	232.4	22.26	9.58	15	0.3386	236.7	23.74	10.03	15	0.6198
BODY WEIGHT AT PPS (g)	A			DUNNETT	242.9	19.92	8.19	15	232.9	22.26	9.58	15	0.3535	236.3	23.74	10.03	15	0.6217
INITIAL BODY WEIGHT (PND 23, g)	U			DUNNETT	59.0	2.72	4.60	15	58.6	2.50	4.26	15	0.8917	58.9	2.54	4.31	15	0.9671
FINAL BODY WEIGHT (PND 53, g)	U			DUNNETT	301.4	17.19	5.70	15	293.6	21.78	7.42	15	0.4901	297.9	19.41	6.52	15	0.8415
FINAL BODY WEIGHT (% OF CONTROL)	U								-2.59					-1.16				
BODY WEIGHT GAIN (g)	U			DUNNETT	242.4	17.12	7.06	15	235.0	20.37	8.67	15	0.4927	239.0	18.17	7.60	15	0.8381
Proportion unseparated (#/N)								0/15				1/15					1/15	

* Means different from controls at p<0.05 are marked by a shaded cell
Transform or nonparam: blank cell = no transformation; log,sqrt,np = log transformation, square root transformation, or nonparametric analysis, respectively
U=Unadjusted, A=Adjusted

D. HORMONE ANALYSIS

There were no test substance-related effects on mean hormone levels noted at 0.25 or 0.5 mg/kg/day. Differences from the control group were not statistically significant, did not correlate with any organ weight or microscopic effects, or effects on male pubertal development (see table below).

Table 3. T_4 and TSH and Testosterone Levels

OXAMYL	Effect	Transform or nonparam	Pairwise test	Vehicle Control				0.25 mg/kg/day					0.5 mg/kg/day				
				MEAN	SD	CV	N	MEAN*	SD	CV	N	P-VALUE	MEAN*	SD	CV	N	P-VALUE
TOTAL T4 (ug/dL)			DUNNETT	5.51	0.536	9.739	15	5.65	0.778	13.771	15	0.8507	5.47	0.794	14.522	15	0.9839
TSH (ng/mL)			DUNNETT	14.1	7.576	53.656	15	12.52	6.867	54.848	15	0.7912	14.27	7.076	49.595	15	0.9977
TESTOSTERONE (ng/mL)			DUNNETT	3.1	1.75	56.98	15	2.5	1.59	64.85	15	0.4159	2.8	2.02	71.77	15	0.8522

* Means different from controls at p<0.05 are marked by a shaded cell
Transform or nonparam: blank cell = no transformation; log,sqrt,np = log transformation, square root transformation, or nonparametric analysis, respectively

E. SERUM CHEMISTRY

There were no test substance-related alterations in the serum chemistry parameters evaluated (urea nitrogen and creatinine). There were no statistically significant differences noted from the control group.

F. SACRIFICE AND PATHOLOGY

1. Organ weight

There were no test substance-related alterations in organ weights. The only significant ($p = 0.0455$) difference from the control group was a higher mean kidney weight relative to final body weight in the 0.25 mg/kg/day group. This small difference was not considered test substance-related because there were no microscopic correlates or dose response profile (see table below).

2. Gross pathology and histopathology

There were no macroscopic findings observed at the scheduled necropsy that could be related to the test substance. There were no test substance-related histologic changes in the thyroid, testes, or kidney.

III. CONCLUSION

Based on the lack of effects on the mean age and body weight at attainment of balanopreputial separation, male reproductive organs, serum testosterone levels, or test substance-related macroscopic and microscopic findings, oxamyl did not alter the hypothalamic-pituitary-gonadal axis function when administered orally to peripubertal male rats at dosage levels of 0.25 and 0.5 mg/kg/day. Based on the absence of effects on serum thyroid hormone levels, thyroid weights, and microscopic alterations in the thyroid, oxamyl did not display the potential to disrupt hypothalamic-pituitary-thyroid axis.

RMS comments and conclusion for renewal

This study is accepted as a key study

Table 2. Organ Weights at Necropsy

OXAMYL					Vehicle Control				0.25 mg/kg/day					0.5 mg/kg/day				
Organ weights		Effect	Transform or Nonparam	Pairwise test	MEAN	SD	CV	N	MEAN*	SD	CV	N	P-VALUE	MEAN*	SD	CV	N	P-VALUE
ADRENAL GLANDS (mg)	U			DUNNETT	44.4	4.29	9.67	15	46.2	7.34	15.90	15	0.6252	43.9	7.32	16.70	15	0.9639
ADRENAL GLANDS (mg)	A			DUNNETT	44.3	4.29	9.67	15	46.3	7.34	15.90	15	0.5661	43.7	7.32	16.70	15	0.9591
ADRENAL GLANDS: BW RATIO (mg/100 g)	R			DUNNETT	14.6	1.65	11.29	15	15.5	1.79	11.56	15	0.3597	14.5	2.28	15.69	15	0.9934
DORSOLATERAL PR (mg)	U			DUNNETT	117.7	17.22	14.63	15	117.3	21.54	18.36	15	0.9969	122.9	20.46	16.64	15	0.6702
DORSOLATERAL PR (mg)	A			DUNNETT	117.3	17.22	14.63	15	117.7	21.54	18.36	15	0.9982	122.5	20.46	16.64	15	0.6736
EPIDIDYMIS, LT (mg)	U			DUNNETT	184.6	30.03	16.27	15	186.7	20.83	11.15	15	0.8472	194.7	23.52	12.08	15	0.3463
EPIDIDYMIS, LT (mg)	A			DUNNETT	183.8	30.03	16.27	15	187.6	20.83	11.15	15	0.8480	193.9	23.52	12.08	15	0.3548
EPIDIDYMIS, RT (mg)	U			DUNNETT	200.2	18.03	9.00	15	199.6	25.80	12.93	15	0.9935	198.1	25.00	12.62	15	0.9462
EPIDIDYMIS, RT (mg)	A			DUNNETT	199.6	18.03	9.00	15	200.0	25.80	12.93	15	0.9977	197.5	25.00	12.62	15	0.9466
KIDNEYS (g)	U			DUNNETT	2.12	0.179	8.453	15	2.16	0.188	8.689	15	0.6506	2.14	0.104	4.845	15	0.8752
KIDNEYS (g)	A			DUNNETT	2.11	0.179	8.453	15	2.16	0.188	8.689	15	0.5913	2.14	0.104	4.845	15	0.8696
KIDNEYS: BW RATIO (g/100 g)	R	*		DUNNETT	0.69	0.040	5.819	15	0.73	0.035	4.805	15	0.0455	0.71	0.037	5.261	15	0.3729
LABC MUSCLE GP (mg)	U			DUNNETT	545.9	78.68	14.41	15	504.2	108.76	21.57	15	0.3582	538.0	66.46	12.35	15	0.9535
LABC MUSCLE GP (mg)	A			DUNNETT	544.2	78.68	14.41	15	506.3	108.76	21.57	15	0.3736	536.2	66.46	12.35	15	0.9522
LIVER (g)	U			DUNNETT	14.32	1.422	9.931	15	13.78	1.430	10.382	15	0.4867	14.20	1.322	9.305	15	0.9638
LIVER (g)	A			DUNNETT	14.30	1.422	9.931	15	13.81	1.430	10.382	15	0.4692	14.18	1.322	9.305	15	0.9514
LIVER: BW RATIO (g/100 g)	R			DUNNETT	4.69	0.288	6.136	15	4.63	0.225	4.852	15	0.6537	4.70	0.246	5.230	15	0.9889
PITUITARY (mg)	U			DUNNETT	11.3	1.11	9.83	15	11.1	2.29	20.64	15	0.9253	12.3	1.68	13.72	15	0.2423
PITUITARY (mg)	A			DUNNETT	11.3	1.11	9.83	15	11.1	2.29	20.64	15	0.9042	12.3	1.68	13.72	15	0.2318
PITUITARY: BW RATIO (mg/100 g)	R			DUNNETT	3.7	0.32	8.58	15	3.8	0.85	22.63	15	0.9653	4.1	0.54	13.30	15	0.1992
SV/CG/ W/O FLUID (mg)	U			DUNNETT	392.2	31.95	8.15	15	390.2	66.18	16.96	15	0.9984	401.1	46.28	11.54	15	0.8176
SV/CG/ W/O FLUID (mg)	A			DUNNETT	390.7	31.95	8.15	15	391.9	66.18	16.96	15	0.9960	399.5	46.28	11.54	15	0.8196
SV/CG/ACC FLUID (mg)	U			DUNNETT	682.6	83.34	12.21	15	666.2	181.30	27.21	15	0.9747	697.4	125.37	17.98	15	0.9234
SV/CG/ACC FLUID (mg)	A			DUNNETT	678.3	83.34	12.21	15	671.0	181.30	27.21	15	0.9811	692.9	125.37	17.98	15	0.9249
TESTIS, LT (mg)	U			DUNNETT	1523.2	253.28	16.63	15	1564.9	401.29	25.64	15	0.8799	1470.3	100.25	6.82	15	0.8287
TESTIS, LT (mg)	A			DUNNETT	1522.6	253.28	16.63	15	1564.8	401.29	25.64	15	0.8889	1469.9	100.25	6.82	15	0.8325
TESTIS, RT (mg)	U			DUNNETT	1512.9	159.92	10.57	15	1502.8	142.46	9.48	15	0.9941	1577.4	346.03	21.94	15	0.6781
TESTIS, RT (mg)	A			DUNNETT	1510.7	159.92	10.57	15	1506.8	142.46	9.48	15	0.9985	1574.8	346.03	21.94	15	0.6704
THYROID GLANDS (mg)	U			DUNNETT	12.02	2.514	20.910	15	12.54	1.585	12.641	15	0.6664	12.13	2.285	18.834	15	0.9858
THYROID GLANDS (mg)	A			DUNNETT	11.99	2.514	20.910	15	12.58	1.585	12.641	15	0.6739	12.09	2.285	18.834	15	0.9861
VENTRAL PROSTATE (mg)	U			DUNNETT	266.7	37.82	14.18	15	243.9	56.42	23.13	15	0.3900	268.7	52.75	19.63	15	0.9906
VENTRAL PROSTATE (mg)	A			DUNNETT	265.7	37.82	14.18	15	245.0	56.42	23.13	15	0.4048	267.6	52.75	19.63	15	0.9911

* Means different from controls at p<0.05 are marked by a shaded cell
U=Unadjusted, A=Adjusted, R=Organ-to-body weight ratio
Transform or nonparam: blank cell = no transformation; log,sqrt,np = log transformation, square root transformation, or nonparametric analysis, respectively

Study submitted to the EU for the first time in this submission.

B.6.8.3/03

Reference: **CA 5.8.3/09**	**Report:**	[redacted] (2012b); Pubertal development and thyroid function in intact juvenile/peripubertal female rats following oral administration of oxamyl (DPX-D1410) technical (98% w/w) **DuPont Report No.:** DuPont-33934 **Guidelines:** OPPTS 890.1450 Deviations: None **Testing Facility:** [redacted] **Testing Facility Report No.:** 189243 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its potential effects on the endocrine system by identifying effects on pubertal development and thyroid function in the juvenile/peripubertal female rat. The female pubertal assay is part of the Tier 1 battery of the United States Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirement of Federal Food, Drug, and Cosmetic Act (FFDCA) Section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that make up the full EDSP Tier 1 screening battery.

The test substance, oxamyl, in the vehicle (deionised water) was administered by oral gavage to two groups of 15 juvenile/peripubertal female Crl:CD(SD) rats, obtained from non-treated time-mated females, once daily during postnatal Day (PND) 22–42 or 43. Dosage levels were 0.25 and 0.5 mg/kg/day administered at a dosage volume of 5 mL/kg. A concurrent control group composed of 15 juvenile/peripubertal females received only the vehicle on a comparable regimen.

All females selected for study were observed twice daily for mortality and moribundity from weaning through study termination. Clinical observations were recorded daily. Animals were weighed on the day of randomisation, daily prior to test substance administration, and on the day of euthanasia. All females were observed daily for vaginal opening. Once vaginal opening was observed, daily vaginal lavages were performed for each female to determine the stage of the oestrous cycle. A complete necropsy was conducted on all rats on PND 42 or 43; selected organs were weighed and preserved. Hormone (thyroxine [T4] and thyroid stimulating hormone [TSH]) and clinical pathology evaluations (urea nitrogen and creatinine) were conducted on all animals. In addition, histopathological evaluation of the thyroid, kidney, ovary, and uterus was performed.

All animals survived to the scheduled necropsy on PND 42 or 43. There were no clinical or macroscopic findings observed at any dosage level.

No test substance-related effects were noted on mean body weights or body weight gains in the 0.25 and 0.5 mg/kg/day groups. Mean ages and body weights at the age of attainment of vaginal opening, the mean age at the first occurrence of oestrus, oestrous cyclicity, and mean oestrous cycle length in these groups were unaffected by oxamyl administration.

No test substance-related effects on serum creatinine, urea nitrogen, T4, or TSH levels were noted at either dosage level.

There were no test substance-related effects noted on organ weights in the 0.25 and 0.5 mg/kg/day groups. No test substance-related microscopic findings were noted in these groups.

Based on the lack of test substance-related effects on the mean age and body weight at attainment of vaginal patency, oestrous cyclicity, wet and blotted uterus weights, ovary weights, and histopathology of the female reproductive organ weights, or test substance-related macroscopic and microscopic findings, oxamyl did not alter the hypothalamic-pituitary-gonadal axis function when administered orally to peripubertal female rats at

dose levels of 0.25 and 0.5 mg/kg/day. Based on the absence of effects on serum thyroid hormone levels, thyroid weights, and microscopic alterations in the thyroid, oxamyl did not display the potential to disrupt the hypothalamic-pituitary-thyroid axis.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.0%
   Description: White powder
   CAS #: 23135-22-0
   Stability of test compound: Not determined
   Vehicle: Deionised water
2. Test animals
   Species: Rat
   Strain: Sprague Dawley (Crl:CD[SD])
   Age at dosing: Approximately 22 days old
   Weight at dosing: 46.7–57.2 g for females
   Source: [redacted]
   Acclimation period: Not applicable, since the test animals were born in the same facility that the testing was being conducted.
   Diet: Harlan Laboratories 2016*CM* Teklad Global (16% Protein Rodent Diet), *ad libitum*
   Water: Reverse osmosis-purified water, *ad libitum*
   Housing: Animals were housed three/cage in plastic maternity cages that contained Aspen bedding. Enrichment (GnawPucks®) was placed in each cage.
3. Environmental conditions
   Temperature: 20.4–21.7°C
   Humidity: 37.6–59.5%
   Air changes: 10 changes/hour
   Photoperiod: Alternating 14-hour light and 10-hour dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

   06-December-2011 to 27-December-2011
2. Animal assignment and treatment

   Two groups of 15 juvenile/peripubertal female Crl:CD(SD) rats, obtained from non-treated time-mated females, were dosed by oral gavage with 0.25 and 0.5 mg/kg/day of the test substance once daily during PND 22–42 or 43. A concurrent control group composed of 15 juvenile/peripubertal females received only the vehicle on a comparable regimen. Animals were assigned to dose and were distributed by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Table 886 Study design: Oral pubertal development study in female rats

Group no.	No./ group	Daily dose (mg/kg/day)	Dose volume (mL/kg)	Treatment
I	15	0 (control)	5	Vehicle control
II	15	0.25	5	Oxamyl
III	15	0.5	5	Oxamyl

3. Dosage preparation and analysis

The test substance formulations were prepared daily as single formulations for each dosage level and stored at room temperature. The test substance formulations were stirred continuously throughout the preparation, sampling, and dose administration procedures. The first dosing formulations were visually inspected by the Study Director and were found to be visibly homogeneous and acceptable for administration. Subsequent dosing formulations were not visually inspected, but were considered homogeneous based on the results of the first inspection.

4. Statistics

Chi-square analysis was used to determine significant difference between the cycling status (cycling vs. not cycling) and percent of animals cycling regularly of the test substance-treated groups from the control group. Oestrous cycle length and the day of first oestrus were subjected to a parametric one−way analysis of variance (ANOVA) to determine intergroup differences. If the ANOVA revealed significant ($p < 0.05$) intergroup variance, Dunnett's test was used to compare the test substance-treated groups to the control group. Histopathology findings presenting as a dichotomous response were analysed with pairwise Fisher's exact tests to compare each test substance-treated group with the control group. The tests were 1-sided at the 0.05 significance level (testing for an increase).

Data that were statistically analysed included the organ weights, organ weight to necropsy body weight ratio (liver, kidneys, pituitary, and adrenal glands), daily body weights (including necropsy body weight), cumulative body weight gain, hormone values, serum chemistries, and vaginal opening data. Each endpoint was tested for homogeneity of variance using Levene's test. If that test was significant at $p = 0.01$, then a log transform was applied and Levene's test conducted on the transformed data. If that test was still significant then the square root transformation was applied to the raw data and Levene's test conducted again. If the test was still significant then a nonparametric test, as described below, was used to analyse the data.

If the variances were homogeneous then an ANOVA was conducted, on the raw or transformed data, as appropriate. The statistical model contained a factor for treatment group and a blocking factor based on the study necropsy date. A 2-sided Dunnett's test was conducted, regardless of the outcome of the ANOVA, looking for significant differences in the test substance-treated groups when compared with the control group. If the transformations were unsuccessful in making the variances homogeneous then the nonparametric Kruskal-Wallis test was used, ignoring the blocking factor, followed by Dunn's test, to compare each of the test substance-treated groups with the control group. Because these are pre-planned pairwise comparisons, Dunn's test was conducted regardless of the outcome of the Kruskal-Wallis test. The tests were 2-sided, at the 0.05 significance level, looking for significant differences from the control group.

In addition, organ weights and vaginal opening data were subject to the following analyses if they met the homogeneity of variance criteria: 1) Analysis of covariance (ANCOVA) with Dunnett's test. The model was as described above for ANOVA with the exception that PND 21 body weight was included as a covariate; 2) linear trend test using the ANOVA model; and 3) linear trend test using the ANCOVA model.

Histopathology findings presented as a graded response were analysed with pairwise Mann-Whitney U tests to compare each test substance-treated group with the control group. The tests were 1-sided at the 0.05 significance level testing for increased severity. The tests were 2-sided for graded responses presented on a Grade 1–5 scale at the 0.05 significance level testing for increased or decreased severity. Exact p-values were calculated for the Mann-Whitney U test.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and morbidity and once daily for signs of abnormal behaviour and appearance. Prior to test substance administration during the treatment period, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations.

2. Body weights

All animals were weighed on the day of randomisation, daily prior to test substance administration, and on the day of euthanasia. All animals were also weighed on the day of achievement for vaginal opening.

3. Vaginal opening

Each female was observed for vaginal perforation beginning on PND 22. In addition, the appearance of a small "pin hole," a vaginal thread, and complete vaginal opening were recorded. The age and body weight on the day at which the vaginal lumen was first observed to open was recorded for each animal. However, if any animal within any group showed incomplete opening (persistent pin holes and/or threads) for greater than 3 days, a separate second analysis was conducted using the age at which incomplete opening was first observed. Examination of the females was continued daily until vaginal opening was present or until euthanasia. When an animal did not attain vaginal opening prior to necropsy, PND 44 and body weight at necropsy were used as the age and body weight at vaginal opening, respectively.

4. Oestrous cyclicity

Beginning on the day vaginal opening was observed, vaginal lavages were performed daily, and the slides were evaluated microscopically to determine the stage of the oestrous cycle of each female until necropsy. The age of first vaginal oestrus after vaginal opening was recorded. The average cycle length was calculated and reported for complete oestrous cycles (*i.e.*, the total number of returns to metoestrus [M] or dioestrus [D] from oestrus [E] or proestrus [P]). Oestrous cycle length was determined by counting the number of days from the first M or D in a cycle to the first M or D in a subsequent cycle. At the end of the study, the overall pattern of each female was characterised as regularly cycling, irregularly cycling, non-cycling, or insufficient data.

5. Clinical pathology (hormone analysis and serum chemistry)

Trunk blood samples were collected from all animals immediately upon sacrifice. Hormone analysis and serum chemistry were all performed on the samples.

6. Sacrifice and pathology

At termination, animals were sacrificed by decapitation without anaesthesia. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 87. Gross lesions were saved in 10% neutral-buffered formalin for possible future histopathological examination.

Table 897 Oral pubertal development study in female rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Microscopic/histopathologic evaluation conducted
Adrenal glands[a]	X	
Kidneys[a]	X	X
Liver	X	
Ovaries[a]	X	X
Pituitary gland	X	
Thyroid[b]	X	X
Uterus	X	X

[a] Paired organs were weighed together.

[b] Fixed with attached trachea. Trimmed and weighed after fixation.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No statistically significant or biologically significant changes in the incidence of clinical signs of toxicity were observed for any concentration.

2. Mortality

Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on body weights or body weight gains.

C. VAGINAL OPENING

A single female (no. 33061-11) in the control group did not attain vaginal opening prior to necropsy on PND 43. The mean age of vaginal opening in the control group was 35.7 days, and when the day of attainment was adjusted for this female with more than 3 days of incomplete opening, the mean day of attainment was 35.0 days. The mean ages of vaginal opening in the 0.25 and 0.5 mg/kg/day groups (33.8 days for both groups) were lower than the control group values (with and without adjustment). The unadjusted differences were significant ($p \leq 0.0461$) for the 0.25 and 0.5 mg/kg/day groups using ANOVA, ANCOVA, and trend tests. The adjusted differences remained slightly significant ($p=0.0453$) using the linear trend test (ANOVA model). However, the values for the mean ages of attainment in the 0.25 and 0.5 mg/kg/day groups were within the [redacted] historical control data range (mean of 35.6 days; range of 32.9-36.6 days) and there was no indication of a dose-response pattern.

In addition, the mean body weights at the age of attainment in the 0.25 and 0.5 mg/kg/day groups were 122.7 g and 115.9 g, respectively, compared to the control group (126.9 g); although significance ($p \leq 0.0308$) was achieved for the 0.5 mg/kg/day group using ANOVA and ANCOVA, there was also a significant ($p \leq 0.0166$) decreasing trend through 0.5 mg/kg/day. However, the mean body weights at attainment in the 0.25 and 0.5 mg/kg/day groups were within the [redacted] historical control data range (mean of 124.1 g; range of 113.5 g-141.6 g), and the higher control value was partly due to the single control female that did not attain complete vaginal opening. The body weight of this control female on PND 43 was 162.4 g, which inflated the mean control value. The body weight for this control female at the time of partial vaginal pening on PND 34 was 119.2 g; adjusting for this body weight results in an adjusted control mean value of 124.1 g.

Therefore, considering the small magnitude of the changes, the higher mean control value resulting partially from a single female that did not obtain complete vaginal opening, and the absence of estrogenic effects on any other parameter (age at first estrus, estrous cyclicity, uterine weights, etc.), the lower mean age and body weight on the day of vaginal opening compared to the concurrent control group were not considered to be test substance-related.

The mean age of attainment of vaginal opening and the CV value in the control group (35.7 days and 7.76, respectively) exceeded the recommended maximum value in the performance criteria (35.62 days and 6.52, respectively). When a second analysis was performed, adjusting the day of attainment to be the first day of incomplete vaginal opening for the female with 3 or more days of incomplete opening, the mean age of attainment of vaginal opening and the cv value range in the control group (35.0 days and 4.45, respectively) were within the performance criteria acceptable range.

D. OESTROUS CYCLES

The mean ages of the first occurrence of estrus in the 0.25 and 0.5 mg/kg/day groups (35.3 days and 34.9 days, respectively) were similar to the control group (35.9 days). There were no test substance-related effects on the percentage of females cycling and cycling regularly in the 0.25 and 0.5 mg/kg/day groups. A lower percentage of females cycling in the 0.25 mg/kg/day group (93.3%) compared to the control group (100.0%) and a higher percentage of females that were cycling regularly in the 0.25 mg/kg/day group (100.0%) compared to the control group (69.2%); however, these differences did not occur in a dose-related manner (100.0% of females were cycling and 54.5% of females were cycling regularly in the 0.5 mg/kg/day group), were not statistically significant, and were not attributed to the test substance.

Due to the number of females with insufficient data, cycling status could be determined for only 13, 13, and 11 females in the control, 0.25, and 0.5 mg/kg/day groups, respectively, and 4, 3, and 5 females in the same respective groups could not have estrous cycle length determined. Mean estrous cycle lengths in the test substance-treated groups were generally similar to the control group.

E. HORMONE ANALYSIS

There were no test substance-related alterations in thyroid hormone (T_4 and TSH) parameters. No statistically significant differences from the control group were noted.

F. SERUM CHEMISTRY

There were no test substance-related alterations in the serum chemistry parameters evaluated (urea nitrogen and creatinine). There were no statistically significant differences noted from the control group.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

No test substance-related changes in mean organ weights or organ weights relative to final body weight were apparent at any concentration.
Although mean uterus weights (blotted and wet) in the 0.25 and 0.5 mg/kg/day groups were significantly ($p \leq 0.0469$) higher than the control group, this was due to the estrous cycle state of the females on the day of necropsy. Table 2 below presents those females that were assumed to be in the proliferative phase for females cycling normally as they were determined to be in diestrus (or proestrus) on the third consecutive day of their estrous cycle, assuming a typical 4-day cycle.
Alternatively, failure to meet these criteria was used to exclude a cycling female with placement into the non-proliferative phase (see Table 3). Therefore, females that were not determined to be normally cycling were excluded. In the control, 0.25, and 0.5 mg/kg/day groups, 1, 8, and 3 females, respectively, were assumed to be in the proliferative phase and 10, 6, and 7 females, respectively, were assumed to be in the non-proliferative phase. The mean uterus weights (blotted and wet) in proliferating females of each group shows an expected increase associated with cell proliferation and luminal fluid accumulation that takes place during the proliferative phase of the estrous cycle in comparison to the non-proliferating females in each group. Specifically, the control group showed an increase of 37.5% and 34.2% (single animal), the 0.25 mg/kg/day group showed an increase of 65.9% and 34.8%, and the 0.5 mg/kg/day group showed an increase of 68.5% and 41.4% in the blotted and wet uterus weights, respectively.

Therefore, the increased number of females in the proliferative phase (0.25 mg/kg/day group only) relative to the increase number of females in the non-proliferative phase (control group), along with the noted increase in blotted and wet uterus weights across each group was responsible for the indicated statistical significance. Furthermore, these changes in organ weights did not fit a dose-response pattern, were not associated with any microscopic change in the uterus, and were considered to be, in part, due to biological variability.
In addition to the concurrent male pubertal study ([redacted] 2012, [redacted]-189244) that did not produce a delay in balanopreputial separation, which would be expected for an estrogenic compound (Biegal et al., 1998 and Tyl et al., 2006), the other EDSP studies conducted by the Sponsor with this test Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats following Oral Administration of Oxamyl (DPX-D1410) Technical (98% w/w) DuPont-33934 [redacted]-18924337 substance, including the estrogen receptor binding and transactivation and uterotrophic assays were negative for estrogenic activity (Willoughby, 2012, DuPont-32073; [redacted] 2011, DuPont-32075; and Snajdr, 2012, DuPont-32074).

Mean thyroid weight in the 0.25 mg/kg/day group was significantly ($p \leq 0.0296$) higher than the control group. Since the organ weight change did not follow a dose-response profile, and there was no histomorphologic or hormonal (T4 or TSH) correlation, this change in mean thyroid weight was considered to be unrelated to test substance treatment.

Text Table 2. Assumed Proliferative Phase Females at the Time of Necropsy

Animal Number	Dose Level (mg/kg/day)	Final Body Weight [g]	Wet Uterus Weight [g]	Blotted Uterus Weight [g]
33087-10	0	180	0.3237	0.2956
33070-09	0.25	179	0.6421	0.4594
33055-14	0.25	177	0.4514	0.3587
33069-09	0.25	170	0.5595	0.4007
33064-10	0.25	161	0.4607	0.3404
33083-12	0.25	168	0.3348	0.3187
33057-12	0.25	147	0.3970	0.3202
33058-13	0.25	170	0.5060	0.3346
33065-04	0.25	161	0.4822	0.3724
	Mean	166.7	0.4792	0.3631
	SD	10.2	0.09444	0.04771
	CV%	6.14	19.71	13.14
33063-09	0.5	153	0.4967	0.3531
33061-15	0.5	152	0.4628	0.3971
33082-13	0.5	165	0.5403	0.4384
	Mean	157	0.4999	0.3962
	SD	7.2	0.03885	0.04266
	CV%	4.62	7.77	10.77

Text Table 3. Assumed Non-Proliferative Phase Females at the Time of Necropsy

Animal Number	Dose Level (mg/kg/day)	Final Body Weight [g]	Wet Uterus Weight [g]	Blotted Uterus Weight [g]
33059-08	0	157	0.2060	0.1924
33091-12	0	189	0.2303	0.2121
33093-12	0	164	0.2021	0.1862
33062-09	0	178	0.2549	0.2349
33057-06	0	160	0.2374	0.2232
33053-07	0	150	0.2001	0.1869
33092-05	0	164	0.3547	0.3419
33083-11	0	134	0.2442	0.2311
33058-14	0	176	0.2069	0.1891
33084-11	0	173	0.2175	0.2055
	Mean	165	0.2354	0.2203
	SD	15.7	0.04603	0.04648
	CV%	9.51	19.55	21.09
33073-08	0.25	183	0.2584	0.2353
33082-09	0.25	174	0.2711	0.2496
33060-08	0.25	162	0.3422	0.3268
33089-13	0.25	180	0.3746	0.3572
33080-08	0.25	160	0.2225	0.2093
33077-11	0.25	202	0.2638	0.2381
	Mean	177	0.2888	0.2694
	SD	15.4	0.05740	0.05857
	CV%	8.73	19.88	21.74
33087-12	0.5	160	0.2541	0.2394
33092-04	0.5	164	0.3250	0.3083
33069-13	0.5	140	0.2880	0.2734
33083-10	0.5	153	0.2885	0.2657
33077-08	0.5	187	0.2165	0.2029
33052-12	0.5	153	0.3006	0.2832
33090-14	0.5	162	0.4045	0.3880
	Mean	160	0.2967	0.2801
	SD	14.4	0.05892	0.05819
	CV%	9.02	19.85	20.77

2. Gross pathology and histopathology

No test substance-related gross lesions were observed at necropsy

III. CONCLUSION

Based on the lack of test substance-related effects on the mean age and body weight at attainment of vaginal patency, oestrous cyclicity, wet and blotted uterus weights, ovary weights, and histopathology of the female reproductive organ weights, or test substance-related macroscopic and microscopic findings, oxamyl did not alter the hypothalamic-pituitary-gonadal axis function when administered orally to peripubertal female rats at dose levels of 0.25 and 0.5 mg/kg/day. Based on the absence of effects on serum thyroid hormone levels, thyroid weights, and microscopic alterations in the thyroid, oxamyl did not display the potential to disrupt the hypothalamic-pituitary-thyroid axis.

RMS comments and conclusion for renewal
This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/04

Reference: CA 5.8.3/05	**Report:**	Snajdr, S.I. (2012a); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* aromatase inhibition using human recombinant microsomes **DuPont Report No.:** DuPont-32072 **Guidelines:** OPPTS 890.1200 (2009) **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-32072 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its ability to inhibit human recombinant microsomal aromatase activity, an enzyme responsible for the conversion of androgens to oestrogens. The *in vitro* aromatase inhibition assay using human recombinant microsomes is part of the Tier 1 battery of the Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

The assay is based upon the $^{3}H2O$-aromatase assay, an *in vitro* method that has been used extensively for the determination of the presence of the aromatase enzyme in multiple target tissues in vertebrates, and has long been used in pharmaceutical research to identify chemicals that can inhibit the catalytic activity of aromatase through an interaction with the substrate binding site on the enzyme. In brief, radioactive substrate (^{3}H-androstenedione) and β-nicotinamide adenine dinucleotide phosphate reduced form, tetrasodium salt (NADPH), are added to microsomes containing the aromatase (CYP19) and reductase complex. $^{3}H2O$ is released during the conversion of androstenedione (ASDN) to oestrone, and can be quantified as a direct measurement of aromatase activity per unit reaction time. Competitive inhibition of aromatase activity by test chemicals can be detected by serial reaction tubes containing increasing concentrations of the chemical of interest.

The positive control, 4-hydroxyandrostenedione (4-OH ASDN), was evaluated to verify test system performance. As expected, 4-OH ASDN showed effects consistent with aromatase inhibition in three independent assays. The estimated logIC50 for 4-OH ASDN was approximately -7.2 logM.

Under the conditions of the study, oxamyl did not inhibit aromatase activity when tested at up to a maximum concentration of 1.0 × 10-3 M. Therefore, oxamyl is classified as a non-inhibitor in this aromatase inhibition assay.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98%
	Description:	Powder
	CAS #:	23135-22-0
	Stability of test compound:	The test substance was stable throughout the exposure phase of the study; no evidence of instability was observed.
	Solvent/ final concentration:	Deionised water/ 1%
2.	Positive control:	4-hydroxyandrostenedione (4-OH ASDN)
	Lot number:	081K2133
	Purity:	99.6%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	566-48-3
	Solvent/ final concentration:	Dimethyl sulfoxide (DMSO), 1%
3.	Substrate:	Non-radiolabelled androstenedione (ASDN)
	Lot number:	L1712
	Purity:	99.8%
	Source:	Steraloids, Newport, Rhode Island, USA
4.	Radiolabelled substrate:	Radiolabelled ASDN ([1β-^{3}H]-Androst-4-ene-3,17-dione, [^{3}H]-ASDN)
	Lot number:	619344
	Purity:	>97%
	Source:	Perkin Elmer, Waltham, Massachusetts, USA
	Specific activity:	26.3 Ci (0.974 TBq)/mmol (adjusted specific activity: 24.7 Ci/mmol)
	Concentration:	1.0 µCi/mL (37 MBq/mL)
5.	Test system (androgen receptor):	Human recombinant microsomes
	Lot number:	03897
	Source:	Gentest™, Woburn, Massachusetts, USA
	Protein content:	7.4 mg protein/mL
	Protein cytochrome c reductase activity:	290 nmole/min/mg
	P450 aromatase activity:	6.0 pmole product/min/pmole P450 aromatase activity

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

02-September-2011 to 10-October-2011

2. Stock and working dose solution preparation and analysis

The positive control, 4-OH ASDN (not adjusted for purity), was used to verify test system performance at eight final concentrations ranging from 1×10^{-10} to 1×10^{-5} M. The stock solution was prepared by adding 2.512 mL of DMSO to 7.6 mg of 4-OH ASDN and was stored frozen at ≥-10°C. Preparations of the working solutions for the positive control were prepared daily by serial dilution from the stock solution, which was also the highest concentration used in the assay.

The test substance (adjusted for purity) was evaluated at eight final concentrations ranging from 1×10^{-10} to 1×10^{-3} M and stored within the period of established stability. No indication of

precipitation of the test substance was observed during the assay. The stock solution for Run 1 was prepared by adding 1.369 mL of deionised water to 30.62 mg of test substance, the solution for Run 2 was prepared by adding 6.024 mL of deionised water to 134.72 mg of test substance, the solution for Run 3 was prepared by adding 2.195 mL of deionised water to 49.09 mg of test substance, and the solution for Run 4 was prepared by adding 2.037 mL of deionised water to 45.55 mg of test substance. Preparations of the working solutions for test substance were prepared daily by serial dilution from the stock solution, which was also the highest concentration used in the assay.

Samples of the test substance stock and working dose solutions were collected near the beginning of the study and stored at room temperature until analysis. The samples were analysed to verify concentration and confirm stability. At the time of analysis, the samples were diluted with an appropriate solvent and analysed by high-performance liquid chromatography (HPLC) with mass spectrometry (LC/MS).

The amount of [^{3}H]-ASDN (measured in disintegrations per minute, DPM) in each standard was determined by subjecting a diluted (up to 1 mL with acetonitrile) 5 µL aliquot of the standard to radioanalysis by liquid scintillation counting (LSC).

3. Aromatase activity

The ability of the test substance to inhibit human recombinant microsomal aromatase activity using a mixture of ASDN and [^{3}H]-ASDN as the substrate was evaluated according to the EPA test guideline.

The recombinant microsomes were diluted to 0.008 mg/mL and pre-incubated at approximately 37°C for approximately 5 minutes before being added to the assay tubes. The final volume per assay tube was 2.0 mL. Each tube contained 680 µL assay buffer (0.1 M sodium phosphate, pH 7.4), 100 µL of propylene glycol, 100 µL of ASDN/[^{3}H]-ASDN substrate solution, 100 µL of NADPH (background control received 100 µL assay buffer), and 20 µL of either the appropriate solvent, the positive control, or test substance. All reagents were added to the assay tubes as described above except for the microsomes, and the tubes were pre-incubated for approximately 5 minutes at approximately 37°C prior to initiation of the reaction. The reaction was initiated by addition of 1000 µL of warmed microsomes (0.008 mg/mL).

The assay tubes were incubated at approximately 37°C for 15 minutes. The reaction was stopped and the ^3H_2O was extracted by the addition of 3 mL of chloroform, vortexing, and placing the tubes on ice for at least 5 minutes. The unbound [^{3}H]-ASDN was extracted with chloroform followed by a charcoal/dextran wash.

The tubes were centrifuged at 200 × g for 10 minutes at approximately 4°C, then 700 µL of the supernatant (aqueous layer) were drawn off and placed in a test tube to which 700 µL of charcoal/dextran solution were added. The extraction tubes were vortexed and placed on ice for 10 minutes. The tubes were centrifuged at 200 × g for 10 minutes at approximately 4°C, and two aliquots of 500 µL of the supernatant were added to respective liquid scintillation ±counting (LSC) vials. The vials were counted for 5 minutes on a LSC. Aromatase activity was reported as nmol ^3H_2O/mg microsomal protein/minute.

Four independent experimental runs were conducted. In the second run, the deionised water full activity controls (FACs) had an activity of approximately 0.06 nmol ^3H_2O/min/mg protein, which was low in comparison to the performance criteria recommended in the test guideline. Therefore, this run was excluded from calculations and a fourth run was performed.

4. Statistics

The concentration that inhibited 50% of maximum radioligand binding (IC_{50}) values was determined as appropriate using Origin 8.5.1. ANOVA analysis for the logIC_{50} and slope values was determined using SAS Version 9.2.

The IC_{50} values were determined by fitting the competitive curves using the following equation:

$$Y = \text{Bottom} + \frac{\text{Top - Bottom}}{1+10^{(\text{Log IC50-X})\text{HillSlope}+\log[(\text{Top-Bottom})/(50-\text{Bottom})-1]}}$$

where X is the logarithm of the concentration of test substance and Y is the percent of radioligand bound to the receptor. LogIC_{50} is X at Y = 50%. “Top” and “Bottom” refer to the value of Y when there was minimal binding by the test chemical, and when there was maximal binding by the test chemical, respectively. A concentration-response model was fitted for each test run for each curve generated.

The average response curves were generated using the average of the above parameters.

The competitive curves were fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y.
In each run, the model parameters were estimated along with their standard errors. The variances (squared standard errors) for each parameter were compared using F-tests to determine which were different. Pooled variance T-tests or Sattherthwaite T-tests were then used to compare the parameter estimates (slope or $logIC_{50}$), according as the corresponding F-test was not or was significant. All statistical significance was at the 0.05 significance level. Normality of the underlying data from each run was assessed from the residuals from the model.

5. Performance criteria

The following performance criteria applied to assess the performance of the inhibition assay. The suggested mean for the FAC in the absence of an inhibitor should be at least 0.1 nmol/mg-protein/min. The ideal background activity control (BAC, without NADPH) should be ≤10% of the FAC (with NADPH) for each respective solvent. The suggested coefficient of variation (CV) for replicates within each sample type and concentration of 4-OH ASDN should be less than 15%. Performance parameters for the positive control serve as guidance in identifying runs that provide parameters in the ideal ranges.

II. RESULTS AND DISCUSSION

A. RADIOCHEMICAL PURITY

The radiochemical purity was measured with a radiochemical liquid chromatographic detector to ensure mass balance. Radiochemical purity of [^{3}H]-ASDN was 99.972%.

B. ANALYTICAL DETERMINATIONS

Oxamyl was present at acceptable concentrations in the dosing solutions (within ±13.7% of nominal concentrations). Oxamyl was shown to be stable in the dosing solutions under the conditions of the study. Oxamyl was not found in the 0 mg/mL samples.

C. AROMATASE INHIBITION ASSAY

Four independent aromatase inhibition runs were performed. In the second run, the deionised water FACs had an activity of approximately 0.06 nmol ^3H_2O/min/mg protein, which did not meet the performance criteria as described in the test guideline. As a result, the second run was excluded from the calculations, and a fourth run was performed.

The $logIC_{50}$ values for 4-OH ASDN for each of the three reported runs were -7.0, -7.2, and -7.3 logM [^{3}H]-ASDN with an average of -7.2 ± 0.05 logM [^{3}H]-ASDN. The $logIC_{50}$ values for each run were all statistically significantly different from each other. The actual estimates were quite close; however, the standard errors were very small, which resulted in the statistical differences. The slope values were statistically indistinguishable from each other with two exceptions, the slope values for Runs 3 and 4 were statistically significantly different from each other. The actual estimates were quite close; however, the standard errors were very small, which resulted in the statistical differences. The actual estimates are within 10% of one another; however the standard errors are very small which results in the statistical differences.

A $logIC_{50}$ was not determined for the test substance since there were no test substance-related effects on aromatase activity up to the concentration of 1 mM, which represents the maximum concentration required by the test guideline.

Table 908 Aromatase activity assay: Mean ± standard deviation of the percent activity (nmol/min/mg protein) for each concentration

	Final conc.	Run 1		Run 3		Run 4	
Compound	**(M)**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
BAC (DMSO)	—	-0.34	0.02	-0.05	0.07	-0.03	0.03
FAC (DMSO)	—	116.46	4.57	103.12	3.46	97.69	8.63
4-OH ASDN	1×10^{-10}	112.81	14.82	107.78	0.89	88.29	4.55
	1×10^{-9}	118.83	6.51	99.71	2.38	94.71	6.22
	1×10^{-8}	99.07	11.74	88.14	4.07	78.45	1.03
	3×10^{-7}	87.35	1.76	68.51	2.67	63.03	1.71
	1×10^{-7}	50.56	2.17	39.86	0.55	35.12	0.81
	3×10^{-6}	23.52	0.51	19.23	0.40	15.21	0.22
	1×10^{-6}	8.94	0.70	7.32	0.14	6.01	0.29
	1×10^{-5}	1.20	0.17	0.92	0.06	0.90	0.22
BAC (DMSO)	—	0.34	NA	0.05	0.01	0.03	0.02
FAC (DMSO)	—	83.54	0.79	96.88	10.39	102.31	1.48
BAC (dH_2O)	—	-0.05	0.15	0.01	0.05	0.05	0.22
FAC (dH_2O)	—	107.47	6.13	107.33	0.70	110.77	2.47
Oxamyl	1×10^{-10}	110.65	0.68	107.77	10.09	105.30	3.04
	1×10^{-9}	100.17	20.96	102.70	4.92	105.42	2.33
	1×10^{-8}	115.30	8.29	98.65	1.55	105.03	1.63
	1×10^{-7}	112.98	1.84	102.22	7.21	106.32	2.35
	1×10^{-6}	110.23	1.47	96.47	5.56	100.56	4.29
	1×10^{-5}	107.89	4.38	87.27	2.04	98.69	0.20
	1×10^{-4}	106.13	1.25	91.33	2.69	98.42	3.16
	1×10^{-3}	102.13	0.17	103.15	0.43	104.06	2.47
BAC (dH_2O)	—	0.05	0.35	-0.01	0.03	-0.05	0.00
FAC (dH_2O)	—	92.53	1.03	92.67	4.68	89.23	0.71

D. ASSAY PERFORMANCE

The assay runs were compared to the performance criteria as described above. The aromatase activity of the FACs were above 0.1 nmol ^3H_2O/min/mg protein except for the second run where the initial deionised water FAC had an activity of approximately 0.06 nmol ^3H_2O/min/mg protein. As noted above, the results from the second run were excluded from the calculations, and a fourth run was performed and included in the calculations. The aromatase activity of the BACs was between 0.34–0.95% and 0.52–0.96% of the FAC with DMSO and deionised water, respectively. The CVs for replicates within each sample type and concentration of 4-OH ASDN were <15%, except for the final concentration of 10 µM 4-OH ASDN in the third run, which was 24.42%. However, this single occurrence did not warrant performing another run since the mean for that concentration was below 1, and since the 15% CV criteria is a suggested acceptable, not a mandatory limit. The performance parameters for the positive control were within the acceptable ranges as specified in the test guideline, with the exception of the top of the curve value for Run 1 was 115%, which was slightly higher than the suggested test guideline range. However, this single occurrence did not warrant performing another run since it was only slightly above the suggested range for the top of the curve (90–110%). The assay drift, a measure of the consistency of the BACs and FACs from the beginning and end of the run for each solvent, was within the recommended test guideline ranges, with the exception of the average initial DMSO FAC value of 116%, which was slightly higher than the suggested test guideline range and the average ending DMSO FAC value of 84% for Run 1, which was slightly lower than the suggested test guideline range. However, this single occurrence did not warrant performing another run since they were only slightly outside the suggested range for the FAC (90–110%). With the exception of Run 1, all runs were within or very close to the suggested performance criteria, and therefore the assay was considered valid.

Table 919 Performance criteria for the positive control, 4-OH ASDN, in the aromatase activity assay

Parameters	Suggested acceptable range according to test guideline		Run 1[a]	Run 3[a]	Run 4[a]	Mean ± SEM[b]
	Lower limit	Upper limit				
Top (%)	90	110	115 ± 3.5[c]	105 ± 1.3	92 ± 1.7	103 ± 4.1
Bottom (%)	-5	+6	0.4 ± 0.64	-0.04 ± 0.23	0.4 ± 0.29	0.3 ± 0.71
Log IC_{50}	-7.3	-7.0	-7.0 ± 0.03[d]	-7.2 ± 0.01[d]	-7.3 ± 0.02[d]	-7.2 ± 0.05
Slope	-1.2	-0.8	-1.0 ± 0.08	-0.9 ± 0.03[e]	-1.0 ± 0.05[f]	-1.0 ± 0.10

[a] Curve fit of the individual replicates (value ± SEM); n = 3.
[b] Curve fit of the of the mean values from each run; n = 3.
[c] Outside of preferred range.
[d] Statistically significant difference from the other runs at p <0.05 by Satterthwaite T-test.
[e] Statistically significant difference from run 4 at p <0.05 by Satterthwaite T-test.
[f] Statistically significant difference from run 3 at p <0.05 by Satterthwaite T-test.

III. CONCLUSION

An *in vitro* aromatase inhibition assay was performed to evaluate the ability of oxamyl to inhibit human recombinant microsomal aromatase activity, an enzyme responsible for the conversion of androgens to estrogens in vitro. A positive control chemical, 4-OH ASDN, was evaluated concurrently to verify test system performance. As expected, 4-OH ASDN showed effects consistent with aromatase inhibition in all 3 independent runs. The mean estimated logIC50 for all 3 runs for 4-OH ASDN was approximately -7.2 logM.

Under the conditions of the study, oxamyl did not inhibit aromatase activity when tested up to a maximum concentration of 1.0×10^{-3} M. Therefore, oxamyl is classified as a non-inhibitor in this aromatase inhibition assay.

RMS comments and conclusion for renewal

This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/05

Reference: CA 5.8.3/10	**Report:**	Willoughby, J.A. (2012); Oxamyl (DPX-D1410) technical: Estrogen receptor transcriptional activation (human cell line (HeLa-9903)) **DuPont Report No.:** DuPont-32073, Revision No. 1 **Guidelines:** OPPTS 890.1300 (2009), OECD 455 (2009) **Deviations:** None **Testing Facility:** CeeTox, Inc., Kalamazoo, MI, USA **Testing Facility Report No.:** 9144V-100361ERTA **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Oxamyl was evaluated for its ability to act as an agonist of human oestrogen receptor alpha (hERα) using the hERα-HeLa-9903 cell line. Preliminary assessments of cytotoxicity and precipitation were conducted in order to identify a suitable top concentration of oxamyl for use in the transcriptional activation assays. Ultrapure water (water purified to 18.2 MΩ-cm) was selected as the vehicle for the test substance and did not have a significant effect on the assay. The final concentrations of oxamyl tested in the transcriptional activation assays were: 10-10.3, 10-9.3, 10-8.3, 10-7.3, 10-6.3, 10-5.3, 10-4.3, and 10-3.3 M for the first and second runs. All concentrations were tested in replicates of six per plate. In addition, for each concentration, two replicates per plate were prepared that incorporated the hERα antagonist ICI 182,780. Replicates incorporating the hERα antagonist allow for the identification of non-specific (*i.e.*, non-hERα-mediated) induction of the luciferase gene. The duration of exposure was 24 hours. A complete concentration response curve for each of four reference compounds (17β-oestradiol, 17α-oestradiol, corticosterone, and 17α-methyltestosterone) was run each time the transcriptional activation assay was performed.

The maximum concentration of oxamyl selected for use in the transcriptional activation assays was 10-4.3 M as no cytotoxicity (≥20% reduction in cell viability) or precipitation was observed in a preliminary cytotoxicity assay at concentrations higher than 10-4.3 M. In two independent runs of the transcriptional activation assay, oxamyl did not result in an increase in luciferase activity (RPCmax <10%) at any of the viable concentrations tested.

Based on the results of this study, oxamyl is not considered an agonist of human oestrogen receptor alpha (hERα) in the HeLa-9903 model system.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Pure oxamyl (PAI)
   Lot/Batch #: D1410-196
   Purity: 98.0%
   Description: White powder
   CAS #: 23135-22-0
   Stability of test compound: Not determined
   Vehicle/ final concentration: Ultrapure water
2. Cell line: hERα-HeLa-9903
   Source: Japanese Collection of Research Bioresources (JCRB) Cell Bank, Ibaraki-shi, Osaka, Japan

B. STUDY DESIGN AND METHODS

1. *Experimental start/completion*

07-December-2011 to 19-December-2011

2. *Cell culture and plating conditions*

Cells were maintained in Eagle's Minimum Essential Medium (EMEM) without phenol red, supplemented with 60 mg/L of Kanamycin (antibiotic) and 10% dextran-coated-charcoal-treated foetal bovine serum (DCC-FBS), in a CO_2 incubator (5% CO_2) at 37 ± 1°C. When the cells reached 75–90% confluency, they were subcultured at 10 mL of 0.4×10^5 to 1×10^5 cells/mL. The cells were suspended with 10% DCC-FBS in EMEM and plated into wells of a 96-well cell culture plate at a density of about 1×10^4 cells/75 µL/well. The cells were then placed into a 5% CO_2 incubator 37 ± 2°C for at least 3 hours prior to chemical exposure.

3. *Chemical exposure and assay plate organisation*

The reference chemicals were dissolved in DMSO, and oxamyl was dissolved in ultrapure water. The reference controls and test substance were then serially diluted as appropriate in the respective vehicle before further dilution in medium to prepare 2× concentrated stock solutions. The final concentration of DMSO and ultrapure water in the medium was held constant at 0.1% (v/v).

After the 3-hour (minimum) post-seeding incubation, the plates were removed from the incubator, and the media were aspirated. A 75-µL aliquot of fresh media, followed by 75 µL of the 2× concentrated stock solutions were added to wells containing about 1×10^4 cells/well for a final volume of 150 µL/well. All test substance and reference control assay plates were organised in 96-well plates with 12 columns and 8 rows.

After adding the reference chemicals/test substance, the plates were incubated in a 5% CO_2 incubator at 37 ± 2°C for 24 ± 2 hours.

All concentrations were tested in replicates of six per plate. In addition, for each concentration, two replicates per plate were prepared that incorporated the hERα antagonist ICI 182,780. Replicates incorporating a hERα antagonist allow for the identification of non-specific (*i.e.*, non-hERα-mediated) induction of the luciferase gene as true hERα-mediated induction is inhibited by addition of an antagonist whereas non-specific induction is not.

4. *Cytotoxicity assay*

Cell viability was monitored by propidium iodide (PI) uptake. As PI is a light sensitive compound, all procedures were conducted under low light conditions.

Cells were seeded and exposed to the test article in replicates of six while the last two rows received 125 µM digitonin as a positive control for cell death. Following chemical exposure, the growth medium was removed, and 50 µL of a PI working solution (44 µM in phosphate buffered saline or cell culture medium) was added to each well. Background fluorescence was evaluated by measuring fluorescence immediately on a Packard Fusion fluorescence plate reader at an excitation wavelength of 544 nm and an emission wavelength of 612 nm. Following this determination, 50 µL of a 2% (v/v) Triton X-100 solution was added to each well and the plate was incubated at room temperature for a minimum of 15 minutes to fully lyse all cells in the wells before measuring fluorescence at the same wavelengths.

The background-corrected fluorescence was calculated for each well by subtracting the results of the first read from the results of the second read. The change in cell viability was determined by comparing treated wells to the vehicle control wells. A ≥20% reduction in cell viability was considered evidence of cytotoxicity.

5. *Precipitation assay*

Limit of solubility was determined by visual inspection.

6. *Transcriptional activation assay*

A luciferase assay was performed as described in CeeTox Standard Operating Protocol (SOP) 2041. Luciferase assay reagent was prepared as described in CeeTox SOP 2041 (proprietary information).

7. *Preliminary range finding*

In order to identify a suitable top concentration for use in the transcriptional activation assays, preliminary cytotoxicity and precipitation assays were conducted with oxamyl. These preliminary

assays assessed cytotoxicity and precipitation, respectively, at the following: $10^{-6.8}$, $10^{-6.3}$, $10^{-5.8}$, $10^{-5.3}$, $10^{-4.8}$, $10^{-4.3}$, $10^{-3.8}$, and $10^{-3.3}$ M (for cytotoxicity) and $10^{-3.3}$ M (for precipitation).

8. *Analysis*

The test substance was considered negative if the maximum response relative to the positive control (RPC_{Max}) was <10% in at least two runs of the transcriptional activation assay. The test substance was considered positive if RPC_{Max} was ≥10% in at least two runs of the transcriptional activation assay.

II. RESULTS AND DISCUSSION

A. CONCENTRATION RANGE FOR THE TEST SUBSTANCE

In order to identify a suitable top concentration for use in the transcriptional activation assays, preliminary assessments of cytotoxicity and precipitation were conducted over a concentration range of $10^{-6.8}$ M to $10^{-3.3}$ M. Results of the cytotoxicity assay and precipitation assessment for oxamyl are shown in Table 92. On the basis of this preliminary assessment, the suitable top concentration of oxamyl for use in the transcriptional activation assays was $10^{-4.3}$ M as no cytotoxicity (>20% reduction in cell viability) or precipitation was observed.

The final concentrations of oxamyl tested in the transcriptional activation assays were: $10^{-10.3}$, $10^{-9.3}$, $10^{-8.3}$, $10^{-7.3}$, $10^{-6.3}$, $10^{-5.3}$, $10^{-4.3}$, and $10^{-3.3}$ M for the first and second runs. The results for oxamyl and the four reference compounds (17β-oestradiol, 17α-oestradiol, corticosterone, and 17α-methyltestosterone) are shown in Table 93 and Table 94.

Table 9210 Results of the preliminary cytotoxicity and precipitation assays

Concentration of oxamyl (M)	Cell viability (% of VC[a])		Precipitation
	Mean	SD[b]	
$10^{-6.8}$	104	3	n.a.[c]
$10^{-6.3}$	100	2	n.a.
$10^{-5.8}$	94	3	n.a.
$10^{-5.3}$	98	2	n.a.
$10^{-4.8}$	99	3	n.a.
$10^{-4.3}$	93	6	n.a.
$10^{-3.8}$	70[e]	3[e]	n.a.
$10^{-3.3}$	52[e]	2[e]	—[d]

[a] Vehicle control
[b] Standard deviation
[c] Not applicable
[d] No precipitation observed
[e] Cytotoxicity observed (viability <80% of control)

Table 9311 Results of the first transcriptional activation assay

Chemical	Concentration (M)	RTA[a] (% of PC[b])		RTA with ICI (% of PC)		Cell viability (% of VC[c])		Precipitation
		Mean	SD[d]	Value 1	Value 2	Mean	SD	
Oxamyl	$10^{-10.3}$	0.7	1.5	0.5	-1.6	91.3	1.6	—[e]
	$10^{-9.3}$	0.3	2.3	-0.6	-0.7	87.3	2.6	—
	$10^{-8.3}$	1.4	2.2	-0.8	-1.6	86.4	2.7	—
	$10^{-7.3}$	-0.5	2.1	-1.4	-2.4	83.3	3.7	—
	$10^{-6.3}$	2.9	1.7	-0.1	-1.7	82.5	4.0	—
	$10^{-5.3}$	2.1	1.7	-0.3	-1.6	82.3	2.7	—
	$10^{-4.3}$	[f]	[f]	-2.3[f]	-2.7[f]	75.5[f]	3.9[f]	—
	$10^{-3.3}$	[f]	[f]	-3.2[f]	-3.3[f]	37.6[f]	1.1[f]	—
17β-Oestradiol	10^{-15}	0.6	1.8	1.6	-1.9	94.0	2.7	—
	10^{-14}	0.0	1.6	2.1	-1.6	92.5	3.7	—
	10^{-13}	1.5	2.1	1.3	-1.2	89.7	3.0	—
	10^{-12}	1.2	2.0	1.8	-2.1	90.3	4.5	—
	10^{-11}	19.4	12.2	3.1	-1.1	90.3	5.1	—
	10^{-10}	83.3	17.1	3.3	-0.8	87.5	1.7	—
	10^{-9}	170.4	43.6	2.8	-1.6	89.9	1.7	—
	10^{-8}	83.2	32.0	1.7	-1.5	81.4	3.0	—
17α-Oestradiol	10^{-13}	0.5	1.7	1.2	-1.2	97.1	5.3	—
	10^{-12}	-0.2	1.3	1.5	-1.5	92.4	5.6	—
	10^{-11}	1.9	1.8	0.9	-1.4	92.9	5.0	—
	10^{-10}	2.3	2.1	-0.5	-1.4	92.4	3.7	—
	10^{-9}	37.4	16.0	1.2	-0.4	90.3	6.0	—
	10^{-8}	116.2	16.2	1.1	-0.9	88.2	5.0	—
	10^{-7}	149.3	27.8	0.1	-0.9	89.6	3.7	—
	10^{-6}	77.4	27.8	0.4	-1.3	82.7	8.3	—
Corticosterone	10^{-11}	1.1	2.2	1.7	-1.9	88.0	2.9	—
	10^{-10}	0.1	1.4	1.8	-1.8	91.4	2.1	—
	10^{-9}	1.6	1.8	1.5	-1.4	90.3	2.3	—
	10^{-8}	-0.5	1.3	0.9	-1.7	86.4	5.9	—
	10^{-7}	3.1	2.4	3.1	-0.1	84.3	1.9	—
	10^{-6}	1.3	0.9	2.7	-0.2	85.0	3.1	—
	10^{-5}	-0.9	1.0	0.0	-1.8	82.7	2.8	—
	10^{-4}	[f]	[f]	-2.0[f]	-2.9[f]	70.6[f]	4.0[f]	—
17α-Methyltestosterone	10^{-12}	0.9	2.7	1.7	-1.5	95.3	3.0	—
	10^{-11}	0.0	1.9	2.1	-1.3	95.1	2.4	—
	10^{-10}	1.5	2.8	2.7	-1.0	94.2	3.5	—
	10^{-9}	-0.3	1.9	2.6	-1.9	94.0	2.5	—
	10^{-8}	3.4	2.4	3.4	-0.7	90.3	2.6	—
	10^{-7}	4.0	0.7	3.4	-0.6	90.1	5.5	—
	10^{-6}	7.3	1.8	2.9	-0.3	97.1	7.2	—
	10^{-5}	24.3	11.3	0.2	-0.9	87.6	3.5	—

[a] Relative transcriptional activation
[b] Positive control (1 nM 17β-oestradiol)
[c] Vehicle control
[d] Standard deviation
[e] No precipitation observed
[f] Cytotoxicity observed data not evaluated
ICI = ICI 182,780 hERα antagonist

Table 9412 Results of the second transcriptional activation assay

Chemical	Concentration (M)	RTA[a] (% of PC[b])		RTA with ICI (% of PC)		Cell viability (% of VC[c])		Precipitation
		Mean	SD[d]	Value 1	Value 2	Mean	SD	
Oxamyl	$10^{-10.3}$	1.9	2.9	2.8	0.2	104.9	4.6	—[e]
	$10^{-9.3}$	0.9	2.7	1.4	-1.7	106.3	3.5	—
	$10^{-8.3}$	2.9	3.5	2.7	-0.7	102.6	5.1	—
	$10^{-7.3}$	1.3	3.0	0.1	-1.5	106.8	4.7	—
	$10^{-6.3}$	4.4	2.6	3.4	1.3	106.3	6.4	—
	$10^{-5.3}$	3.0	2.3	3.5	3.7	111.1	4.1	—
	$10^{-4.3}$	-4.4	0.6	-2.6	-3.0	94.8	2.8	—
	$10^{-3.3}$	[f]	[f]	-4.7[f]	-4.8[f]	41.1[f]	4.9[f]	—
17β-Oestradiol	10^{-15}	3.4	3.8	2.9	-0.2	97.3	4.4	—
	10^{-14}	2.9	3.9	1.4	-0.1	94.4	3.7	—
	10^{-13}	4.1	3.9	3.2	-1.5	92.2	7.3	—
	10^{-12}	5.6	5.5	1.0	-0.2	96.3	4.1	—
	10^{-11}	30.7	16.8	4.2	1.0	95.2	2.4	—
	10^{-10}	97.4	30.9	4.6	3.8	96.9	3.0	—
	10^{-9}	153.8	27.0	4.2	0.4	99.4	6.6	—
	10^{-8}	93.9	29.6	1.0	-2.4	101.3	4.9	—
17α-Oestradiol	10^{-13}	1.6	2.2	1.9	-0.4	96.3	3.9	—
	10^{-12}	1.2	2.1	1.8	-0.1	97.2	3.9	—
	10^{-11}	2.5	2.5	3.5	-0.7	94.0	4.6	—
	10^{-10}	1.3	2.3	0.5	-1.4	96.6	3.1	—
	10^{-9}	16.2	4.7	3.4	0.7	96.7	3.0	—
	10^{-8}	78.8	22.1	4.3	5.9	98.6	4.8	—
	10^{-7}	106.7	23.7	3.2	1.9	104.1	4.7	—
	10^{-6}	77.1	16.2	4.7	-0.9	104.0	5.9	—
Corticosterone	10^{-11}	2.0	2.8	1.3	-0.4	99.4	3.1	—
	10^{-10}	1.3	2.6	1.5	-0.8	99.1	2.2	—
	10^{-9}	2.7	2.9	3.5	-0.8	94.6	2.9	—
	10^{-8}	0.7	2.0	0.3	-0.9	98.5	2.7	—
	10^{-7}	2.5	1.7	1.9	1.3	95.1	4.0	—
	10^{-6}	0.2	1.2	1.5	3.1	99.8	4.7	—
	10^{-5}	-0.9	1.0	-0.8	-1.8	98.3	5.3	—
	10^{-4}	-3.1	0.8	-2.6	-3.3	90.0	4.6	—
17α-Methyltestosterone	10^{-12}	2.8	4.5	3.0	-0.3	96.7	4.9	—
	10^{-11}	0.8	2.7	2.7	-0.2	95.7	3.9	—
	10^{-10}	1.9	2.6	3.9	-0.3	91.3	3.9	—
	10^{-9}	0.6	2.5	2.0	0.5	96.2	3.3	—
	10^{-8}	4.9	3.3	5.4	3.0	95.1	3.6	—
	10^{-7}	3.2	2.6	4.4	5.9	97.4	4.6	—
	10^{-6}	7.4	5.3	4.4	1.1	98.9	3.2	—
	10^{-5}	26.6	10.4	1.2	-0.4	87.7	5.3	—

[a] Relative transcriptional activation
[b] Positive control (1 nM 17β-oestradiol)
[c] Vehicle control
[d] Standard deviation
[e] No precipitation observed
[f] Cytotoxicity observed data not evaluated
ICI = ICI 182,780 hERα antagonist

B. TRANSCRIPTIONAL ACTIVATION ASSAY ACCEPTANCE CRITERIA

In two independent runs of the assay, oxamyl did not result in an increase in luciferase activity at any of the concentrations tested as the RPC_{max} values in both independent runs of the assay were <10% (mean values of $2.9 \pm 1.7\%$ and $4.4 \pm 2.6\%$ in the first and second independent runs, respectively) (Table 93 and Table 94).

III. CONCLUSIONS

The suitable top concentration of oxamyl for use in the transcriptional activation assays was $10^{-4.3}$ M as cytotoxicity (≥20% reduction in cell viability) was observed at concentrations higher than 10-4.3 M. Cytotoxicity was also observed at concentrations ≥ $10^{-4.3}$ M oxamyl in the first run. There was no precipitation observed with oxamyl.

In two independent runs of the transcriptional activation assay, oxamyl did not result in an increase in luciferase activity (RPCmax<10%) at any of the viable concentrations tested.

Based on the results of this study, oxamyl is not considered an agonist of human oestrogen receptor alpha (hERα) in the HeLa-9903 model system.

RMS comments and conclusion for renewal
This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/06

Reference: CA 5.8.3/06	**Report:**	Snajdr, S.I. (2012b); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* estrogen receptor binding assay using rat uterine cytosol (ER-RUC) **DuPont Report No.:** DuPont-32074 **Guidelines:** OPPTS 890.1250 (2009) **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-32074 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its ability to bind to the oestrogen receptors in rat uterine cytosol. The *in vitro* oestrogen receptor binding assay using rat uterine cytosol is part of the Tier 1 battery of the Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay was intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

Saturation binding assays measure the affinity of a radiolabelled oestrogen ligand, 17β-oestradiol ([^{3}H-]E2), (Kd) for the oestrogen receptor and the concentration of the oestrogen receptors (Bmax) present in the cytosol. This is determined by measuring specific binding of increasing concentrations of radioligand under conditions of equilibrium. Three independent runs were performed using hexatritiated 17β-oestradiol ([^{3}H]-E2) as the radioligand to characterise the rat uterine cytosol. The Kd was approximately 0.081 nM [^{3}H]-E2, and the Bmax was approximately 32.5 fmol/100 µg protein, which is consistent with the acceptable range listed in the test guideline.

Competitive binding assays measure the binding of the radioligand to the receptors with increasing concentrations of a test substance. The concentration at which the test substance displaces half of the bound

radioligand is the IC_{50} (often expressed as $logIC_{50}$). Three independent runs were performed to evaluate oxamyl for its ability to compete with [³H]-E2 in binding to rat uterine oestrogen receptors *in vitro*. Oxamyl was evaluated at eight concentrations ranging 1.0×10^{-10} to 1×10^{-3} M. Radioinert E2, the oestrogen receptor agonist reference standard, 19-norethindrone, a weak oestrogen receptor agonist used as the positive control, and octyltriethoxysilane, a non-oestrogen receptor agonist used as the negative control, were used to verify test system performance. As expected, radioinert E2 and 19-norethindrone showed effects consistent with strong and weak competitive binding, respectively, and octyltriethoxysilane did not compete for binding to the oestrogen receptor in all three runs. The $logIC_{50}$ was determined to be approximately -9.16 and -4.85 for radioinert E2 and 19-norethindrone, respectively. The relative binding affinity of 19-norethindrone compared to radioinert E2 was approximately 0.0054%. The $logIC_{50}$ value for the radioinert E2 is consistent with those previously reported in the EPA's Integrated Summary Report for the validation of the oestrogen receptor binding assay; no logIC50 values for 19-norethindrone were reported.

Under the conditions of the study, the test substance, oxamyl, did not competitively bind to the oestrogen receptor when tested up to a maximum concentration of 1.0×10^{-3} M. Therefore, oxamyl is classified as a non-inhibitor in the oestrogen receptor binding assay.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Powder
	CAS #:	23135-22-0
	Stability of test compound:	The test substance was stable throughout the exposure phase of the study; no evidence of instability was observed
	Solvent/ final concentration:	Deionised water/ 2%
2.	Positive control:	19-norethindrone
	Lot number:	019K1735
	Purity:	100%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	68-22-4
	Solvent/ final concentration:	Ethanol, 2%
3.	Negative control:	Octyltriethoxysilane
	Lot number:	XD0346
	Purity:	100%
	Source:	Spectrum Chemicals and Laboratory Products, Gardena, California, USA
	CAS #:	2943-75-1
	Solvent/ final concentration:	Ethanol, 2%
4.	Radioligand:	Hexatritiated 17β-oestradiol ([3H]-E2)
	Lot number:	650702
	Purity:	97%
	Source:	Perkin Elmer, Waltham, Massachusetts, USA
	Specific Activity:	130.2 Ci/mmol (4817 GBq/mmol)
	Concentration:	1.0mCi/mL (37 MBq/mL)
	Solvent/final concentration:	Ethanol, 2%
5.	Reference oestrogen receptor agonist standard:	Radioinert 17β-oestradiol
	Lot number:	21K1267
	Purity:	99%
	Source:	Sigma Aldrich, St. Louis, Missouri, USA
	CAS #:	50-28-2
	Solvent/final concentration:	Ethanol, 2%

B. STUDY DESIGN AND METHODS

1. *Experimental start/completion*

16-August-2011 to 25-August-2011

2. *Rat uterine cytosol preparation*

Uteri were thawed on ice, homogenised (0.1 g of tissue per 1.0 mL cold TEDG + PMSF buffer [10 mM Tris, 1.5 mM EDTA, and 10% glycerol + 1 mM dithiothreitol and 1 mM phenylmethylsulfonyl fluoride]), centrifuged for 10 minutes at approximately 2500 × g at 4°C, and the supernants were centrifuged at approximately 105000 × g for 60 minutes at 4°C. The resulting supernants were then pooled, aliquoted, and stored frozen (approximately -60°C to -80°C) until use.
The protein concentration of the uterine cytosol was determined prior to performing the receptor assays. Samples for the standard curve were prepared using bovine serum albumin with final concentrations ranging from 0.01–0.1 mg/mL. The protein concentration of the uterine cytosol was determined to be 3.5 mg/mL.

3. *Dose preparations and analysis*

The reference standard, E_2 (not adjusted for purity), was used to verify test system performance at seven final concentrations ranging from 1.0×10^{-11} to 1.0×10^{-7} M. The positive control, 19-norethindrone (not adjusted for purity), was used to verify test system performance at eight final concentrations ranging from 3.16×10^{-9} to 1.0×10^{-4} M. The negative control, octyltriethoxysilane (not adjusted for purity), was used to verify test system performance at eight final concentrations ranging from 1.0×10^{-10} to 1.0×10^{-3} M. The test substance (adjusted for purity) was evaluated at eight targeted final concentrations ranging from 1.0×10^{-10} to 1.0×10^{-3} M.
Preparations of the initial solutions for the reference standard, positive control, negative control, and test substance were prepared by serial dilution from a stock solution, which was also the respective high initial concentration. No indication of precipitation of the reference standard, positive control, negative control, or test substance was observed during the assay.
Samples of each test substance dosing preparation were collected near the beginning of the study and stored at room temperature until analysis. The samples were analysed to verify concentration and confirm stability. At the time of analysis, the samples were diluted with an appropriate solvent and analysed by high-performance liquid chromatography (HPLC) with a mass spectrometer detector (LC/MS). Samples of the reference standard, positive control, and negative control were not evaluated for concentration or stability.

4. *Verification of scintillation counting efficiency accuracy*

The scintillation counting efficiency was determined by calculating the expected DPMs when counting target concentrations of 3, 1, 0.6, 0.3, 0.1, 0.08, 0.06, and 0.03 nM [^{3}H]-E_2. The calculated values ranged from 103 to 116% of the counts returned by the liquid scintillation counter (LSC).

5. *Oestrogen receptor saturation assay*

Briefly, [^{3}H]-E_2 was diluted in cold TEDG + PMSF buffer to final concentrations of 3, 1, 0.6, 0.3, 0.1, 0.08, 0.06, and 0.03 nM. Radioinert E_2 was diluted first in ethanol, then in cold TEDG + PMSF buffer to final concentrations of 300, 100, 60, 30, 10, 8, 6, and 3 nM, respectively, (*i.e.*, 100× the concentration of [^{3}H]-E_2).
Uterine cytosol was thawed on ice then kept on ice prior to being diluted in cold TEDG + PMSF buffer to a concentration of approximately 0.4 mg protein/mL, which was shown previously to bind to approximately 27% of 0.03 nM [^{3}H]-E_2.
After addition of all assay components, tubes were incubated at approximately 4°C with gentle mixing for approximately 20 hours, with the exception of the total [^{3}H]-E_2 tubes, which were counted without further processing. Hydroxyapatite slurry (HAP) was washed three times in cold TEDG + PMSF buffer and resuspended to a ratio of 60% HAP and 40% TEDG + PMSF buffer by volume. The bound [^{3}H]-E_2 was separated from free by adding 250 µL of cold resuspended HAP slurry to each assay tube, vortexing for 10 seconds every 5 minutes for 15 minutes, centrifuging for approximately 10 minutes at 4°C at 1000 × g, and then discarding the supernatant. The tubes were washed three times by adding 2.0 mL of cold (approximately 4°C) TEDG + PMSF buffer, briefly vortexing, and centrifuging at 4°C for 10 minutes at 1000 × g. The supernatant from each wash was discarded. The bound [^{3}H]-E_2 was extracted by adding 1.5 mL ethanol and vortexing for approximately 10 seconds at 5-minute intervals for approximately 20 minutes at room temperature. The tubes were then centrifuged for 10 minutes at 1000 × g, and 0.5 mL of the supernatant was added to 7 mL Ultima Gold XR liquid scintillation fluid and counted in a LSC for 5 minutes.

6. *Oestrogen receptor competitive binding assay*

The reference standard was evaluated in triplicate at final concentrations of 0.01, 0.1, 0.316, 1.0, 3.16, 10, and 100 nM. The positive control was evaluated in triplicate at final concentrations of 3.16, 31.6, 100, 316, 1000, 3160, 31600, and 100000 nM. The negative control was evaluated in triplicate at final concentrations of 0.1, 1, 10, 100, 1000, 10000, 100000, and 1000000 nM. The test substance was evaluated in triplicate at final concentrations of 0.1, 1, 10, 100, 1000, 10000, 100000, and 1000000 nM. Uterine cytosol was diluted in cold TEDG + PMSF buffer to approximately 1.2 mg protein/mL, which was shown previously to bind to approximately 7% of 1 nM [^{3}H]-E_2. The average estimated K_d of radioinert E_2 for the batch of uterine cytosol used in this study was 0.081 nM as determined by the saturation binding assay.

The final volume per assay tube was 500 µL. Each assay tube contained 390 µL of 1 nM [^{3}H]-E_2 in cold TEDG + PMSF buffer, 10 µL of either solvent or prediluted reference standard, positive control, negative control, or test substance, and 100 µL of diluted rat uterine cytosol. Each total [^{3}H]-E_2 tube contained 50 µL of 1 nM [^{3}H]-E_2 for a total volume of 50 µL and was counted without further processing.

After addition of all assay components, the tubes were incubated at approximately 4°C with gentle mixing for approximately 20 hours, with the exception of the total [^{3}H]-E_2 tubes, which were counted without further processing. HAP was washed three times in cold TEDG + PMSF buffer and resuspended to a ratio of 60% HAP and 40% TEDG + PMSF buffer by volume. The bound [^{3}H]-E_2 was separated from free by adding 250 µL of cold resuspended HAP slurry to each assay tube, vortexing for 10 seconds every 5 minutes for 15 minutes, centrifuging for approximately 10 minutes at 4°C and 1000 × g and discarding the supernatant. The tubes were washed three times by adding 2.0 mL of cold (approximately 4°C) TEDG + PMSF buffer, vortexing, and centrifuging at 4°C for 10 minutes at 1000 × g. The supernatant from each wash was discarded. The bound [^{3}H]-E_2 was extracted by adding 1.5 mL ethanol and vortexing for approximately 10 seconds at 5-minute intervals for approximately 20 minutes at room temperature. The tubes were then centrifuged for 10 minutes at 1000 × g and 0.5 mL of supernatant was added to 7 mL Ultima Gold XR liquid scintillation fluid and counted in a LSC for 5 minutes.

7. Statistics

The dissociation constant (K_d), maximal binding capacity (B_{max}), and the concentration that inhibits 50% of maximum radioligand binding ($logIC_{50}$) values were determined as appropriate using Origin 8.5.1.

The K_d and B_{max} values for the uterine cytosol were determined by fitting the saturation curves using the following equation:

$$Y = \frac{B_{max} \times X}{X + K_d} + (a \times X)$$

where Y = total binding, a = ratio between non-specific bound ligand and free ligand, and X = concentration of [^{3}H]-E_2.

The IC_{50} values were determined by fitting the competitive curves using the following equation:

$$Y = Bottom + \frac{Top - Bottom}{1+10^{(Log\ IC50-X)HillSlope+log[(Top-Bottom)/(50-Bottom)-1]}}$$

where X is the logarithm of the concentration of test substance and Y is the percent of radioligand bound to the receptor. $LogIC_{50}$ is X at Y = 50%. “Top” and “Bottom” refer to the value of Y when there is minimal binding by the test substance, and when there is maximal binding by the test substance, respectively. A concentration-response model was fitted for each test run for each curve generated.

8. Performance criteria

The following criteria were applied to assess the performance of the competitive binding assays. Increased concentrations of radioinert E_2 displace [^{3}H]-E_2 from the receptor in a manner consistent with one-site competitive binding. Ligand depletion was no greater than 15%. The parameter values (top, bottom, and slope) for reference standard and positive control were within the tolerance bounds provided. The solvent control did not alter the sensitivity or reliability of the assay. The negative control substance (octyltriethoxysilane) did not displace more than 25% of the radioligand from the oestrogen receptor on average across all concentrations. The test substance was tested over a concentration range that fully defined the top of the curve (*i.e.*, a range that showed that a top plateau

was achieved), and the top is within 25% of either the solvent control or the value for the lowest concentration of the oestradiol standard for that run. In addition to meeting the criteria for individual runs, consistency occurs across runs of the top plateau level, Hill slope, placement along the X-axis, and the bottom plateau (where defined).

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Oxamyl was present at acceptable concentrations in the dosing solutions (within 12.6% of nominal concentrations). Oxamyl was shown to be stable in the dosing solutions under the conditions of the study. Oxamyl was not found in the 0 mg/mL samples.

B. OESTROGEN RECEPTOR SATURATION BINDING ASSAY

Three independent oestrogen receptor saturation binding runs were completed using [^{3}H]-E_2 as the radioligand and radioinert E_2 as the ligand. The K_d values for each of the three runs were 0.077, 0.081, and 0.085 nM [^{3}H]-E_2 with an average of 0.081 nM [^{3}H]-E_2. The B_{max} values for each of the three runs were 28.0, 35.1, and 34.5 fmol/100 μg protein with an average of 32.5 fmol/100 μg protein. Confidence in these numbers is high as the adjusted coefficient of determination (adjusted R^2) was 0.913, 0.880, and 0.889 for each of the respective runs, with small variations between runs. The K_d and B_{max} were within the range provided by the test guideline.

Table 953 Saturation binding assay: K_d and B_{max} values for uterine cytosol

Parameter	Run S1[a]	Run S2[a]	Run S3[a]	Mean[b]	±	SE
Adjusted R^2 (unweighted)	0.913	0.880	0.889	0.894	±	0.010
B_{max} (nM)	0.020	0.024	0.024	0.023	±	0.001
B_{max} (fmol/100 µg protein)	28.0	35.1	34.5	32.5	±	2.25
K_d (nM)	0.077	0.081	0.085	0.081	±	0.002
Time since specific activity certification (days)	83	87	90	-	-	-
Adjusted specific activity on date of run (Ci/mmol)	128.5	128.5	128.4	-	-	-
Percent NSB of TB	4–11	4–12	4–11	-	-	-
Percent ligand depletion of [^{3}H]-E_2 conc (nM)						
0.03	19	22	19	-	-	-
0.06	15	17	17	-	-	-
0.08	12	15	15	-	-	-
0.1	11	14	13	-	-	-
0.3	5.6	6.0	5.6	-	-	-
0.6	2.9	3.5	3.4	-	-	-
1	1.9	2.4	2.2	-	-	-
3	0.64	0.82	0.79	-	-	-
Scintillation counting efficiency accuracy - Percent difference (DPMs counted/DPMs calculated)						
0.03	110	112	116	-	-	-
0.06	111	114	110	-	-	-
0.08	114	113	115	-	-	-
0.1	110	104	112	-	-	-
0.3	105	108	112	-	-	-
0.6	106	106	110	-	-	-
1	103	105	112	-	-	-
3	104	106	111	-	-	-

[a] N = 3

[b] Mean ± SE for all three runs

C. OESTROGEN RECEPTOR COMPETITIVE BINDING ASSAY

Three independent oestrogen receptor competitive binding runs were completed using [^{3}H]-E_2 as the radioligand and radioinert E_2 as the reference standard, 19-norethindrone as the positive control, octyltriethoxysilane as the negative control or the test substance.

The logIC_{50} values for radioinert E_2 for each of the three runs were -9.43, -9.03, and -9.02 logM [^{3}H]-E_2 with an average of -9.16 logM [^{3}H]-E_2. The logIC_{50} values for 19-norethindrone for each of the three runs were -4.92, -4.70, and -4.95 logM [^{3}H]-E_2 with an average of -4.85 logM [^{3}H]-E_2. The relative binding affinity (RBA) of 19-norethindrone as compared to radioinert E_2 for each of the three runs was 0.0031, 0.0046, and 0.0085% with an average of 0.0054%. As expected, neither a logIC_{50} nor RBA were able to be determined for octyltriethoxysilane as no competitive binding was observed.

A logIC_{50} was not determined for the test substance since there were no test substance-related effects on oestrogen receptor binding up to the concentration of 1 mM represents the highest concentration required by the test guideline.

Table 964 Competitive binding assay: Mean ± standard error of the percent [^{3}H]-E_2 bound for each concentration

Competitor	Final conc. (M)	Run C1[a] Mean	Run C1[a] SE	Run C2[a] Mean	Run C2[a] SE	Run C3[a] Mean	Run C3[a] SE
SC (ethanol)	—	92.33	8.57	98.97	4.65	100.60	4.10
SC (dH_2O)	—	85.16	3.17	98.96	1.83	99.57	4.08
Radioinert E_2 (NSB)	1×10^{-07}	-0.35	0.18	0.00	0.08	0.16	0.22
Radioinert E_2	1×10^{-08}	4.53	0.47	8.93	0.60	7.65	0.30
	3.16×10^{-09}	16.23	1.58	21.68	0.24	24.63	0.86
	1×10^{-09}	31.70	2.73	48.10	0.80	49.59	0.92
	3.16×10^{-10}	53.13	0.46	76.56	2.93	72.48	1.40
	1×10^{-10}	66.22	2.05	86.60	1.06	88.97	4.70
	1×10^{-11}	74.93	1.67	100.81	1.46	96.78	4.29
Octyltriethoxysilane	1×10^{-03}	88.83	2.39	99.26	0.66	105.39	5.41
	1×10^{-04}	84.61	0.70	96.18	1.28	100.38	1.98
	1×10^{-05}	100.52	3.61	101.94	1.25	101.44	6.26
	1×10^{-06}	103.27	6.71	98.77	1.40	102.99	2.73
	1×10^{-07}	103.47	4.75	98.94	4.99	103.66	2.95
	1×10^{-08}	94.12	12.57	103.29	0.92	104.47	4.34
	1×10^{-09}	92.66	13.34	97.40	1.55	98.10	0.41
	1×10^{-010}	93.09	9.58	92.99	2.47	103.52	4.00
Norethindrone	1×10^{-04}	14.18	2.08	13.25	0.41	13.60	0.35
	3.16×10^{-05}	29.01	1.81	38.92	1.63	28.26	0.41
	3.16×10^{-06}	77.26	7.50	85.39	3.48	75.61	2.02
	1×10^{-06}	88.97	1.54	91.83	2.98	95.66	4.75
	3.16×10^{-07}	90.10	5.79	100.39	3.85	96.33	0.86
	1×10^{-07}	90.14	5.03	102.99	2.66	92.47	2.99
	3.16×10^{-08}	97.43	7.64	102.96	0.79	99.05	3.24
	3.16×10^{-09}	99.18	5.53	101.21	2.68	109.37	2.96
Oxamyl	1×10^{-03}	85.86	10.59	106.35	1.08	98.91	1.49
	1×10^{-04}	99.37	6.08	99.56	3.70	93.30	2.54
	1×10^{-05}	98.31	3.12	97.05	2.30	88.43	2.97
	1×10^{-06}	100.56	10.44	98.66	1.83	94.54	2.86
	1×10^{-07}	110.42	2.94	104.55	3.63	84.53	1.28
	1×10^{-08}	110.82	1.86	96.28	1.63	90.66	1.99
	1×10^{-09}	113.57	0.31	96.58	0.63	89.50	3.76
	1×10^{-10}	110.14	5.84	97.41	2.47	93.50	5.09
SC (ethanol)	—	107.67	2.71	101.03	1.36	99.40	4.88
SC (dH_2O)	—	114.84	3.14	101.04	2.12	100.43	4.46
Radioinert E_2 (NSB)	1×10^{-07}	0.35	0.03	0.00	0.13	-0.16	0.07

[a] N = 3

D. ASSAY PERFORMANCE

The performance parameters for radioinert E_2, as listed in Table 97, were within the acceptable ranges as specified in the test guideline, with the exception of the top of the curve (%) of 76 and 92 for run C1 and the average for all runs, which was slightly below the suggested range as specified in the test guideline. The performance parameters for 19-norethindrone, as listed in Table 97, were within the acceptable ranges as specified in the test guideline, with the exception of the bottom of the curve (%) of -22.1 for run C2, which was well below the suggested range as specified in the test guideline, and the bottom of the curve (%) of 3.0 and -5.5 for run C3 and the average for all runs, which were slightly above and below the suggested

range as specified in the test guideline, respectively. All runs were within or very close to the suggested performance criteria, and therefore the assay was considered valid.

Confidence in these numbers is high due to the small variation between runs. There was no observed precipitation at any of the concentrations tested. The solvent control responses indicated no drift in any of the runs. The $logIC_{50}$ value for the radioinert E_2 is consistent with those previously reported in the EPA's Integrated Summary Report; no $logIC_{50}$ values for 19-norethindrone were reported.

Table 975 Competitive binding assay: $LogIC_{50}$ values, relative binding affinity, performance standards, and assay drift

Competitor	Parameter	Lower limit[a]	Upper limit[a]	Run C1[b]	Run C2[b]	Run C3[b]	Mean[c]	±	SE
Radioinert E_2	Top (%)	94	111	76[d]	101	98	92[d]	±	7.7
	Bottom (%)	-4.0	+1.0	-1.3	-1.1	-1.7	-1.4	±	0.2
	Slope	-1.1	-0.7	-0.9	-1.0	-0.9	-0.9	±	0.01
	Adjusted R^{2e}	—	—	0.992	0.994	0.988	0.991	±	0.002
	Log_e (S_{yx})	—	2.35	0.95	1.06	1.40	1.14	±	0.13
	$LogIC_{50}$ (M)	—	—	-9.43	-9.03	-9.02	-9.16	±	0.13
19-norethindrone	Top (%)	90	110	96	103	101	100	±	2.27
	Bottom (%)	-5	+1.0	2.6	-22.1[d]	3.0[d]	-5.5[d]	±	8.3
	Slope	-1.1	-0.7	-1.0	-0.8	-0.9	-0.9	±	0.06
	Adjusted R^{2f}	—	—	0.931	0.983	0.967	0.960	±	0.015
	Log_e (S_{yx})	—	2.60	2.14	1.45	1.83	1.81	±	0.20
	$LogIC_{50}$ (M)	—	—	-4.92	-4.70	-4.95	-4.85	±	0.08
	RBA (%)[g]	—	—	0.0031	0.0046	0.0085	0.0054	±	0.0016
Oxamyl[h]		—	—	—	—	—	—		
Percent ligand depletion		—	>15	8.2	11	10	—		
Percent end solvent control of initial solvent control									
	Ethanol (%)	—	—	116	102	99	—		
	dH_2O (%)	—	—	134	102	101	—		
Time since specific activity certification (days)									
		—	—	102	104	109	—		
Adjusted specific activity on date of run (Ci/mmol)									
		—	—	128.2	128.1	128.0	—		

[a] Suggested acceptable range according to test guideline
[b] N = 3
[c] Mean ± SE for all three runs
[d] Parameter was outside the performance criteria
[e] With no weighting as bottom of the curve was below 0
[f] With no weighting
[g] Relative binding affinity (RBA) relative to radioinert E_2
[h] No competitive binding observed, therefore no parameters could be calculated

III. CONCLUSION

An *in vitro* estrogen receptor binding assay was performed to evaluate oxamyl for its ability to compete with [^{3}H]-E2 in binding to rat uterine estrogen receptors *in vitro*. Oxamyl was evaluated at 8 concentrations ranging from 1.0×10^{-10} to 1.0×10^{-3} M.

A saturation binding assay (3 independent runs) was used to characterize the rat uterine cytosol. The Kd was approximately 0.081 nM [^{3}H]-E2 and the Bmax was approximately 32.5 fmol/100 µg protein was generated.

Radioinert E2, as the estrogen receptor agonist reference standard, 19-norethindrone, as the positive control, and octyltriethoxysilane, as the negative control, were used to verify the test system performance in the competitive binding assay (3 independent runs). As expected, radioinert E2 showed effects consistent with strong competitive binding, 19-norethindrone weak competitive binding and octyltriethoxysilane no competitive binding of the estrogen receptor in all 3 independent runs. The logIC_{50} was determined to be approximately -9.16 and -4.85 for radioinert E2 and 19-norethindrone, respectively. The relative binding affinity of 19-norethindrone compared to radioinert E2 was approximately 0.0054%.

Under the conditions of the study, the test substance, oxamyl, did not bind to the oestrogen receptor when tested up to a maximum concentration of 1.0×10^{-3} M. Therefore, oxamyl is classified as a non-inhibitor in this oestrogen receptor binding assay.

RMS comments and conclusion for renewal
This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/07

Reference: CA 5.8.3/03	**Report:**	[redacted] (2011a); Oxamyl (DPX-D1410) technical (98% w/w/): 3-Day uterotrophic assay for detecting estrogenic activity **DuPont Report No.:** DuPont-32075 **Guidelines:** OPPTS 890.1600 (2009) **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** DuPont-32075 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its potential oestrogenic effects when administered by oral gavage to ovariectomised rats for 3 days. The uterotrophic assay employed in this study is part of Tier 1 of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) of a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

Four groups of young adult ovariectomised Crl:CD(SD) rats (six/group) were dosed by oral gavage with 0, 0.1, 0.25, or 0.5 mg/kg/day of the test substance for 3 consecutive days and sacrificed approximately 24 hours after the last administered dose. A separate ovariectomised positive control group, administered 0.1 mg/kg/day of the oestrogen receptor agonist 17α-ethynyl oestradiol, was included to verify test system performance. Body weights, food consumption, and clinical observations were recorded daily. Vaginal cytology was evaluated daily to assess the potential of the test substance to induce cytological changes consistent with those observed with the positive control. At necropsy, uterine weights were collected in order to assess the ability of the test substance to induce uterine growth.

No test substance-related deaths occurred, and there were no clinical observations noted during the in-life phase of the study. No test substance-related effects on body weight or nutritional parameters were observed. There were no test substance-related effects indicative of oestrogenic activity. All animals receiving the test substance remained in dioestrus for the duration of the study, and at necropsy, there were no gross observations noted or effects on uterine weight.

Although there were no test substance-related effects observed in the current studies, the doses used for this study reached a maximum-tolerated dose as determined in previous studies in adult rats. In those studies, there was decreased cholinesterase activity and clinical signs of toxicity observed at dose levels ranging from 0.75 mg/kg to 2.0 mg/kg/day. Therefore, while no test substance-related effects were observed in the current study, cholinesterase activity should be decreased at dosages of 0.25 and 0.5 mg/kg/day.

In rats administered the positive control chemical, 17α-ethynyl oestradiol, no deaths occurred, and there were no clinical observations noted during the in-life phase of the study. Mean body weight gain and mean final body weights were decreased. The body weight effects were accompanied by decreased food consumption and food efficiency. As expected, rats administered 17α-ethynyl oestradiol showed effects consistent with an oestrogen receptor agonist. Five of six rats administered 17α-ethynyl oestradiol showed effects on the stage of oestrous, and on test Day 3, five of the six rats administered 17α-ethynyl oestradiol showed cytological markers indicative of either pro-oestrus or oestrus. At necropsy, all rats showed the presence of uterine fluid within the uterus. The increased presence of fluid within the uterus was accompanied by increased uterine weights. Absolute uterine wet weight and blotted weight were increased to 308 and 239% of the negative control, respectively. Relative (to final body weight) uterine wet weight and blotted weight were increased to 331 and 256% of control, respectively. The results with 17α-ethynyl oestradiol are consistent with an oestrogen receptor agonist.

In conclusion, the test substance, oxamyl, did not induce changes on any parameters consistent with the potential to act as an oestrogen agonist in ovariectomised adult female rats. Under the conditions of this study, oxamyl did not induce oestrogenic effects in the uterotrophic assay when administered up to 0.5 mg/kg/day for 3 consecutive days.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Solid, powder
	CAS #:	23135-22-0
	Stability of test compound:	The test substance was stable throughout the exposure phase of the study; no evidence of instability was observed.
	Vehicle:	Deionised water
2.	Positive control:	17α-ethynyl oestradiol
	Lot number:	028K1411
	Purity:	99%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	57-63-6
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD(SD)
	Age at initial dosing:	Approximately 67 days old
	Weight at initial dosing:	278.3–320.3 g for females
	Source:	[redacted]
	Acclimation period:	At least 6 days
	Diet:	Harlan Teklad certified feed (2016), *ad libitum*.
	Water:	Filtered tap water, *ad libitum*
	Housing:	Animals were housed in pairs in solid bottom caging with bedding mixed with nestlets provided as enrichment.
4.	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. *In-life initiated/completed*

20-June-2011 to 23-June-2011

2. *Animal assignment and treatment*

Four groups of six animals/concentration were dosed by oral gavage with 0, 0.1, 0.25, and 0.5 mg/kg/day of the test substance for 3 consecutive days. A separate ovariectomised positive control group, administered 0.1 mg/kg/day of the oestrogen receptor agonist 17α-ethynyl oestradiol, was included to verify test system performance. Animals were assigned to dose groups based on adequate body weight gain and freedom from any clinical signs of disease or injury, and were distributed by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (NRC 1996).

Table 986 Study design: 3-Day uterotrophic assay for detecting oestrogenic activity in female rats

Group no.	No./ group	Daily dose (mg/kg/day)[a]	Dose volume (mL/kg/day)[a]	Test substance	Vehicle
1	6	0 (control)	5	None (negative control)	Deionised water
2	6	0.1	5	Oxamyl	Deionised water
3	6	0.25	5	Oxamyl	Deionised water
4	6	0.5	5	Oxamyl	Deionised water
5	6	0.1	2	17α-Ethynyl oestradiol (positive control)	Corn oil with 1% ethanol

[a] Test substance or positive control administered once daily by oral gavage on test Days 0–2.

3. *Dose preparation and analysis*

The dose volume for the groups receiving the test substance was 5 mL/kg bw/day. The test substance was dissolved in the vehicle (deionised water) and was adjusted for purity. The route of administration was oral gavage. The same volume of vehicle was given to the negative control group. The dosing solutions for the test substance were prepared daily.

The dose concentration of the positive control, 17α-ethynyl oestradiol, selected for this study, 0.1 mg/kg/day, was based on the recommend dosage specified by the test guideline. The dose volume for the group receiving the positive control, 17α-ethynyl oestradiol, was 2 mL/kg bw/day. The positive control was dissolved in ethanol prior to dilution in corn oil and was not adjusted for purity. The final concentration of ethanol in the vehicle was 1%. The route of administration was oral gavage. The dosing solution for the 17α-ethynyl oestradiol was prepared once and used within the period of established stability.

Samples of each test substance dosing preparation were collected near the beginning of the study. Analysis of the samples verified the concentration. The samples from the beginning of the study were stored at room temperature for approximately 5 hours, then analysed to establish the stability. At the time of analysis, the samples were diluted with an appropriate solvent and analysed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection.

4. *Statistics (see table 117)*

Table 997 Statistics: 3-Day uterotrophic assay for detecting oestrogenic activity in female rats

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance using final body weight as covariate followed by Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

C. METHODS

1. *Observations*

Animals were observed at least twice daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations. Rats were individually observed for acute signs of toxicity approximately 25–35 minutes post-dose based on the time of peak effect established in previous studies.

2. *Body weights*

All animals were weighed daily to the nearest 0.1 g.

3. *Food consumption and food efficiency*

The amount of food consumed by each cage of animals over test Days 0–3 was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight. Only spillage >25 grams was recorded. Cage food consumption was divided by the number of animals in the cage to calculate individual animal food consumption. From these measurements, mean daily food consumption over the interval was determined. From the food consumption and body weight data, the mean daily food efficiency was calculated.

4. *Oestrous cycle evaluation*

All rats were evaluated for vaginal cytology on test Days -5 through 3. Vaginal washes were collected using saline and evaluated using established cytological markers for evidence of conversion out of dioestrus. All rats were in dioestrus throughout the acclimation period, indicating complete ovariectomy of all animals.

5. *Sacrifice and pathology*

Approximately 24 hours after the last dose, animals were sacrificed by exsanguination under isofluorane anaesthesia. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 100. Relative organ weights (% final body weight) were calculated.

Table 1008 3-Day uterotrophic assay for detecting oestrogenic activity in female rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Tissues saved for potential microscopic evaluation
Uterus (including uterine horns and cervix)	X	X
Vagina		X
Ovarian stumps		X

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. *Clinical signs of toxicity*

There were no test substance-related clinical signs of toxicity in any treatment group, including those administered the test substance or 17α-ethynyl oestradiol (positive control).

2. *Mortality*

Test substance-related mortality did not occur during the course of this study.

B. BODY WEIGHT AND BODY WEIGHT GAIN

There were no test substance-related effects on mean body weight. Mean body weight gain was slightly increased in oxamyl treated groups at 0.25 and 0.5 mg/kg/day by 10.4% and 7.6% relative to control. These numbers were not statistically significant.

Rats administered 0.1 mg/kg/day 17α-ethynyl oestradiol (positive control) showed a statistically significant decrease in mean final body weight, which was accompanied by a statistically significant decrease in mean body weight gain over the duration of the study (test Days 0–3). Mean final body weight was

approximately 7% lower than the negative control group. Rats in the 17α-ethynyl oestradiol group lost an average of 7.6 g body weight compared to a gain of 12.2 g in the negative control group.

Table 1019 3-Day uterotrophic assay for detecting oestrogenic activity in female rats: Body weights (g)

Day	0 mg/kg bw/day	0.1 mg/kg bw/day	0.25 mg/kg bw/day	0.5 mg/kg bw/day	0.1 mg/kg bw/day oestradiol
Day 0	294.3 ± 14.6	292.7 ± 10.3	292.2 ± 9.6	293.2 ± 10.7	292.2 ± 11.0
Day 1	298.2 ± 15.9	298.0 ± 11.2	296.4 ± 9.0	298.1 ± 11.5	293.9 ± 11.8
Day 2	303.1 ± 15.2	301.8 ± 12.4	303.2 ± 9.8	303.8 ± 10.0	290.0 ± 11.4
Day 3	306.5 ± 16.4	305.5 ± 10.4	305.7 ± 10.5	306.4 ± 9.3	284.6 ± 9.4[a]

[a] Significantly different from control by the Dunnett 2-sided test criteria, p <0.05.

Table 10220 3-Day uterotrophic assay for detecting oestrogenic activity in female rats: Body weight gain (g)

Day	0 mg/kg bw/day	0.1 mg/kg bw/day	0.25 mg/kg bw/day	0.5 mg/kg bw/day	0.1 mg/kg bw/day oestradiol
Days 0–3	12.2 ± 4.2	12.8 ± 1.9	13.5 ± 3.0	13.2 ± 3.8	-7.6 ± 3.9[a]

[a] Significantly different from control by the Dunnett non-parametric 2-sided test criteria, p <0.05.

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

In the 0.25 mg/kg/day dose group for the test substance, there was a statistically significant increase in mean daily food consumption, which was approximately 7% higher than the negative control group. This increase was considered not to be test substance-related since the degree of change was small and lacked a dose response. There were no test substance-related effects on mean daily food consumption at any other dose level or on food efficiency at any dose level.

Rats administered 0.1 mg/kg/day 17α-ethynyl oestradiol (positive control) showed a statistically significant decrease in mean daily food consumption and mean daily food efficiency compared to the negative control group. Mean daily food consumption was 29% lower than the negative control group.

Table 121 3-Day uterotrophic assay for detecting oestrogenic activity in female rats: Food consumption/ food efficiency

Parameter	0 mg/kg bw/day	0.1 mg/kg bw/day	0.25 mg/kg bw/day	0.5 mg/kg bw/day	0.1 mg/kg bw/day oestradiol
Food consumption, Days 0–3 (g/animal/day)	25.8 (—)[b]	26.7 (3.3)[b]	27.6[a] (6.7%)[b]	25.5 (-1.1%)[b]	18.4[a] (-28.6)[b]
Food efficiency, Days 0–3 (avg wt gain/avg food consumed)	0.157	0.160	0.162	0.172	-0.140[a]

[a] Significantly different from control by the Dunnett non-parametric 2-sided test criteria, p <0.05.
[b] % of control.

D. OESTROUS CYCLE EVALUATION

There were no test substance-related effects on the stage of oestrous over the duration of the study. All rats in the negative control group and the groups receiving the test substance were determined to be in dioestrus during test Days 0–3. Rats administered 0.1 mg/kg/day 17α-ethynyl oestradiol (positive control) showed effects on the stage of oestrous on test Days 2 and 3. On test Day 2, two of six rats administered 17α-ethynyl oestradiol showed cytological markers indicative of pro-oestrus. On test Day 3, two rats administered 17α-ethynyl oestradiol showed cytological markers indicative of oestrus, three rats showed cytological markers of pro-oestrus, and one rat showed cytological markers indicative of dioestrus. Cytological evidence of increased conversion out of dioestrus was consistent with an oestrogen receptor agonist.

E. SACRIFICE AND PATHOLOGY

1. Organ weights and gross observations

At necropsy, there were no gross observations noted for any animal in any dose group, and no ovarian tissue was observed in the gross evaluation of the ovarian stumps for any animals in any dose group.
There were no test substance-related effects on mean absolute uterus (wet weights or blotted weights) or uterus wet or blotted weights relative to body weight.
Rats administered 0.1 mg/kg/day 17α-ethynyl oestradiol (positive control) showed a statistically significant increase in weights of mean absolute uterine weight and mean relative (to final body weight) uterine weights. Absolute uterine wet weight and blotted weight were increased to 308% and 239% of the negative control, respectively. Relative (to final body weight) uterine wet weight and blotted weight were increased to 331% and 256% of negative control, respectively. This increase in uterine weight was accompanied by the presence of fluid in the uterine lumen in all animals. The effects on uterine weight with the positive control chemical were consistent with an oestrogen receptor agonist.

Table 10322 3-Day uterotrophic assay for detecting oestrogenic activity in female rats: Mean absolute and relative organ weights

Parameter	0 mg/kg bw/day	0.1 mg/kg bw/day	0.25 mg/kg bw/day	0.5 mg/kg bw/day	0.1 mg/kg bw/day oestradiol
Absolute uterus (wet) weight (% control)	0.0986 (—)	0.0939 (95.2)	0.0995 (101)	0.0942 (95.5)	0.3033[b] (308)
Relative[a] uterus (wet) weight (% control)	0.032 (—)	0.031 (96.9)	0.033 (103)	0.031 (96.9)	0.106[c] (3.31)
Absolute uterus (blotted) weight (% control)	0.0973 (—)	0.0927 (95.3)	0.0984 (101)	0.0909 (93.4)	0.2325[d] (239)
Relative[a] uterus (blotted) weight (% control)	0.032 (—)	0.030 (93.8)	0.032 (100)	0.030 (93.8)	0.082[d] (256)

[a] Relative weight is defined as the organ to body weight ratio.
[b] Significantly different from control by the Dunnett non-parametric one sided positive criteria, p <0.002.
[c] Significantly different from control by the Dunnett non-parametric one sided positive criteria, p <0.05.
[d] Significantly different from control by the Dunnett one sided positive criteria, p <0.05.

III. CONCLUSION

The test substance, oxamyl, did not induce changes on any parameters consistent with the potential to act as an oestrogen agonist in ovariectomised adult female rats. Under the conditions of this study, oxamyl did not induce oestrogenic effects in uterotrophic assay when administered up to 0.5 mg/kg/day of the test substance for 3 consecutive days.

RMS comments and conclusion for renewal

This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/08

Reference: **CA 5.8.3/04**	**Report:**	[redacted] (2011b); Oxamyl (DPX-D1410) technical (98% w/w): 10-Day Hershberger bioassay for detecting androgenic activity **DuPont Report No.:** DuPont-32076 **Guidelines:** OPPTS 890.1400 (2009) **Deviations:** None **Testing Facility:** [redacted] **Testing Facility Report No.:** DuPont-32076 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for the potential androgenic and antiandrogenic effects when administered by oral gavage to castrated rats for 10 days. The Hershberger Bioassay employed in this study is part of Tier 1 of the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) of a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

The study was separated into two separate studies, one designed to detect androgenic (*i.e.*, androgen receptor agonist-like activity) and one to detect antiandrogenic (*i.e.*, androgen receptor antagonist-like activity). In the androgenic study, four groups of young adult castrated Crl:CD(SD) rats (six/group) were dosed by oral gavage with 0, 0.1, 0.25, or 0.5 mg/kg/day of the test substance for 10 consecutive days and sacrificed approximately 24 hours after the last administered dose. A separate castrated positive control group, administered 0.4 mg/kg/day of the androgen receptor agonist testosterone propionate (TP), was included to verify test system performance. In the antiandrogenic study, four groups of young adult castrated Crl:CD(SD) rats (six/group) were dosed by oral gavage with 0, 0.1, 0.25, or 0.5 mg/kg/day of the test substance for 10 consecutive days and sacrificed approximately 24 hours after the last administered dose. A separate castrated positive control group, administered 3 mg/kg/day of the androgen receptor antagonist flutamide (FT), was included to verify test system performance. In addition to the test substance or positive control treatment, all treatment groups in the antiandrogenic study also received a daily injection of 0.4 mg/kg of the reference androgen receptor agonist, TP.

Body weights and clinical observations were recorded daily; food consumption was recorded once over the duration of the study (test Days 9–10). At necropsy, organ weights (liver, ventral prostate, seminal vesicle [plus fluids and coagulating glands], levator ani-bulbocavernosus muscle, paired Cowper's glands, and the glans penis) were collected. The Cowper's glands and ventral prostate were saved for possible microscopic evaluation, and serum was saved for possible hormonal analysis. Microscopic evaluation and hormonal analysis were deemed unnecessary. The potential androgenic activity of the test substance was evaluated by its ability to increase weights for the androgen-dependent tissues, similar to the effects induced by the positive control, TP. The potential antiandrogenic activity of the test substance was evaluated by its ability to decrease the TP-stimulated weight increases for the androgen-dependent tissues, similar to the effects induced by the positive control, FT.

In both the androgenic and antiandrogenic studies, no test substance-related deaths occurred, and there were no clinical observations noted during the in-life phase of the study. Although not statistically significant, reductions in body weight gain were observed over the duration of the study in all oxamyl-treated groups (6.7–9.5%). Food consumption was similarly decreased in all oxamyl-treated groups in the antiandrogenic study (5–9.8%) and was statistically significant in the 0.25 mg/kg/day test group. There were no test substance-related effects

indicative of androgenic or antiandrogenic activity. At necropsy, there were no test substance-related effects on organ weights, and there were no gross observations noted.

Although there were no test substance-related effects observed in the current studies, the doses used for this study reached a maximum-tolerated dose as determined in previous studies in adult rats. In those studies, there were decreased cholinesterase activity and clinical signs of toxicity observed from 0.75 to 2.0 mg/kg/day and there was decreased cholinesterase activity in the absence of clinical signs of toxicity at 0.25 mg/kg/day. Therefore, while no test substance-related effects were observed in the current study, cholinesterase activity should be decreased at dosages of 0.25 and 0.5 mg/kg/day.

For the androgenic study in rats administered 0.4 mg/kg/day of the positive control chemical, TP, no deaths occurred, and there were no clinical observations noted during the in-life phase of the study. Mean final body weight was statistically significantly increased on test Days 9 and 10, and mean body weight gain was statistically significantly increased over the duration of the study (test Days 0 to 10). The increases in body weight and body weight gain were accompanied by an increase in mean daily food consumption over the duration of the study (test Days 0 to 10). There were no effects on food efficiency. As expected, rats administered TP showed effects consistent with an androgen receptor agonist. There were statistically significant increases in the weights for all the androgen-dependent tissues evaluated (ventral prostate, seminal vesicle [plus fluids and coagulating glands], levator ani-bulbocavernosus muscle, paired Cowper's glands, and the glans penis). There were no gross observations noted at necropsy.

For the antiandrogenic study in rats administered 3 mg/kg/day of the positive control chemical, FT administered concurrently with 0.4 mg/kg/day of the reference chemical TP, no deaths occurred, and there were no clinical observations noted during the in-life phase of the study. No effects on body weight or nutritional parameters were observed. As expected, rats administered FT showed effects consistent with an androgen receptor antagonist. There were statistically significant decreases in the TP-stimulated weights for all the androgen-dependent tissues evaluated (ventral prostate, seminal vesicle [plus fluids and coagulating glands], levator ani-bulbocavernosus muscle, paired Cowper's glands, and the glans penis). There were no gross observations noted at necropsy. Microscopic evaluation was deemed unnecessary

In conclusion, the test substance, oxamyl, did not show any effects on any parameters that were consistent with potential androgenic or antiandrogenic effects in castrated male rats. Therefore, under the conditions of this study, oxamyl did not induce androgenic or antiandrogenic activity in the Hershberger Bioassay when administered up to 0.5 mg/kg/day for 10 consecutive days.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Solid, powder
	CAS #:	23135-22-0
	Stability of test compound:	Analyses confirmed that test material was stable for at least 5 hours at room temperature followed by 66 hours refrigerated and was present in the feed at targeted concentrations. Dosing solutions were prepared daily.
	Vehicle:	Deionised water
2.	Reference androgen receptor agonist:	Testosterone propionate (TP)
	Lot number:	B0562
	Purity:	96.5%
	Source:	Steraloids, Newport, Rhode Island, USA
	CAS #:	57-85-2
	Vehicle:	Corn oil with 1% ethanol
3.	Reference androgen receptor antagonist:	Flutamide (FT)
	Lot number:	099K1112
	Purity:	>99%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	13311-84-7
	Vehicle:	0.1% Tween® 80 in 0.5% methylcellulose
3.	Test animals	
	Species:	Rat
	Strain:	Crl:CD(SD)
	Age at dosing:	Approximately 56 days old
	Weight at dosing:	241.8–282.2 g
	Source:	[redacted]
	Acclimation period:	At least 3 days
	Diet:	PMI® Nutrition International, LLC Certified Rodent LabDiet® (#5002), *ad libitum*.
	Water:	Tap water, *ad libitum*
	Housing:	Animals were housed in pairs in solid bottom caging with bedding (Shepherd's™ ALPHA-dri®) with nestlets for enrichment.
4.	Environmental conditions	
	Temperature:	18–26°C
	Humidity:	30–70%
	Air changes:	Not recorded
	Photoperiod:	Alternating 12-hour light and dark cycles

B. STUDY DESIGN AND METHODS

1. In-life initiated/completed

10-June-2011 to 23-June-2011

2. Animal assignment and treatment

In the androgenic study, four groups of young adult castrated Crl:CD(SD) rats (six/group) were dosed by oral gavage with 0, 0.1, 0.25, or 0.5 mg/kg/day of the test substance for 10 consecutive days. A separate castrated positive control group, administered 0.4 mg/kg/day of the androgen receptor agonist TP *via* subcutaneous injection, was included to verify test system performance. In the antiandrogenic study, four groups of young adult castrated Crl:CD(SD) rats (six/group) were dosed by oral gavage with 0, 0.1, 0.25, or 0.5 mg/kg/day of the test substance for 10 consecutive days. A separate castrated

positive control group, administered 3 mg/kg/day of the androgen receptor antagonist FT, was included to verify test system performance. In addition to the test substance or positive control treatment, all treatment groups also received a daily injection of 0.4 mg/kg of the reference androgen receptor agonist, TP. Animals were distributed by computerised, stratified randomisation so that there were no statistically significant differences among group body weight means. Animal housing and husbandry were in accordance with the provisions of the *Guide for the Care and Use of Laboratory Animals* (NRC 1996).

Table 123 Study design: 10-Day Hershberger Bioassay for detecting androgenic activity in male rats

Group no.	No./ group	Daily dose (mg/kg/day)[a]	Dose volume (mL/kg/day)[a]	Test substance	Vehicle
I	6	0 (control)	5	None (negative control)	Deionised water
II	6	0.1	5	Oxamyl	Deionised water
III	6	0.25	5	Oxamyl	Deionised water
IV	6	0.5	5	Oxamyl	Deionised water
V	6	0.4	0.5	Testosterone propionate (positive control)	Corn oil with 1% ethanol

[a] Administered by gavage (test substance) or subcutaneous injection (positive control) once daily on test Days 0–9.

Table 1044 Study design: 10-Day Hershberger Bioassay for detecting antiandrogenic activity in male rats

Group no.	No./ group	TP dose level (mg/kg/day)[a]	Daily dose (mg/kg/day)[b]	Dose volume (mL/kg/day)[b]	Test substance	Vehicle
VI	6	0.4	0 (control)	5	None (negative control)	Deionised water
VII	6	0.4	1	5	Oxamyl	Deionised water
VIII	6	0.4	0.25	5	Oxamyl	Deionised water
IX	6	0.4	0.5	5	Oxamyl	Deionised water
X	6	0.4	3	5	Flutamide (positive control)	Methylcellulose with 0.1% Tween® 80

[a] TP administered once daily by subcutaneous injection on test Days 0–9 in 0.1% ethanol in corn oil at a dose volume of 0.5 mL/kg.
[b] Administered by gavage (test substance and positive control) once daily on test Days 0–9.

3. Dosage solution preparation and analysis

The test substance was dissolved in deionised water. The dose solution was prepared daily. The reference androgen receptor agonist, TP, was dissolved in ethanol prior to dilution in corn oil. It was prepared once prior to study start, aliquoted, stored at room temperature and used for all daily doses. The reference androgen receptor antagonist, FT, was dissolved in 0.1% Tween® 80 in 0.5% methylcellulose (prepared in deionised water). It was prepared twice over the duration of the study, once prior to study start and once on test Day 3. Each dose solution was aliquoted, stored refrigerated, and used for all daily doses.

The stability and concentration of oxamyl in the dosing vehicle were checked by analysis using high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection from samples prepared at the beginning and end of the study. The test substance was at target concentrations ±9.5% and was stable for up to 5 hours at room temperature followed by 66 hours refrigerated. Based on this information, it can be concluded that the animals received the targeted concentrations of test substance during the study.

4. Statistics (see table 125)

Table 1055 Statistics: 10-Day Hershberger Bioassay for detecting androgenic activity in male rats

Parameter	Preliminary test	If preliminary test is not significant	If preliminary test is significant
Body weight Body weight gain Food consumption Food efficiency Organ weight	Levene's test for homogeneity and Shapiro-Wilk test for normality	One-way analysis of variance followed by Dunnett's test	Transforms of the data to achieve normality and variance homogeneity were used. The order of transforms attempted was log, square-root, and rank-order. If the log and square-root transforms failed, the rank-order was used.

C. METHODS

1. Observations

Animals were observed at least twice daily for mortality and morbidity and for signs of abnormal behaviour and appearance. On days when they were weighed, each animal was individually handled, examined for abnormal behaviour and appearance, and subjected to detailed clinical observations.

2. Body weights

All animals were weighed daily to the nearest 0.1 g.

3. Food consumption and food efficiency

Food consumption was recorded for each animal over test Days 0–10. During the test period, the amount of food consumed by each rat over test Days 0–10 was determined by weighing each feeder at the beginning and end of the interval and subtracting the final weight and the amount of spillage from the feeder during the interval from the initial weight. Only spillage >25 grams was recorded. Food efficiency was calculated from food consumption and body weight data.

4. Sacrifice and pathology

Approximately 24 hours after the last dose, animals were sacrificed by exsanguination under isofluorane anaesthesia. Gross examinations were performed on all main study animals. Organs that were weighed are listed in Table 100. Relative organ weights (% final body weight) were calculated.

Table 1066 10-Day Hershberger Bioassay for detecting androgenic activity in male rats: Organs/tissues collected for pathological examination

Organ	Organs weighed	Tissues saved for potential microscopic evaluation
Liver	X	
Levator ani-bulbocavernosus muscle	X	
Cowper's glands	X	X
Ventral prostate	X	X
Seminal vesicles plus fluids and coagulating glands	X	
Glans penis	X	

5. Hormonal evaluation

Blood was collected at the time of sacrifice from all animals. The blood was placed in a serum separator tube on ice until the serum was prepared. Serum was stored between -60 and -80°C for possible hormonal analysis. Analyses were deemed unnecessary.

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

There were no test substance-related clinical signs of toxicity in any treatment group, including those administered the test substance, TP, or FT.

2. Mortality

Test substance-related mortality did not occur during the course of this study

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B. BODY WEIGHT AND BODY WEIGHT GAIN

In the androgenic study, there were no test substance-related effects on mean body weight on any test day or on mean body weight gain over the duration of the study (test Days 0–10). However, mean body weight gains were slightly decreased in all oxamyl treated groups (6.7% to 9.5% compared to the negative control group (Table 107 and Table 108).

In the androgenic study, there were statistically significant effects on mean body weight (Table 107) and mean body weight gain (Table 108) in rats administered 0.4 mg/kg/day TP (positive control). On test Days 9 and 10, mean final body weight was increased to approximately 107% of control compared to the negative control group. The increased body weight was accompanied by a statistically significant increase in mean body weight gain over the duration of the study (test Days 0–10), with approximately a 25% increase in body weight gain compared to the negative control group.

Table 1077 10-Day Hershberger Bioassay for detecting androgenic activity in male rats: Body weight (g) – Androgenic

Day	Oxamyl 0 mg/kg bw/day[a]	Oxamyl 0.1 mg/kg bw/day[a]	Oxamyl 0.25 mg/kg bw/day[a]	Oxamyl 0.5 mg/kg bw/day[a]	TP 0.4 mg/kg bw/day[b]
Males:					
Day 0	255.0	256.8	254.0	255.6	257.6
Day 1	264.0	264.8	262.6	264.5	266.9
Day 2	273.0	272.9	268.8	273.4	275.5
Day 3	278.6	279.2	277.4	279.6	283.1
Day 4	287.5	288.2	283.7	284.8	294.5
Day 5	294.7	292.3	290.4	295.0	305.3
Day 6	301.4	301.3	296.6	301.4	313.6
Day 7	309.0	308.4	301.6	307.7	325.0
Day 8	316.7	312.6	309.3	316.1	335.2
Day 9	324.3	321.5	316.4	322.8	345.5[c]
Day 10	336.5	332.9	327.7	331.3	359.5[c]

[a] Oxamyl
[b] Reference androgen receptor agonist, TP = Testosterone propionate
[c] Significantly different from control by the Dunnett 2-sided criteria, $p < 0.05$.

Table 1088 10-Day Hershberger Bioassay for detecting androgenic activity in male rats in rats: Body weight gain (g) – Androgenic

Day	Oxamyl 0 mg/kg bw/day[a]	Oxamyl 0.1 mg/kg bw/day[a]	Oxamyl 0.25 mg/kg bw/day[a]	Oxamyl 0.5 mg/kg bw/day[a]	TP 0.4 mg/kg bw/day[b]
Males:					
Days 0–10	81.5	76.1	73.8	75.8	102.0[c]

[a] Oxamyl
[b] Reference androgen receptor agonist, TP = Testosterone propionate
[c] Significantly different from control by the Dunnett 2-sided criteria, p <0.05.

In the antiandrogenic study, there were no statistically significant effects on mean body weight on any test day in rats administered 3 mg/kg/day FT (positive control). Mean body weight gain over the duration of the study (test Days 0–10) was slightly decreased (16%) relative to the negative control group (not statistically significant).

C. FOOD CONSUMPTION AND FOOD EFFICIENCY

In the androgenic study, there were no statistically significant effects on mean daily food consumption or mean daily food efficiency over the duration of the study (test Days 0–10) in rats administered the test substance.

In the androgenic study, there was a statistically significant increase in mean daily food consumption (Table 109) over the duration of the study (test Days 0–10) in rats administered 0.4 mg/kg/day of TP (positive control). Mean daily food consumption was increased to approximately 110% of the negative control group. Mean daily food efficiency was increased 114% relative to the negative control group.

In the antiandrogenic study, there were decreases in food consumption (Table 110) in all oxamyl-treated groups (5–9.8%) over the duration of the study, and the decrease in rats administered 0.25 mg/kg/day of the test substance was statistically significant.

There were no test substance-related effects on mean food efficiency in rats administered 3 mg/kg/day FT (positive control) over the duration of the study (test Days 0–10). In the antiandrogenic study, there were no statistically significant effects on mean daily food consumption. Mean food efficiency over the duration of the study (test Days 0–10) was decreased by 14% relative to the negative control group (not statistically significant).

Table 1099 10-Day Hershberger Bioassay for detecting androgenic activity in male rats: Food consumption/ food efficiency – Androgenic

Parameter	0 mg/kg bw/day[a]	Oxamyl 0.1 mg/kg bw/day[a]	Oxamyl 0.25 mg/kg bw/day[a]	Oxamyl 0.5 mg/kg bw/day[a]	TP 0.4 mg/kg bw/day[b]
Males:					
Food consumption, Days 0–10 (g/animal/day) (% control)	25.8 (control)	25.4 (-1.6)	25.3 (-1.9)	25.9 (0.3)	28.4[c] (9.8)
Food efficiency, Days 0–10 (avg wt gain/avg food consumed)	0.316	0.300	0.291	0.292	0.360

[a] Oxamyl
[b] Reference androgen receptor agonist, TP = Testosterone propionate
[c] Significantly different from control by the Dunnett 2-sided criteria, p <0.05.

Table 11030 10-Day Hershberger Bioassay for detecting androgenic activity in male rats: Food consumption/ food efficiency – Antiandrogenic

Parameter	0 mg/kg bw/day + 0.4 mg/kg bw/day TP	Oxamyl 0.1 mg/kg bw/day+ 0.4 mg/kg bw/day TP	Oxamyl 0.25 mg/kg bw/day+ 0.4 mg/kg bw/day TP	Oxamyl 0.5 mg/kg bw/day+ 0.4 mg/kg bw/day TP	FT 3 mg/kg bw/day[b] + 0.4 mg/kg bw/day TP
Males:					
Food consumption, Days 0–10 (g/animal/day) (% control)	27.8 (control)	26.4 (-5)	25.1[c] (-9.8)	25.6 (-7.8)	27.1 (-2.5)
Food efficiency, Days 0–10 (avg wt gain/avg food consumed)	0.360	0.351	0.364	0.357	0.308

[b] Reference androgen receptor antagonist, FT = Flutamide
[c] Significantly different from control by the Dunnett 2-sided criteria, p <0.05.
TP = Testosterone propionate

D. ORGAN WEIGHTS AND GROSS OBSERVATIONS

In the androgenic study, there were no statistically significant effects on mean absolute or relative (to final body weight) organ weights (ventral prostate, seminal vesicles [plus fluids and coagulating glands], levator ani-bulbocavernosus muscle, glans penis, and Cowper's glands) in rats administered the test substance (Table 111). There were no gross observations noted.

In the androgenic study, there were statistically significant effects on the weights for all of the androgen-sensitive tissues in rats administered 0.4 mg/kg/day TP (positive control) (Table 111). Absolute and relative (to final body weight) paired weights for the Cowper's gland were increased to 565% and 520% of the negative control, respectively. Absolute and relative (to final body weight) weights for the glans penis were increased to 137% and 128% of the negative control, respectively. Absolute and relative (to final body weight) weights for the levator ani-bulbocavernosus muscle were increased to 296% and 275% of the negative control, respectively. Absolute and relative (to final body weight) weights for the seminal vesicles (plus fluids and coagulating glands) were increased to 799% and 744% of the negative control, respectively. Absolute and relative (to final body weight) weights for the ventral prostate were increased to 868% and 798% of the negative control, respectively. There were no statistically significant effects on mean absolute or relative (to final body weight) liver weight. There were no gross observations noted at necropsy.

In the antiandrogenic study, there were no statistically significant effects on mean absolute or relative (to final body weight) organ weights (ventral prostate, seminal vesicles [plus fluids and coagulating glands], levator ani-bulbocavernosus muscle, glans penis, and Cowper's glands) in rats administered the test substance (Table 112). There were no gross observations noted at necropsy.

In the antiandrogenic study, there were statistically significant effects on the weights for all of the androgen-sensitive tissues in rats administered 3 mg/kg/day of FT (positive control) (Table 112). Absolute and relative (to final body weight) paired weights for the Cowper's gland were both decreased to 41% and 42% of the negative control, relatively. Absolute and relative (to final body weight) weights for the glans penis were decreased to 75% and approximately 78% of the negative control, respectively. Absolute and relative (to final body weight) weights for the levator ani-bulbocavernosus muscle were decreased to 52% and approximately 54% of the negative control, respectively. Absolute and relative (to final body weight) weights for the seminal vesicles (plus fluids and coagulating glands) were decreased to 27% and 28% of the negative control, respectively. Absolute and relative (to final body weight) weights for the ventral prostate were decreased to 31% and 33% of the negative control, respectively. There were no statistically significant effects on mean absolute or relative (to final body weight) liver weight. There were no gross observations noted at necropsy.

Table 11131 10-Day Hershberger Bioassay for detecting androgenic activity in male rats in rats: Mean absolute and relative organ weights – Androgenic

Parameter	Oxamyl 0 mg/kg bw/day[a]	Oxamyl 0.1 mg/kg bw/day[a]	Oxamyl 0.25 mg/kg bw/day[a]	Oxamyl 0.5 mg/kg bw/day[a]	TP 0.4 mg/kg bw/day
Males:					
Absolute Cowper's glands weight (% control)	0.0066 (control)	0.0063 (-4.55)	0.0068 (3.03)	0.0055 (-16.7)	0.0373[d] (465)
Relative[c] Cowper's glands weight (% control)	0.0020 (control)	0.0019 (-5.00)	0.0021 (5.00)	0.0017 (-15.0)	0.0104[e] (420)
Absolute glans penis weight (% control)	0.0590 (control)	0.0488 (-17.3)	0.0499 (-15.4)	0.0537 (-8.98)	0.0807[f] (36.8)
Relative[c] glans penis weight (% control)	0.0176 (control)	0.0147 (-16.5)	0.0152 (-13.6)	0.0162 (-7.95)	0.0225[f] (27.8)
Absolute LABC weight (% control)	0.1913 (control)	0.1765 (-7.74)	0.1933 (1.05)	0.1833 (-4.18)	0.5653[f] (196)
Relative[c] LABC weight (% control)	0.0571 (control)	0.0532 (-6.83)	0.0590 (3.33)	0.0557 (-2.45)	0.1572[f] (175)
Absolute liver weight (% control)	14.68 (control)	14.58 (-0.68)	13.80 (-5.99)	13.74 (-6.40)	15.56 (5.99)
Relative[c] liver weight (% control)	4.361 (control)	4.381 (0.46)	4.209 (-3.49)	4.134 (-5.21)	4.330 (-0.71)
Absolute seminal vesicles weight (% control)	0.0932 (control)	0.0686 (-26.4)	0.0887 (-4.83)	0.0701 (-24.8)	0.7447[f] (699)
Relative[c] seminal vesicles weight (% control)	0.0279 (control)	0.0207 (-25.8)	0.0271 (-2.87)	0.0213 (-23.7)	0.2077[f] (644)
Absolute ventral prostate weight (% control)	0.0195 (control)	0.0131 (-32.8)	0.0155 (-20.5)	0.0133 (-31.8)	0.1693[f] (768)
Relative[c] ventral prostate weight (% control)	0.0059 (control)	0.0039 (-33.9)	0.0047 (-20.3)	0.0040 (-32.2)	0.0471[f] (698)

[a] Oxamyl
[b] Reference androgen receptor agonist, TP = Testosterone propionate
[c] Relative weight is defined as the organ to body weight ratio.
[d] Significantly different from control by the Dunnett 2-sided criteria, p <0.001.
[e] Significantly different from control by the Dunnett non-parametric 2-sided criteria, p <0.05.
[f] Significantly different from control by the Dunnett 2-sided criteria, p <0.05.

Table 11232 10-Day Hershberger Bioassay for detecting androgenic activity in male rats in rats: Mean absolute and relative organ weights – Antiandrogenic

Parameter	0 mg/kg bw/day + 0.4 mg/kg bw/day TP	Oxamyl 0.1 mg/kg bw/day+ 0.4 mg/kg bw/day TP	Oxamyl 0.25 mg/kg bw/day+ 0.4 mg/kg bw/day TP	Oxamyl 0.5 mg/kg bw/day+ 0.4 mg/kg bw/day TP	FT 3 mg/kg bw/day[b] + 0.4 mg/kg bw/day TP
Males:					
Absolute Cowper's glands weight (% control)	0.0363 (control)	0.0392 (7.99)	0.0358 (-1.38)	0.0363 (0.00)	0.0148[d] (-59.2)
Relative[c] Cowper's glands weight (% control)	0.0100 (control)	0.0110 (10.0)	0.0102 (2.00)	0.0102 (2.00)	0.0042[e] (-58.0)
Absolute glans penis weight (% control)	0.0900 (control)	0.0920 (2.22)	0.0906 (0.67)	0.0860 (-4.44)	0.0679[e] (-24.6)
Relative[c] glans penis weight (% control)	0.0248 (control)	0.0258 (4.03)	0.0258 (4.03)	0.0242 (-2.42)	0.0194[e] (-21.8)
Absolute LABC weight (% control)	0.5210 (control)	0.5517 (5.89)	0.5313 (1.98)	0.5176 (-0.65)	0.2719[e] (-47.8)
Relative[c] LABC weight (% control)	0.1440 (control)	0.1553 (7.85)	0.1512 (5.00)	0.1455 (1.04)	0.0774[e] (-46.3)
Absolute liver weight (% control)	14.69 (control)	15.18 (3.34)	14.17 (-3.54)	14.35 (-2.31)	14.95 (1.77)
Relative[c] liver weight (% control)	4.036 (control)	4.276 (5.95)	4.003 (-0.82)	4.048 (0.30)	4.280 (6.05)
Absolute seminal vesicles weight (% control)	0.7619 (control)	0.7077 (-7.11)	0.7318 (-3.95)	0.7446 (-2.27)	0.2060[e] (-73.0)
Relative[c] seminal vesicles weight (% control)	0.2116 (control)	0.1994 (-5.77)	0.2093 (-1.09)	0.2100 (-0.76)	0.0588[e] (-72.2)
Absolute ventral prostate weight (% control)	0.1657 (control)	0.1664 (0.42)	0.1794 (8.27)	0.1968 (18.8)	0.0517[e] (-68.8)
Relative[c] ventral prostate weight (% control)	0.0458 (control)	0.0465 (1.53)	0.0513 (12.0)	0.0556 (21.4)	0.0149[e] (-67.5)

[b] Reference androgen receptor antagonist, FT = Flutamide
[c] Relative weight is defined as the organ to body weight ratio.
[d] Significantly different from control by the Dunnett 2-sided criteria, p <0.001.
[e] Significantly different from control by the Dunnett 2-sided criteria, p <0.05.

III. CONCLUSION

Under the conditions of this study, oxamyl did not affect androgen sensitive organ weights consistent with potential androgenic or antiandrogenic activity in the Hershberger Bioassay when administered to castrated male rats up to 0.5 mg/kg/day for 10 days. As expected, the positive control chemicals showed effects consistent with their mode-of-action in the Hershberger Bioassay. Rats administered 0.4 mg/kg/day TP showed increased organ weights for all androgen-dependent tissues, consistent with an androgen receptor agonist. Rats administered 3 mg/kg/day FT showed effects consistent with an androgen receptor antagonist. When administered to rats receiving a TP as a reference agonist, FT administration decreased organ weights for all androgen-dependent tissues. All performance criteria were met for a valid study.

RMS comments and conclusion for renewal

This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/09

Reference: CA 5.8.3/02	**Report:**	Nabb, D.L. (2012); Oxamyl (DPX-D1410) technical (98% w/w): H295R steroidogenesis assay **DuPont Report No.:** DuPont-32077 **Guidelines:** OPPTS 890.1550 (2009) **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-32077 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its potential to interact with the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors (follicle stimulating hormone [FSHR] and lutinising hormone [LHR]) through the production of testosterone and oestradiol/oestrone using the H295R steroidogenesis assay. The steroidogenic assay is not intended to identify substances that affect steroidogenesis due to effects on the hypothalamus or pituitary gland.

This *in vitro* steroidogenesis assay using the human cell line, H295R, is part of the Tier 1 battery of the Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay is intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

In a steroidogenesis assay, H295R cells cultured *in vitro* in 24-well plates were incubated with oxamyl at concentrations of 100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 μM in triplicate for 48 hours. The test chemical's vehicle was deionised water, and its final concentration was 0.05%.

Testosterone and 17β-oestradiol levels were measured by high pressure liquid chromatography/mass spectrometry/mass spectrometry (HPLC/MS/MS). Three independent runs were performed. A Quality Control (QC) plate was included with each independent run of the test chemical to demonstrate that the assay responded properly to positive control agents at two concentration levels. Positive controls included a known inducer (forskolin) and inhibitor (prochloraz) of testosterone and 17β-oestradiol production.

Oxamyl did not cause biologically relevant changes in testosterone or 17β-oestradiol synthesis relative to the vehicle control in three independent experimental runs. A slight, yet statistically significant, decrease in testosterone levels at 1000 μM (the highest concentration tested) was accompanied by a reduction in cell viability. As expected, forskolin showed effects consistent with testosterone and 17β-oestradiol induction, and prochloraz showed effects consistent with testosterone and 17β-oestradiol inhibition. Performance criteria for a valid assay were met.

Under the conditions of this study, the test substance was judged to be negative for the induction or inhibition of steroid biosynthesis.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	White solid, powder
	CAS #:	23135-22-0
	Stability of test compound:	Results from analysis of the dosing solutions from all trials indicated that the test substance was present at acceptable concentrations in the stock and treatment solutions, and was stable under the conditions of the study.
	Solvent:	Deionised water
2.	Positive control:	Forskolin
	Lot number:	109K50571V
	Purity:	≥98%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	66575-29-9
	Solvent:	Dimethyl sulfoxide (DMSO)
3.	Positive control:	Prochloraz
	Lot number:	SZE6220X
	Purity:	99.1%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	67747-09-5
	Solvent:	Dimethyl sulfoxide (DMSO)
4.	Test system:	H295R human adrenocortical carcinoma cells
	Source:	ATCC®, Manassas, Virginia, USA

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

14-November-2011 to 09-December-2011

2. Plating and pre-incubation of cells

The H295R cells were plated in supplemented media at a density of 300000 cells/mL in a 24-well plate (1 mL of cell suspension per well) to achieve 50–60% confluency in the wells at 24 hours.

3. Exposure of cells - Test chemical

Prior to dosing, a mastermix of each concentration of test substance or positive control stock solution was prepared by adding 2 µL of the test substance stock solution to 4 mL of supplemented medium with charcoal stripped Nu-Serum. Also, a mastermix containing 2 µL of the vehicle/solvent and 4 mL of supplemented medium was prepared. This solution was used to dose the solvent control wells. The final solvent concentration in all dosing solutions was 0.05%. 22(R)- Hydroxycholesterol was added to the supplemented medium to increase basal production of 17β-oestradiol. The final concentration used was 10 µM.

At the time of dosing, the dilutions were visually observed for precipitation; no precipitation was observed.

After a 24-hour pre-incubation of plated cells, the plates were removed from the incubator and checked for attachment and morphology prior to dosing. Observations were recorded.

The cell medium was removed, dosing medium (1 mL/well of the appropriate mastermix) was added, and the plates were returned to the incubator (37°C, 5% CO_2) and incubated for 48 ± 2 hours.

At the end of the exposure period, the plates were removed from the incubator and every well checked under the microscope for cell condition and signs of cytotoxicity. No precipitation or signs of toxicity were noted.

The media from each well was removed and divided into two aliquots, transferred to microcentrifuge tubes, and either analysed immediately or stored frozen at approximately -60 to -80°C until further processing for hormone measurements.

Immediately after removing media, 300 µL of phosphate buffered saline (PBS, with Ca^{2+} and Mg^{2+}) was added to each well to prevent drying, and a live/dead cell viability test was conducted on each exposure plate.

4. *Exposure of cells - Quality control plate*

The quality control (QC) plate was incubated, dosed, and assessed in the same manner as test plates for each test run. The QC plate was dosed with a known inducer (forskolin) and inhibitor (prochloraz) of 17β-oestradiol and testosterone biosynthesis. Dosing exposure, concentrations for forskolin were 1 and 10 µM, and concentrations for prochloraz were 0.1 and 1 µM. Analyses of the production of testosterone and oestradiol by H295R cells were conducted, and performance criteria were met for each of the three independent test runs.

5. *Live/dead viability assay*

Cell viability and cytotoxicity were evaluated using the LIVE/DEAD Viability/Cytotoxicity assay immediately after removal of the culture medium. The plate wells were rinsed twice with 300 µL of phosphate buffered saline (PBS, with Ca^{2+} and Mg^{2+}). Following removal of the cell culture medium and rinse with PBS, 0.5 mL viability reagent containing the polyanionic dye calcein AM and Ethidium homodimer 1 (EthD-1) was added to each well. After a 1-hour incubation at room temperature, the plate was scanned with a fluorometer. Calcein AM is retained within live cells and produces an intense uniform green fluorescence (Ex/Em ~495/~515 nm) when it is enzymatically converted by intracellular esterase to calcein. EthD-1 enters the cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (Ex/Em ~495/~635 nm); EthD-1 is excluded by the intact plasma membrane of live cells. The measurements of calcein AM and EthD-1 for triplicate wells at each test concentration were averaged. The average calcein AM fluorescence for each sample was divided by its EthD-1 fluorescence to obtain a live/dead ratio.

6. *Hormone extraction - 17β-Oestradiol*

Frozen samples were thawed at room temperature for approximately 45 minutes prior to extraction. Samples were protected from light throughout the extraction procedure. Each sample tube containing cell culture media was vortexed for approximately 1 minute at 2300 rpm and then centrifuged at 30 × g, for approximately 20 seconds to remove any medium from the lid. Sample (500 µL) was transferred to a large glass test tube (15 mm × 125 mm, 18.75 mL capacity) and 10 µL of internal standard (10 ppb D4-oestradiol) was added to the tube. After addition of internal standard, 6 mL of methylene chloride was added. The tubes were capped and vortexed for 10 minutes at 2300 rpm. The aqueous layer (upper layer) was then removed to waste, and the methylene chloride layer was transferred to a small glass test tube (13 mm × 100 mm, 13 mL capacity). The methylene chloride was evaporated to dryness under nitrogen in a 45°C water bath for approximately 20 minutes, after which 100 µL of sodium bicarbonate buffer (100 mM, pH 10.5) was added to solubilise the oestradiol, and 100 µL dansyl chloride (1000 ppm) was added to derivatise the oestradiol. Sample tubes were vortexed by hand for 1 minute and then on a multi-tube mixer for 10 minutes at 2300 rpm and incubated at 60°C for 5 minutes. Samples were transferred to HPLC vials with limited volume glass inserts prior to analysis by LC/MS. Standards were prepared by adding 490 µL of media (90% charcoal stripped/ 10% non-stripped), 10 µL of standard stock solution (17β-oestradiol) and 10 µL of internal standard (10 ppb D4-oestradiol) to a large glass test tube following the same extraction procedure as described above. Stock standard dilutions were prepared in methanol and the final concentration range was 0.25–500 ppb. The blanks were prepared in this same manner using 100% methanol in place of the standard stock solution and did not include internal standard.

7. *Hormone extraction - Testosterone*

Frozen samples were thawed at room temperature for approximately 45 minutes prior to extraction. Each sample tube containing cell culture media was vortexed for approximately 1 minute at 2300 rpm. Acetonitrile (1.2 mL) was transferred to wells in a 96-well protein precipitation plate for all test samples and standard stock solutions. A 300-µL aliquot of test sample or stock standard solutions (testosterone) were then added to corresponding wells containing acetonitrile. Internal standard (6 µL D2Testosterone - 150 ppb) was added to all test sample and stock standard wells. The plate was vortexed for 2 minutes at 500 rpm, allowed to stand for 10 minutes, then placed in a vacuum manifold with a 96-well collection plate to collect the eluent. Vacuum was applied for approximately 7 minutes until no liquid was visible in drain spouts. The samples in the collection plate were evaporated to dryness under nitrogen at 35°C for approximately 1 hour and 20 minutes. The samples were

resuspended with 300 µL 50% acetonitrile/ 50% HPLC water. The plate was vortexed for 10 minutes at 500 rpm before analysis by LC/MS.

Standards were prepared by adding 980 µL of media (90% charcoal stripped/ 10% non-stripped) and 20 µL of standard stock solution (testosterone) to a microcentrifuge tube following the same extraction procedure as described above. Stock standard dilutions were prepared in acetonitrile, and the final concentration range was 5–1250 ppb. The blanks were prepared in this same manner using 100% acetonitrile in place of the standard stock solution and did not include internal standard.

8. Statistics

Normality was evaluated using standard probability plots or any other appropriate statistical method (*e.g.*, Shapiro-Wilk's test). If the data were not normally distributed, the data were transformed to approximate a normal distribution. If the data were normally distributed or approximately normally distributed, differences between chemical treatments and solvent controls (SCs) were analysed using a parametric test (*e.g.*, Dunnett's Test). If data were not normally distributed, an appropriate non-parametric test was used (*e.g.*, Kruskal Wallis, Steel's Many-one rank test). Differences were considered significant at $p \leq 0.05$.

9. Performance criteria

The minimum basal level production of testosterone and 17β-oestradiol should be ≥5× and ≥2.5× the minimum detection level, respectively. The minimum basal hormone production should be met in both the solvent control and blank wells. The induction level of testosterone and 17β-oestradiol for the positive control inducer (forskolin, 10 µM) should be ≥1.5× and ≥7.5× the solvent control, respectively. The inhibition level of testosterone and 17β-oestradiol for the positive control inhibitor (prochloraz, 1 µM) should both be ≤0.5× the solvent control.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Oxamyl was present at acceptable concentrations in the dosing solutions (within 10% of targeted concentrations). Oxamyl was shown to be stable in the dosing solutions under the conditions of the study. Oxamyl was not found in the 0 mg/mL samples.

B. CYTOTOXICITY

Viability was greater than 80% in all three trials at all concentrations tested and therefore, all results were included in the final interpretation.

C. TESTOSTERONE

Although a statistically significant decrease in testosterone level relative to the solvent control was observed at test-substance concentration level of 100 µM (Table 113), the test wells at that dose level contained an average of 90% viable cells. The decreased viability would account for the slightly lower testosterone produced from those wells. Due to the evidence of cytotoxicity at the highest dose level and the small magnitude of change compared to the positive controls, it was determined that the test chemical was negative for induction or inhibition of testosterone biosynthesis in this assay.

The positive control substances, forskolin and prochloraz, produced the expected induction and inhibition of testosterone synthesis, respectively. The magnitude of the increases met the assay performance criteria indicated in the test guideline.

Table 1133 Summary of testosterone results

Nominal concentration (μM)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Mean	± SD	Statistical significance
	Testosterone (ng/mL)			Fold difference					
Solvent control	1.480	1.413	1.029	1.000	1.000	1.000	1.000	0.077	
0.0001	1.497	1.363	0.982	1.011	0.965	0.954	0.977	0.087	0.9700
0.001	1.477	1.500	1.170	0.998	1.061	1.137	1.065	0.079	0.2863
0.01	1.327	1.340	0.941	0.896	0.948	0.915	0.920	0.051	0.1381
0.1	1.587	1.380	0.959	1.072	0.976	0.932	0.993	0.067	1.0000
1	1.480	1.427	1.039	1.000	1.009	1.010	1.007	0.057	1.0000
10	1.537	1.530	1.090	1.038	1.083	1.060	1.060	0.079	0.3631
100	1.203	1.237	0.826	0.813	0.875	0.803	0.830	0.050	0.0007[a]

[a] Statistically significant compared to control values by Dunnett's test $p \leq 0.05$.

D. 17β-OESTRADIOL

There were no statistically significant increases or decreases in 17β-oestradiol levels at any test substance concentration level compared to solvent control or any indication of a dose-related response (Table 114).

The positive control substances, forskolin and prochloraz, produced the expected induction and inhibition of 17β-oestradiol synthesis, respectively. The magnitude of the increases met the assay performance criteria indicated in the test guideline.

Table 1144 Summary of 17β-oestradiol results

Nominal concentration (μM)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Mean	± SD	Statistical significance
	Oestradiol (pg/mL)			Fold difference					
Solvent control	50.530	25.330	30.063	1.000	1.000	1.000	1.000	0.096	
0.0001	49.163	24.930	32.063	0.973	0.984	1.067	1.008	0.049	1.0000
0.001	48.030	26.763	30.363	0.951	1.057	1.010	1.006	0.075	1.0000
0.01	45.663	26.863	29.797	0.904	1.061	0.991	0.985	0.078	0.9998
0.1	46.230	28.030	29.897	0.915	1.107	0.994	1.005	0.106	1.0000
1	50.130	24.097	30.697	0.992	0.951	1.021	0.988	0.069	0.9999
10	48.297	27.063	30.363	0.956	1.068	1.010	1.011	0.076	1.0000
100	42.297	24.130	26.463	0.837	0.953	0.880	0.890	0.085	0.1725

E. ASSAY PERFORMANCE

The results of exposure to the positive controls, as listed in Table 115, were within the acceptable ranges as specified in the test guideline and the assay was considered valid.

Table 1155 Assay performance

Nominal concentration (μM)	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Mean	± SD
	Testosterone (ng/mL)			Fold difference				
Blank	1.767	1.560	1.067	1.150	1.047	1.041	1.079	0.074
DMSO	1.537	1.490	1.025	1.000	1.000	1.000	1.000	0.054
1 μM Forskolin	2.450	2.687	1.670	1.594	1.600	1.629	1.676	0.133
10 μM Forskolin	2.740	2.383	1.800	1.783	1.803	1.756	1.713	0.187
0.1 μM Prochloraz	1.257	1.310	0.884	0.818	0.879	0.863	0.853	0.047
1.0 μM Prochloraz	0.535	0.641	0.436	0.348	0.430	0.425	0.401	0.044
	Oestradiol (pg/mL)			Fold difference				
Blank	51.23	32.20	30.43	1.107	1.061	1.028	1.065	0.090
DMSO	46.30	34.13	29.60	1.000	1.000	1.000	1.000	0.095
1 μM Forskolin	1109	790.6	630.6	23.95	23.16	21.31	22.81	2.365
10 μM Forskolin	2367	1277	1162	51.13	37.42	39.27	42.13	6.773
0.1 μM Prochloraz	40.13	26.23	20.96	0.867	0.769	0.708	0.771	0.090
1.0 μM Prochloraz	5.880	2.123	4.527	0.099	0.062	0.229	0.105	0.096

III. CONCLUSION

An *in vitro* H295R steroidogenesis assay was performed to evaluate the potential for oxamyl to interact with the steroidogenic pathway beginning with the sequence of reactions occurring after the gonadotropin hormone receptors (follicle stimulating hormone [FSHR] and lutinizing hormone [LHR]) through the production of testosterone and estradiol biosynthesis.

Oxamyl did not cause statistically significant or biologically relevant changes in testosterone or 17β-estradiol synthesis relative to the vehicle control in three independent experimental runs. Positive control chemicals, forskolin and prochloraz, were evaluated concurrently to verify the test system performance. As expected, forskolin treatment showed effects consistent with testosterone and 17β-estradiol induction, and prochloraz treatment showed effects consistent with testosterone and 17β-estradiol inhibition. Performance criteria for a valid assay were met.

Under the conditions of this study, the test substance was judged to be negative for the induction or inhibition of steroid biosynthesis.

RMS comments and conclusion for renewal

This study is accepted as a key study

Study submitted to the EU for the first time in this submission.

B.6.8.3/10

Reference: CA 5.8.3/07	**Report:**	Snajdr, S.I. (2012c); Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* androgen receptor binding assay using rat prostate cytosol **DuPont Report No.:** DuPont-32153 **Guidelines:** OPPTS 890.1150 (2009) **Deviations:** None **Testing Facility:** DuPont Haskell Laboratory, Newark, Delaware, USA **Testing Facility Report No.:** DuPont-32153 **GLP:** Yes **Certifying Authority:** Laboratories in the USA are not certified by any government agency, but are subject to regular inspections.

Executive summary:

Pure oxamyl (PAI) was evaluated for its ability to bind to the androgen receptors in rat prostate cytosol. The *in vitro* androgen receptor binding assay using rat prostate cytosol employed in this study is part of the Tier 1 battery of the Endocrine Disruptor Screening Program (EDSP), a 2-tiered approach to implement the statutory testing requirements of FFDCA section 408(p) (21 U.S.C. 346a). This assay was intended to be used in conjunction with other guidelines in the OPPTS 890 series that comprise the full screening battery under the EDSP.

Saturation binding assays measure the affinity of a radiolabelled androgen ligand ([^{3}H]-R1881) (Kd) for the androgen receptor and the concentration of the androgen receptors (Bmax) present in the cytosol. This is determined by measuring specific binding of increasing concentrations of radioligand under conditions of equilibrium. Three independent runs were performed using hexatritiated R1881 ([^{3}H]-R1881) as the radioligand to characterise the rat prostate cytosol. The Kd was approximately 0.903 nM [^{3}H]-R1881, and the Bmax was approximately 4.5 fmol/100 µg protein which are consistent with those in the Integrated Summary Report for the androgen receptor binding assay.

Competitive binding assays measure the binding of the radioligand to the receptors with increasing concentrations of a test substance. The concentration at which the test substance displaces half of the bound radioligand is the IC_{50} (often expressed as $logIC_{50}$). Three independent runs were performed to evaluate oxamyl for its ability to compete with [^{3}H]-R1881 in binding to rat prostate androgen receptors *in vitro*. Oxamyl was evaluated at eight concentrations between 1.0×10^{-10} and 1.0×10^{-3} M. Radioinert R1881, the androgen receptor agonist reference standard, and dexamethasone, a weak androgen receptor agonist used as the positive control, were used to verify test system performance. As expected, radioinert R1881 showed effects consistent with strong competitive binding, and dexamethasone showed effects consistent with weak competitive binding to the androgen receptor in all three runs. The $logIC_{50}$ was determined to be approximately -9.64 and -4.37 for radioinert R1881 and dexamethasone, respectively. The relative binding affinity (RBA) of dexamethasone compared to radioinert R1881 was approximately 0.0022%. These values are consistent with those in the Integrated Summary Report.

Under the conditions of the study, oxamyl did not competitively bind to the androgen receptor when tested up to a maximum concentration of 1.0×10^{-3} M. Therefore, oxamyl is classified as a non-inhibitor in the androgen receptor binding assay.

I. MATERIALS AND METHODS

A. MATERIALS

1.	Test material:	Pure oxamyl (PAI)
	Lot/Batch #:	D1410-196
	Purity:	98.0%
	Description:	Powder
	CAS #:	23135-22-0
	Stability of test compound:	Results from analysis of the dosing solutions from the high, intermediate, and low concentrations indicated that the test substance was present at acceptable concentrations in the treatment solutions and was stable when stored at room temperature for approximately 3 hours.
	Solvent/ final concentration:	Deionized water, 3%
2.	Positive control:	Dexamethasone
	Lot number:	0001419230
	Purity:	98.9%
	Source:	Sigma-Aldrich, St. Louis, Missouri, USA
	CAS #:	50-02-2
	Solvent/final concentration	Ethanol, 3%
3.	Radioligand:	Hexatritiated R1881 ([^{3}H]-R1881)
	Lot number:	614814
	Purity:	>97%
	Source:	PerkinElmer, Waltham, Massachusetts, USA
	Specific activity:	85.1 Ci (3.15 TBq)/mmol
	Concentration:	1.0 mCi/mL (37 MBq/mL)
4.	Reference androgen receptor agonist standard:	Radioinert R1881
	Lot number:	590822
	Purity:	>97%
	Source:	PerkinElmer, Waltham, Massachusetts, USA
	CAS #:	965-93-5
	Solvent/ final concentration:	Ethanol/3%
5.	Test system (androgen receptor):	Prostate cytosol from male Crl:CD(SD) rats
	Source:	Charles River Laboratories, Inc., Raleigh, North Carolina, USA

B. STUDY DESIGN AND METHODS

1. Experimental start/completion

13-June-2011 to 14-July-2011

2. Rat prostate cytosol preparation

Prostates were homogenised (1 g of tissue per 10 mL cold TEDG + PMSF buffer (10 mM Tris, 1.5 mM EDTA, and 10% glycerol, 1 mM dithiothreitol, 1 mM sodium molbdate, and 1 mM phenylmethylsulfonyl fluoride, pH 7.4) and centrifuged for 30 minutes at approximately 30000 × g and 4°C. The resulting supernatants were then pooled, aliquoted, and stored frozen (approximately -60°C to -80°C) until use.

The protein concentration of the prostate cytosol was determined prior to performing the receptor assays. Samples for the standard curve were prepared using bovine serum albumin with final concentrations ranging from 0.01–0.1 mg/mL. The protein concentration of the prostate cytosol was determined to be 6 mg/mL.

3. Dose preparations and analysis for the competitive binding assay

The reference standard, R1881 (not adjusted for purity), was used to verify test system performance at six final concentrations ranging from 1.0×10^{-11} to 1.0×10^{-6} M. The positive control, dexamethasone (not adjusted for purity), was used to verify test system performance at eight final concentrations ranging from 1.0×10^{-10} to 1.0×10^{-3} M. The test substance (adjusted for purity) was evaluated at eight final concentrations ranging from 1.0×10^{-10} to 1.0×10^{-3} M.

Preparations of the initial solutions for the reference standard, positive control, and test substance were prepared by serial dilution from a stock solution, which was also the respective high initial concentration. The dilution scheme for the reference standard, positive control, and test substance followed the recommendation specified in the test guideline, with the exception two additional dilutions for the positive control and the test substance: a high concentration of 3.0×10^{-2} M and a low concentration at 3.0×10^{-9} M. No indication of precipitation of the reference standard, positive control, or test substance was observed during the assay.

Samples of the test substance dose preparations were collected near the beginning of the study and stored at room temperature until analysed. The samples were analysed to verify concentration and confirm stability. At the time of analysis, the samples were diluted with an appropriate solvent and analysed by high-performance liquid chromatography (HPLC) with a mass spectrometer detector (LC/MS). Samples of the reference standard and positive control were not evaluated for concentration or stability.

4. *Androgen receptor saturation binding assay*

The androgen receptor binding capacity of the prostate cytosol was characterised with three independent androgenic receptor saturation binding runs using [^{3}H]-R1881 as the radioligand.

[^{3}H]-R1881 was diluted in ethanol, added to the total binding, non-specific binding, and total (count for radioactivity) tubes for final concentrations of 0.25, 0.5, 0.7, 1, 1.5, 2.5, 5, and 10 nM. The total tubes were counted without further processing. Non-radiolabelled R1881 was diluted in ethanol, then added to the non-specific binding tubes for final concentrations of 25, 50, 70, 100, 150, 250, 500, and 1000 nM, respectively (*i.e.*, 100× the concentration of [^{3}H]-R1881). Triamcinolone acetonide was diluted in ethanol to 60 µM and 50 µL added to the total binding and non-specific binding tubes, which were then dried on a speedvac before addition of remaining assay constituents.

Prostate cytosol was thawed on ice, and then kept on ice prior to being used undiluted at a concentration of approximately 6 mg protein/mL, which was shown previously to bind to approximately 23% of [^{3}H]-R1881 when assayed with 0.25 nM [^{3}H]-R1881. Then 300 µL of the undiluted cytosol was added to each tube, which was vortexed and incubated at approximately 4°C with gentle mixing for approximately 20 hours. The total volume in each tube during the incubation was 300 µL. Hydroxyapatite slurry (HAP) was washed three times in 50 mM Tris buffer and resuspended to a ratio of 60% HAP and 40% 50 mM Tris buffer by volume. The bound [^{3}H]-R1881 was separated from free by adding 100 µL of each sample to 500 µL of cold, resuspended HAP, vortexing every 5 minutes for 20 minutes, centrifuging for approximately 3 minutes at 4°C and 600 × g, and then discarding the supernatant. The tubes were washed three times by adding 2.0 mL of cold (4°C) 50 mM Tris buffer, vortexing, and centrifuging at 4°C for approximately 3 minutes at 600 × g. The supernatant from each wash was discarded. The bound [^{3}H]-R1881 was extracted by adding 2 mL ethanol and vortexing at 5-minute intervals three times at room temperature. The tubes were centrifuged for 10 minutes at 600 × g, and 0.5 mL of supernatant was added to 7 mL Ultima Gold XR liquid scintillation fluid, and counted in a liquid scintillation counter (LSC) for 5 minutes.

5. *Androgen receptor competitive binding assay*

The ability of the test substance to competitively bind to androgen receptors in rat prostate cytosol with three independent runs using [^{3}H]-R1881 as the radioligand was evaluated.

The reference standard was assayed in triplicate at final target concentrations of 0.01, 0.1, 1.0, 10, 100, and 1000 nM. The positive control was assayed in triplicate at final target concentrations of 0.1, 1, 10, 100, 1000, 10000, 100000, and 1000000 nM. Test substance samples were assayed in triplicate at final target concentrations of 0.1, 1, 10, 100, 1000, 10000, 100000, and 1000000 nM of the test substance.

All tubes received 30 µL of 100 nM [^{3}H]-R1881 and 50 µL of 60 µM triamcinolone acetonide. In addition, the non-specific binding tubes (1000 nM radioinert R1881) received 30 µL 10000 nM radioinert R1881. The tubes were then dried on a speedvac before addition of remaining assay constituents.

Prostate cytosol was diluted in cold TEDG + PMSF buffer to approximately 4 mg protein/mL, which was shown previously to bind to approximately 11% of 1 nM [^{3}H]-R1881. The average estimated K_d of radioinert R1881 for the batch of prostate cytosol used in this study was 0.903 nM as determined by the saturation binding assay.

The final volume per assay tube was 310 µL. Each prepared and dried assay tube received 10 µL of either solvent or pre-diluted reference standard, positive control, or test substance and 300 µL of diluted rat prostate cytosol. After addition of all components, the tubes were incubated at approximately 4°C with gentle mixing for approximately 20 hours. HAP was washed three times in 50 mM Tris buffer and resuspended to a ratio of 60% HAP and 40% 50 mM Tris buffer by volume.

The bound [^{3}H]-R1881 was separated from free by adding 100 µL of each sample to 500 µL of cold, resuspended HAP, vortexing every 5 minutes for 20 minutes, centrifuging for approximately 3 minutes at 4°C and 600 × g, and then discarding the supernatant. The tubes were washed three times by adding 2.0 mL of cold (4°C) 50 mM Tris buffer, vortexing, and centrifuging at 4°C for approximately 3 minutes at 600 × g. The supernatant from each wash was discarded. The bound [^{3}H]-R1881 was extracted by adding 2 mL ethanol and vortexing at 5-minute intervals three times at room temperature. The tubes were centrifuged for 10 minutes at 600 × g, and 0.5 mL of supernatant was added to 7 mL Ultima Gold XR liquid scintillation fluid and counted in a LSC for 5 minutes.

6. Statistics

The dissociation constant (K_d), maximal binding capacity ($B_{max)}$, and the concentration that inhibited 50% of maximum radioligand binding ($logIC_{50}$) values were determined as appropriate using Origin 8.5.1.

The K_d and B_{max} values for the prostate cytosol were determined by fitting the saturation curves using the following equation:

$$Y = \frac{B_{max} \times X}{X + K_d} + (a \times X)$$

where Y = total binding, a = ratio between non-specific bound ligand and free ligand, and X = concentration of [^{3}H]-R1881.

The IC_{50} values were determined by fitting the competitive curves using the following equation:

$$Y = Bottom + \frac{Top - Bottom}{1+10^{(Log\ IC50-X)HillSlope+log[(Top-Bottom)/(50-Bottom)-1]}}$$

where X is the logarithm of the concentration of test substance and Y is the percent of radioligand bound to the receptor. $LogIC_{50}$ is X at Y = 50%. "Top" and "Bottom" refer to the value of Y when there is minimal binding by test chemical, and when there is maximal binding by test chemical, respectively. A concentration-response model was fitted for each test run for each curve generated.

The competitive curves were fitted by weighted least squares nonlinear regression analysis with weights equal to 1/Y when possible. In the case of negative or zero values at the bottom of a curve, no weighting was used.

7. Performance criteria

The following criteria were applied to assess the performance of the competitive binding assays. Increasing concentrations of radioinert R1881 displaced [^{3}H]-R1881 from the receptor in a manner consistent with one-site competitive binding. Ligand depletion was no greater than 15%. The parameter values (top, bottom, and slope) for the reference standard and positive control were within the tolerance bounds provided. The solvent control did not alter the sensitivity or reliability of the assay. For the test substance, ideally the top of the curve fell within 80–115% binding. If a curve started at a much lower or higher % binding, then a repeat run was considered. In addition to meeting the criteria for individual runs, consistency occurred across runs of the top plateau level, Hill slope, placement along the X-axis, and the bottom plateau (where defined).

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

Oxamyl was present at acceptable concentrations in the dosing solutions (within 8.7% of nominal concentrations). Oxamyl was shown to be stable in the dosing solutions stored at room temperature for approximately 3 hours. Oxamyl was not found in the 0 mg/mL samples.

B. ANDROGEN RECEPTOR SATURATION BINDING ASSAY

Three independent androgen receptor saturation binding runs were completed using [^{3}H]-R1881 as the radioligand and radioinert R1881 as the ligand. The K_d values for each of the three runs were 0.883, 1.015, and 0.811 nM [^{3}H]-R1881 with an average of 0.903 nM [^{3}H]-R1881. The B_{max} values for each of the three runs were 4.7, 4.6, and 4.4 fmol/100 µg protein with an average of 4.5 fmol/100 µg protein.

Confidence in the assay results was high as the adjusted coefficient of determination (adjusted R^2) was 0.865, 0.872, and 0.821 for each of the respective runs, with small variations between runs.

Table 1166 Saturation binding assay: K_d and B_{max} values for prostate cytosol

Parameter	Run S1[a]	Run S2[a]	Run S3[a]	Mean[b]	±	SE
R^2 (unweighted)	0.865	0.872	0.821	0.853	±	0.016
B_{max} (nM)	0.282	0.275	0.265	0.274	±	0.005
B_{max} (fmol/100 µg protein)	4.7	4.6	4.4	4.5	±	0.08
K_d (nM)	0.883	1.015	0.811	0.903	±	0.060
Time since specific activity certification (days)	350	354	357	—	—	—
Adjusted specific activity on date of run (Ci/mmol)	80.6	80.6	80.5	—	—	—
% NSB of TB[d]	8–23[c]	6–20	7–22[c]	—	—	—

[a] N = 3.
[b] Mean ± SE (standard error) for all three runs.
[c] Outside test guideline tolerance of 20% at the 10 nM [^{3}H]-R1881 concentration. All other values were within suggested range.
[d] NSB = non-specific binding; TB = total binding

C. ANDROGEN RECEPTOR COMPETITIVE BINDING ASSAY

Three independent androgen receptor competitive binding runs were completed using [^{3}H]-R1881 as the radioligand and radioinert R1881 as the reference standard, dexamethasone as the positive control, or the test substance.

The logIC_{50} values for radioinert R1881 for each of the three runs were -9.12, -9.01, and -9.01 logM [^{3}H]-R1881 with an average of -9.05 logM [^{3}H]-R1881. The logIC_{50} values for dexamethasone for each of the three runs were -4.34, -4.47, and -4.32 logM [^{3}H]-R1881 with an average of -4.37 logM [^{3}H]-R1881. The relative binding affinity (RBA) of dexamethasone as compared to radioinert R1881 for each of the three runs was 0.0017, 0.0028, and 0.0020% with an average of 0.0022%.

A logIC_{50} was not determined for the test substance since there were no test substance-related effects on androgen receptor binding up to the concentration of 1 mM, which represents the highest concentration.

D. ANDROGEN RECEPTOR COMPETITIVE BINDING ASSAY PERFORMANCE

The performance parameters for radioinert R1881 were within the acceptable ranges as specified in the test guideline, with the exception of the bottom of the curves (%) of -2.6 and -2.5 for Runs C1 and C2, which was slightly lower, and the slope of Run C1, which as -0.7, was slightly greater. The performance parameters for dexamethasone were within the acceptable ranges as specified in the test guideline, with the exception of the top of the curve (%) of 115 for Run C2, which was slightly above the suggested range. All runs were within or very close to the suggested performance criteria, and therefore the assay was considered valid.

Confidence in the assay results was high due to the small variation between runs. There was no observed precipitation at any of the concentrations tested. The solvent control responses indicated no drift in any of the runs.

Table 1177 Competitive binding assay: Mean ± standard error of the percent [^{3}H]-R1881 bound for each concentration

	Final conc.	Run C1[a]		Run C2[a]		Run C3[a]	
Competitor	**(M)**	**Mean**	**SE[b]**	**Mean**	**SE**	**Mean**	**SE**
SC[c] (Ethanol)	—	101.90	9.16	106.18	18.50	97.20	4.06
SC (dH_2O)	—	107.30	11.77	109.59	6.68	95.30	4.14
R1881 (NSB)	1×10^{-6}	0.00	0.53	0.00	0.14	0.00	0.26
	1×10^{-7}	1.22	0.62	-0.86	0.32	2.11	0.64
	1×10^{-8}	8.43	1.38	8.74	1.07	10.86	1.51
	1×10^{-9}	49.11	2.99	51.49	6.84	48.28	2.93
	1×10^{-10}	81.51	12.37	90.80	7.79	95.41	5.80
	1×10^{-11}	106.11	8.25	110.06	5.79	99.39	5.89
Dexamethasone	1×10^{-3}	4.52	0.72	4.53	0.68	5.23	0.06
	1×10^{-4}	31.16	3.18	30.91	4.03	31.91	2.14
	1×10^{-5}	81.67	2.89	73.75	6.97	91.51	9.19
	1×10^{-6}	99.86	7.52	113.62	2.37	89.38	3.38
	1×10^{-7}	88.74	5.64	103.72	5.13	110.78	1.86
	1×10^{-8}	111.80	9.39	116.88	5.93	117.58	5.34
	1×10^{-9}	85.70	4.65	118.64	6.89	108.65	4.99
	1×10^{-10}	103.81	11.64	119.00	13.20	105.62	11.46
Oxamyl	1×10^{-3}	106.41	11.91	109.13	8.81	83.79	2.51
	1×10^{-4}	81.47	1.82	96.30	5.93	82.54	3.40
	1×10^{-5}	88.10	5.02	107.66	12.79	94.60	4.88
	1×10^{-6}	86.13	7.13	117.02	8.99	96.49	6.56
	1×10^{-7}	106.36	4.76	97.61	3.54	95.91	5.43
	1×10^{-8}	101.72	2.17	101.55	7.34	89.99	0.15
	1×10^{-9}	93.43	3.10	83.61	10.67	97.38	6.45
	1×10^{-10}	96.83	6.36	83.50	6.51	94.71	1.44
SC (Ethanol)	—	98.10	12.27	93.82	8.38	102.80	3.93
SC (dH_2O)	—	92.70	2.76	90.41	15.28	104.70	10.27
R1881 (NSB)	1×10^{-6}	-0.59	0.55	-0.55	0.50	2.00	0.09

[a] N = 3
[b] SE = standard error
[c] SC = solvent control
[d] NSB = non-specific binding

Table 1188 Competitive binding assay: LogIC$_{50}$ values, relative binding affinity, performance standards, and assay drift

Competitor	Parameter	Lower limit[a]	Upper limit[a]	Run C1[b]	Run C2[b]	Run C3[b]	Mean[c]	±	SE
Radioinert R1881	Top (%)	82	114	111	112	102	108	±	3.4
	Bottom (%)	-2.0	+2.0	-2.6[d]	-2.5[d]	1.5	-1.2	±	1.3
	Slope	-1.2	-0.8	-0.7[d]	-0.8	-1.1	-0.8	±	0.12
	Adjusted R^{2e}	—	—	0.941	0.969	0.980	0.964	±	0.012
	$LogIC_{50}$ (M)	—	—	-9.12	-9.01	-9.01	-9.05	±	0.04
Dexamethasone	Top (%)	87	106	96	115[d]	106	106	±	5.6
	Bottom (%)	-12	+12	-4.3	-3.8	0.5	-2.5	±	1.5
	Slope	-1.4	-0.6	-1.1	-0.7	-1.0	-0.9	±	0.12
	Adjusted R^{2f}	—	—	0.966	0.977	0.975	0.973	±	0.003
	$LogIC_{50}$ (M)	—	—	-4.34	-4.47	-4.32	-4.37	±	0.05
	RBA (%)[g]	—	—	0.0017	0.0028	0.0020	0.0022	±	0.0003
Oxamyl[h]	—	—	—	—	—	—	—		
Percent ligand depletion		—	>15	0.85	0.70	0.68	—		
Percent end solvent control of initial solvent control									
	Ethanol (%)	—	—	97	89	105	—		
	dH_2O (%)	—	—	87	84	109	—		
Time since specific activity certification (days)									
		—	—	363	375	382	—		
Adjusted specific activity on date of run (Ci/mmol)									
		—	—	80.5	80.3	80.2	—		

[a] Suggested acceptable range according to test guideline.
[b] N = 3.
[c] Mean ± SE (standard error) for all three runs.
[d] Parameter was outside the performance criteria.
[e] With no weighting as bottom of the curve was below 0.
[f] With weights equal to 1/Y.
[g] RBA (relative binding affinity) relative to radioinert R1881.
[h] No competitive binding observed; therefore, no parameters could be calculated.

III. CONCLUSION

An *in vitro* androgen receptor binding assay was performed to evaluate oxamyl for its ability to compete with [^{3}H]-R1881 in binding to rat prostate androgen receptors *in vitro*. Oxamyl was evaluated at 8 concentrations ranging from 1.0 x 10^{-10} to 1.0 x 10^{-3} M

A saturation binding assay (3 independent runs) was used to characterize the rat prostate cytosol. The Kd was approximately 0.903 nM [^{3}H]-R1881 and the Bmax was approximately 4.5 fmol/100 µg protein.

Radioinert R1881, the androgen receptor agonist reference standard, and dexamethasone, the positive control, were used to verify test system performance in the competitive binding assay (3 independent runs). As expected, radioinert R1881 showed effects consistent with strong competitive binding and dexamethasone showed effects consistent with weak competitive binding to the androgen receptor in all 3 runs. The $\log IC_{50}$ was determined to be approximately -9.05 and -4.37 for radioinert R1881 and dexamethasone, respectively. The relative binding affinity of dexamethasone compared to radioinert R1881 was approximately 0.0022%.

Under the conditions of the study, oxamyl did not bind to the androgen receptor when tested up to a maximum concentration of 1.0×10^{-3} M. Therefore, oxamyl is classified as a non-inhibitor in this androgen receptor binding assay.

RMS comments and conclusion for renewal

This study is accepted as a key study

RMS comments and conclusion on oxamyl potential for endocrine activity

Oxamyl was negative for endocrine effects in the Tier 1 Endocrine Disruption Screening Program (EDSP).

On the basis of the available studies within the endocrine assessment conducted for oxamyl, it can be concluded that oxamyl does not have the potential to be active on the endocrine system.

B.6.8.4 Summary of studies on metabolites and supplementary studies on the active substance

B.6.8.4.1 Summary of studies with metabolites

Table 139 Summary of studies on metabolites

Type of study	Test substance	Test system	Concentration/ Dose range tested	Result	Reference[a]
Acute oral (ALD)	IN-A2213	Male rats	90–11000 mg/kg bw	ALD = 11000 mg/kg bw	HLR 300–68
10-Dose subacute oral	IN-A2213	Male rats	1100 and 2200 mg/kg bw/day	Mortality, histopathological changes at 2200 mg/kg bw/day; histopathological changes at 100 0mg/kg bw/day; clinical signs and body weight loss	HLR 228–71
Oral LD_{50} study	IN-L2953	Male rats	4000–7000 mg/kg bw	LD_{50} = 6675 mg/kg bw (6370–6990 mg/kg bw); clinical signs at all doses; tremors at 6000 mg/kg bw; ↓bw	HLR 126–73
Acute oral study (ALD)	IN-N0079	Male rats	90–1000 mg/kg bw	ALD = 450 mg/kg bw; abnormal posture, salivation, hyper-responsiveness to noise, weight loss at lethal doses.	HLR 585–74
10-Dose subacute oral study	IN-N0079	Male rats	90 mg/kg bw/day	↓ bw/bw gain; ↓abs. liver and kidney wt., ↓ rel. spleen and thymus wt., ↑ rel. testes wt.; reversed spleen, thymus and bone marrow atrophy; partially reversed cytoplasmic de-vacuolation of centrilobular hepatocytes	HLR 390–76
90-day oral study	IN-N0079	Rat	0, 4.0, 11.4, 34.3 mg/kg bw/day ♂; 0, 4.2, 12.6, 35.7 mg/kg bw/day ♀	Clinical signs; ↓ bw gain; haematology, clinical chemistry and urinalysis effects; ↓ bw F1 pups	HLR 630–76
Ames study	IN-N0079	(*S.typhimurium*)	250–10,000 µg/plate ±S9	No increase in revertants	HLR 284–78
Oral LD_{50} study	IN-D2708	Male rats	2500 and 5000 mg/kg bw	LD_{50} = 3540 mg/kg bw; Clinical signs including irregular respiration, abnormal posture and weakness; ↓ bw	HLR 399–72

[a] Summarised in Point CA 5.8.1 in this document.

CONCLUSIONS:

All the available studies are quite old and areconsidered as supporting studies. The % of excretion in the urine or faeces for IN-A2213 (I); IN-L2953 (II); IN-D2708 (III); IN-KP532 (IV) which were found as major metabolites also in livestock, crop and soil, water and sediments indicate that they are in the range of 27% to 46%. Therefore they are covered by the parent studies and new studies (both for toxicity and genotoxicity) were not requested.

IN-N0079 was demonstrated to be degraded and eliminated mainly as conjugates of IN-D2708 and IN-KP532, and for this reason, corroborated by the results from the supporting studies provide evidence that the toxicity is

much lower than the parent. However, **data are missing for IN-T2921, which was never detected in metabolism studies with rodents. This is identified as a data gap**.

B.6.8.4.2 SUMMARY OF ENDOCRINE STUDIES

A summary of the results of endocrine testing is given in Table .

Table 140 Summary of endocrine studies with oxamyl

Type of study	Species	Concentration range tested	Result	Reference[a]
Intact male juvenile/ peripubertal male rat	Juvenile/ peripubertal male Crl:CD(SD) rats	0.25 and 0.5 mg/kg/day	Negative	DuPont-33933
Intact female juvenile/ peripubertal male rat	Juvenile/ peripubertal female Crl:CD(SD) rats	0.25 and 0.5 mg/kg/day	Negative	DuPont-33934
Aromatase inhibition assay	Human recombinant microsomes	1.0×10^{-10} M to 1.0×10^{-3} M	Negative	DuPont-32072
Estrogen receptor transcriptional assay	hERα-HeLa-9903 cell line	$1 \times 10^{-6.8}$ M to $1 \times 10^{-3.3}$ M	Negative	DuPont-32073, Revision No. 1
ER binding assay	Estrogen receptors in rat uterine cytosol	1.0×10^{-10} to 1.0×10^{-3} M	Negative	DuPont-32074
Uterotrophic assay	Ovariectomised adult Crl:CD(SD) rats	0, 0.1, 0.25, or 0.5 mg/kg/day	Negative	DuPont-32075
Hershberger assay	Young adult castrated Crl:CD(SD) rats	0, 0.1, 0.25, or 0.5 mg/kg/day	Negative	DuPont-32076
Steroidogenesis assay	Human cell line, H295R	100, 10, 1, 0.1, 0.01, 0.001, and 0.0001 μM	Negative	DuPont-32077
AR binding assay	Rat prostate cytosol	1.0×10^{-10} to 1.0×10^{-3} M	Negative	DuPont-32153

[a] Summarised in Point CA 5.8.1 in this document.

In summary, oxamyl was not found to be endocrine active in any of the studies conducted to fulfil the Tier 1 Endocrine Disruption Screening Program. Therefore, oxamyl is concluded not to have any endocrine disrupting potential.

B.6.9 Medical data

B.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

The manufacturing sites of oxamyl technical and the representative formulations are considered confidential information and are reported in the Oxamyl EU Renewal Dossier, Document J, Part 1, DuPont-40926 EU. During the past 14 years there have been no incidents or accidents involving oxamyl that have been reported at the formulations sites of the finished product.

The risk of inhaling oxamyl is very well managed and minimised by the wearing of protective respiratory equipment. To minimise dermal exposure, protective clothing, *e.g.*, Tyvek coverall and nitrile gloves are worn.

Fume hoods have been installed in the work areas where oxamyl-containing material is loaded for the manufacturing of Oxamyl 10GR, and local air monitoring, which is carried out on a routine basis, has shown that the AEL is not exceeded. The AEL at the facility is 0.5 mg/m^3 (8-hour time weighted average) and 1.0 mg/m3 (15-minute time weighted average). To prevent exposure of plant personnel, periodic inspection of process equipment is routinely undertaken. Additional protective measures include air monitoring for dust and solvent at the workplaces with the highest potential for exposure.

As reported for the Oxamyl 10GR formulation, there are no records of accidents involving workers involved in the final packaging or handling of Oxamyl 10SL. As with Oxamyl 10GR, the appropriate personal protective equipment, including ventilation and local air monitoring procedures, are in place in work areas to prevent accidental exposure. Periodically and routinely, inspection of the process equipement is conducted.

At both manufacturing locations, there were established procedures to ensure that in the event of an accidental exposure to oxamyl, appropriate medical treatment would be given and the accident recorded.

The notifier reports that an accident occurred recently at the site where the synthesis of oxamyl is made involving a fatal exposure of some workers to a raw material used for the synthesis of carbamates. This incident is not linked directly to exposure of finished product, and investigation is ongoing to correct the mechanical and procedural problems that resulted in the incident.

B.6.9.2 Data collected on humans

No cases of accidental poisoning were identified in the literature search that was conducted in conjunction with this EU Renewal submission (see the Oxamyl EU Renewal Dossier, Document M-CA, Section 9, DuPont−40936 EU).

The literature search indicated no available reports on incidents or accidents related to the agricultural use of oxamyl.

B.6.9.3 Direct observations

Although no reports were found in the open literature to confirm the validity of extrapolating from animal to human data, a human volunteer study (DuPont report no. HLO-1998-01505) has been carried out. The results of the study are summarised under Point CA 5.8.2 in this document. In conclusion, no other adverse toxic effects were observed in humans than those described in the animal studies. Furthermore, this study confirms the most sensitive effect in humans is related to the known mode of action, acetylcholinesterase inhibition. Specifically, increased salivation and blood pressure changes were measured changes associated with low oral doses of oxamyl.

B.6.9.4 Epidemiological studies

Mammalian toxicology data available for oxamyl regarding these endpoints in the open literature were reviewed and found not to be relevant to the risk assessment in the context of this assessment. A reference for the articles reviewed can be found in Appendix 1.

Neither data related to exposure of the general public to oxamyl nor epidemiological studies are available. The open literature search conducted as described under Point CA 5.9.2 with relation to epidemiological studies did not result in the identification of any relevant publications.

B.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Poisoning due to overexposure to oxamyl is related to inhibition of cholinesterase activity.

Acute poisoning may arise very quickly, sometimes within minutes, and may encompass one or more different symptoms. Malaise, muscle weakness, dizziness, and sweating are commonly reported early symptoms. Headache, excessive salivation, nausea, vomiting, abdominal pain, and diarrhoea are often prominent.

In severe cases of poisoning, dyspnea, bronchspasm, and chest tightness may eventuate in pulmonary oedema. Blurred vision, muscle twitching, and spasms may occur. Miosis, incoordination, and slurred speech are

reported. Bradycardia occurs infrequently. Severe neurological manifestations, including convulsions and coma, are less commonly observed than with organophosphate poisoning.

Long-term overexposure of laboratory animals to oxamyl may cause non-specific effects such as weight loss and irritation, as well as signs of cholinesterase inhibition.

B.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First aid measures

If any symptom of acute poisoning occurs, the work must be stopped at once and all exertion prohibited. Contaminated clothing should be removed, and exposed skin, eyes, and hair washed. A physician should be consulted at once.

The airway should be cleared of secretion, if necessary. Artificial pulmonary ventilation, intubation, or tracheotomy may be necessary in case of severe acute poisoning. If indicated, pulmonary ventilation can be provided mechanically as long as respiratory drive is depressed. Complete rest for 24 hours at least, *e.g.*, in hospital, is recommended.

Therapeutic regimes, antidote and medical treatment

Atropine sulphate is an antidote, whereas morphine and oximes, *e.g.*, pralidoxime (2-PAM), are contra-indicated. Atropine does not reactivate the cholinesterase enzyme or accelerate excretion or breakdown of oxamyl. The objective of atropine treatment is to antagonise the effects of excessive concentrations of acetylcholine at end organs having muscarinic receptors. Atropine is ineffective against nicotinic actions, specifically muscle weakness and respiratory depression.

Depending on the severity of poisoning, doses of atropine ranging from small to very large may be required. Tissue oxygenation should be improved as much as possible before administering atropine to minimise the risk of ventricular fibrillation. Atropine sulphate can be administered intravenously or intramuscularly until atropinisation is adequate. Atropinisation is maintained by repeated doses for 2-12 hours or longer, if necessary, depending on the severity of poisoning. Rales in lung bases, miosis, nausea, bradycardia, and other cholinergic manifestations nearly always indicate inadequate atropinisation. Severely poisoned individuals may exhibit remarkable tolerance to atropine.

B.6.9.7 Expected effects of poisoning

Expected effects and duration of poisoning as a function of the type, level, and duration of exposure or ingestion

The toxic effects of acute poisoning are reversible and decline rapidly. As mentioned above, the inhibition of acetylcholinesterase leads to a specific effect in humans that is characterized by increased salivation and measureable acetylcholinesterase depression that progresses with increasing exposure concentration to headache, dizziness, chest tightness, and possible convulsions. Atropine treatment would be indicated and is expected to result in a complete reversal of these effects.

Reduced body weight gain and temporary alterations in clinical chemistry including liver enzymes and glucose levels may be observed with chronic low level exposure.

Tests in laboratory animals demonstrate no serious health effects of long term exposure. Oxamyl is neither a carcinogen nor reproductive or developmental toxin in animals, nor is it a genotoxin in *in vitro* tests.

Expected effects and duration of poisoning as a function of varying time periods between exposure or ingestion and commencement of treatment

Sublethal effects of exposure to carbamate pesticides are rapidly reversible and resolves within a few hours, typically 2–4 based on the reversibility data available in mammals.

B.6.10 Summary of mammalian toxicology and overall evaluation

Toxicokinetics

In vitro and *in vivo* rat metabolism studies have been conducted with ^{14}C-oxamyl and the plant metabolites, ^{14}C –IN-N0079, and/or ^{14}C -IN-A2213 glucoside.

Two rat metabolism studies are available to address the absorption, distribution, excretion, and metabolism of ^{14}C -oxamyl in rats. The most recent study (summarised in B.6.1.1/02 and accepted as a key study), evaluated oxamyl metabolism after administration of a single oral dose (1 mg/kg bw) in five male and five female rats. The single oral dose was approximately 30–40% of the LD_{50} in male and female rats (3.1 and 2.5 mg/kg bw, respectively, see below B6.2.1.). Oxamyl is acutely toxic by the oral route, and the studied dose is considered the highest oral dose that can be administered without causing significant toxicity or death. The majority (>90%) of the dose was excreted in the urine of both genders within 168 hours of dosing; approximately 80% was eliminated within 0-24 hours indicating a rapid and nearly quantitative oral absorption of the test substance. Faecal excretion was 3% and 2% for males and females, respectively; approximately 1% of the dose was retained in the carcass (after removal of the tissues) expired air was a very minor elimination route at ≤0.5%, and tissue residues were low (0.03 to 0.09 mg/kg). There were no significant differences in tissue concentrations between males and females. The highest concentration of radioactivity was found in whole blood (approximately 0.1 µg equivalents/g) and in heart, liver, kidney, lungs, spleen, and the gastro-intestinal tract (approximately 0.04 to 0.09 µg equivalents/g). Bile cannulated rats were not used in the study, but the low % of faecal excretion indicates that the information is not crucial to determine the oral absortion value. Therefore the oral absorption value is >90% (summing up the radioactivity found in the urine, tissues and carcass).

Although a single dose was tested, it is reasonable that a lower dose would be similarly absorbed and eliminated. The metabolism of rats dosed with ^{14}C-IN-A2213 (a less toxic hydrolysis product and principal oxamyl metabolite) was also studied The excretion patterns after single oral doses of 14C-IN-A2213 at 1 mg/kg bw and 100 mg/kg bw were similar to that recorded after Oxamyl administration at 1 mg/kg bw. In addition sufficient metabolites for identification were obtained, thus establishing the metabolic pathway for oxamyl in rats (Fig. 1). The major urinary metabolite in Oxamyl and IN-A2213-dosed rats was the beta-glucuronide of the oxime (IN-A2213). Minor unidentified metabolites were considered to be conjugates of demethylated compounds (e.g., IN-L2953 or IN-D2708). The major route of Oxamyl biotransformation was hydrolysis to IN-A2213 followed by conjugation

The other available study (summarised in B.6.1.1/01**),** is dated and was conducted prior to the adoption of any metabolism test guidelines was adopted. It has some limitations, therefore was accepted as supporting study. It evaluated the metabolism of oxamyl in two male rats (one for each treatment regimen) after oral administration (2.5–4.4 mg/kg bw) of ^{14}C-oxamyl following preconditioning with repeated doses of oxamyl (50 and 150 mg/kg diet corresponding approximately to 2.5–7.5 mg/kg bw daily. The higher dose is expected to give rise to some toxic effects, based on the acute toxicity of oxamyl, therefore value obtained at that dose are not fully reliable. The patterns of absorption, distribution, excretion, and metabolism were consistent with the single dose study. The majority (around 70%) of the dose was eliminated within 72 hours of dosing (most in the urine), and low levels of radioactivity were distributed throughout the body. ^{14}C-IN-N0079 and ^{14}C-IN-A2213 glucoside were also studied in *in vivo* (oral administration to preconditioned rats) experiments. In addition, the metabolism of ^{14}C-oxamyl, ^{14}C-IN-N0079, and ^{14}C-IN-A2213 glucoside (main oxamyl plant metabolites) were examined in *in vitro* (rat liver microsomes) experiments. The results of the *in vitro* experiments were consistent with the whole animal studies and further substantiated the metabolic pathway in rats.

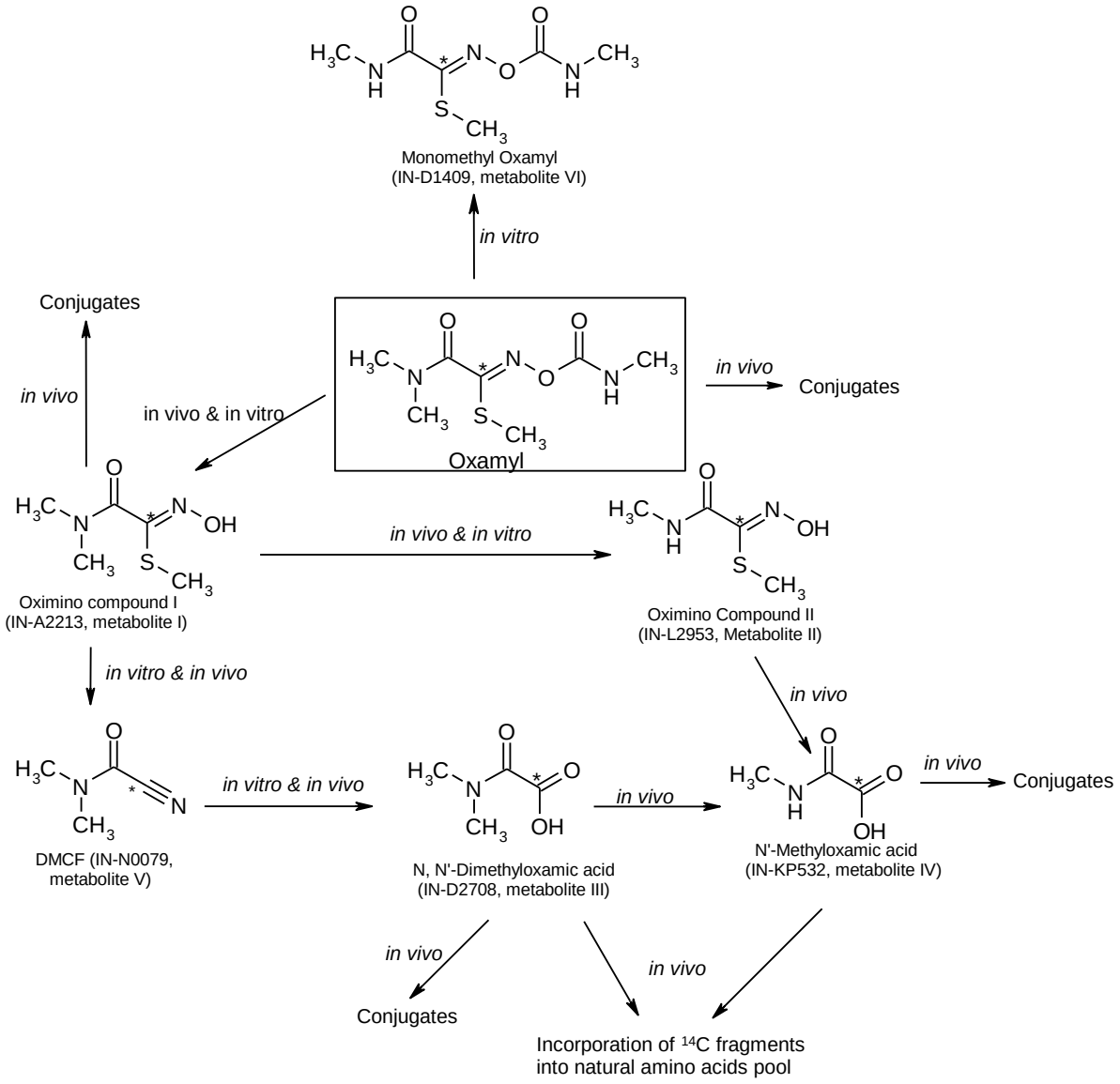
The combination of these studies adequately addresses the regulatory requirements for oxamyl metabolism in rats. Additional studies conducted with higher and/or lower single oral doses of oxamyl would provide no additional information on the absorption, distribution, excretion, and metabolism of ^{14}C -oxamyl in rats.

Further, according to regulation (EU) No 283/2013 comparative *in vitro* metabolism studies are required. For oxamyl, metabolic differences among species have been assessed: (O/ME 33 [summarised in Point CA 5.1.1]; AMR 1226-88 [summarised in Point CA 5.1.1]; Chang and Knowles, 1979 [Arch. Environm. Contam. Toxicol. 8: 499–508]).

In the Chang and Knowles study 1979 [Arch. Environm. Contam. Toxicol. 8: 499–508]), *in vitro* and *in vivo* assessments were reported. In the *in vivo* portion of the study, twenty-nine mice were injected intraperitoneally with a saline solution of [^{14}C]-oxamyl corresponding to a dose of 1.16 mg/kg bw. Mice were sacrificed 96 hours post dosing. Approximately 89% of the dose was eliminated in the urine in 96 hours. Unconjugated urine metabolites included IN-A2213, IN-D2708, IN-N0079, IN-L2953, IN-KP532, and IN-D1409. Minor amounts of conjugated (glucuronide or sulphate) IN-A2213, IN-L2953, IN-D2708, and IN-KP532 were also present. In an *in vitro* experiment, incubation of oxamyl with mouse liver subcellular fractions gave IN-N0079, IN-D2708, IN-KP532, IN-A2213, and IN-D1409.

The most sensitive endpoint in all species is acute neurotoxicity observed due to inhibition of acetylcholinesterase. This effect is rapidly reversible and oxamyl is rapidly hydrolysed. The metabolic pathway of oxamyl in rats (and mice) is consistent with the pathways seen in plants and livestock. Oxamyl is hydrolysed rapidly to IN-A2213, an oxime, then a glucuronide is formed either early or late in the pathway. There are no significant differences in the rate of the initial conversion of oxamyl to the oxime metabolite, which is the key event in the detoxification process. Moreover, based on a scientifically sound and ethically conducted volunteer study (HLO−1998-01505, summarised in Point CA 5.7.1), it can be concluded that the metabolism of oxamyl by humans is also rapid.

Figure 2 Proposed metabolic pathway of oxamyl and its metabolites *in vitro* and *in vivo* in rats



The dermal penetration of oxamyl (250 g oxamyl/L aqueous formulation) has been measured *in vitro* through human and rabbit skin mounted in a static diffusion cell model, in two recent GLP studies carried out according to OECD TG n°428. Dermal absorption of pure oxamyl (PAI) at 18 hours post-exposure, defined as the percent of applied dose detected in the receptor fluid (0.597 ±0.379 %) and stripped skin, (0.456 ± 0.282), was 1.7%, and 2.7% in human and rabbit skin, respectively, calculated by summing up the SD to the mean value (when >25% of the mean value) according to the EFSA guidance for dermal absorption (EFSA Journal 2012;10(4):2665).

Acute toxicity

High purity oxamyl (~97–98%) has been evaluated in acute oral, inhalation, and dermal toxicity studies. The results indicate that oxamyl has high acute oral and inhalation toxicity, but relatively low acute dermal toxicity. High purity oxamyl was not irritating to either the skin or eyes of rabbits. Toxicity precluded testing of pure oxamyl for skin sensitisation in guinea pigs by both the Maximisation and Buehler methods when the test substance was administered at doses recommended by the test guidelines. However, negative results were obtained in a study with oxamyl technical (42%) , which represented the approximate maximum concentration that could be evaluated without significant clinical signs of toxicity or mortality. In addition, oxamyl was determined not to have the potential to be phototoxic based on the mouse 3T3 assay.

Table 141 Summary of acute toxicity studies with oxamyl

Type of study	Purity	Species	Result	Reference[a]
Acute oral LD_{50}	98.1%	Rat	LD_{50} 2.5 mg/kg bw (female)	DuPont-26931
Acute dermal LD_{50}	97.1%	Rabbit	LD_{50} 5027 mg/kg bw (male) LD_{50} >5000 mg/kg bw (female)	HLR 114-88
Acute inhalation LC_{50} (4 hr)	98.1%	Rat	LC_{50} 56 mg/m^3 (equivalent to 0.056 mg/L)	DuPont-6331
Acute skin irritation	98.1%	Rabbit	Not irritating	DuPont-7060
Acute eye irritation	98.2%	Rabbit	Not irritating	DuPont-7059
Skin sensitisation	96.9%	Guinea pig	Study discontinued due to toxicity	DuPont-3021
Skin sensitisation	42%[b]	Guinea pig	Not a sensitiser	HLR 179-88
Phototoxicity	99.1%	Mouse fibroblast cell line, Balb/3T3, clone A31	Not phototoxic	DuPont-42100

[a] Summarised in Point CA 5.2 in this document.
[b] Oxamyl technical 42% (42 a.s. in cyclohexanone and water)

Subchronic toxicity

It has to be underlined that the standard of these available studies varied considerably. Some of them were dated: they were not conducted in compliance with GLP principles and deviated substantially from the recommendations of current EU /OECD guidelines (deviations are listed above). They were therefore considered as supporting studies or not used at al for the evalutation.

In a 13-week rat study, decreased body weight gain; decreased kidney, heart, thymus, spleen, liver, and lung weights; and increased stomach weights were noted in males and/or females at the mid- and/or high-dose level. At the high dose, clinical signs of toxicity (fasciculations, ruffled fur, mild diarrhoea, bulging eyes, and lacrimation), decreased food consumption, and an increased incidence of protein and blood in the urine were observed. In a 13-week dog study, affected by a number of limitations in the study design, when compared with the current reuest of the guidelines for this kind of studies, no treatment-related effects were noted at any dose level up to the highest dose tested of 150 ppm. It was therefore not considered valid in the present evaluation However, since 1-years dog studies are available it was considered not appropriate to ask for the re-conduct of the study, also considering that the mechanism of action of carbamates (cholinesterase inhibition) is well understood and the most relevant effect is the acute neurotoxic effect (e.g. there are no significant variation depending on the duration of the study) which is rapidly reversible and similar across species. The conduct of an additional subchronic study in the dog, beside being not in line with the EU animal welfare policy, would not contribute to the understanding of the hazards or add relevant information to the risk assessment.

The results of one oral 12-month dog study showed significant and biologically relevant dose-dependent effects on body weight, body weight gain, food consumption and food efficiency, and clinical signs of toxicity related

to cholinesterase inhibition in males and/or females at the mid- and high-dose levels (150 and 250 ppm, respectively). Body weight effects and plasma and brain cholinesterase inhibition were also observed in males at the lowest dose tested (50 ppm; equivalent to 1.56 mg/kg bw/day): being consistent with the mechanism of action of the a.s. and being part of the dose-dependentce of the effects they were considered relevant and therefor a NOAEL could not be derived. For this reason, a second oral 12-month study was specifically designed to establish a NOEL in male dogs, by using 4 doses (plus the control) ≤ 50 ppm. No treatment-related findings, including cholinesterase inhibition were noted even at the highest dose tested (50 ppm , equivalent to 1.36 mg/kg bw/day). Based on the results of these two studies, since at 50 ppm controversial results were obtained (significant quite relevant brain cholinesterase inhibition was observed in the first study when male dogs consumed a diet of 50 ppm at 1.56 mg/kg bw/day, whereas no toxicity was observed in the second study when male dogs consumed a similar diet at 1.36 mg/kg bw/day) it is consider appropriate to set **an overall subchronic oral NOAEL** at 35 ppm, corresponding to 0.930 mg/kg bw/day, rounded to **1 mg/kg bw/day**.

In a 21-day dermal toxicity study in rabbits, plasma, RBC, and/or brain cholinesterase activities were decreased at the mid- and high-doses (nominal value 50 and 250 mg/kg bw/day, respectively). Mild hyperglycemia and an accumulation of an eosinophilic material in the duodenal submucosa were also noted at the high dose. However a high incidence of mortality was observed in the high dose group, and the quantification of the treatment dose was questionable: the actual treatment was considered to be almost 2-fold higher than the nominal one. In addition, the test sites were wrapped with an impervious (plastic film) wrap, which would have enhanced test substance absorption. For this reason it was not considered valid in the present evaluation. A second 21-day study was conducted to more precisely define the NOEL in rabbits by the dermal route of exposure, in which the test sites were wrapped with a porous, semi−occlusive (gauze) wrap as requested by the test guideline. Although the treatment has a shorter duration with respect to the requested 28 days, the mechanism of action of carbamates (cholinesterase inhibition) is well understood and the most relevant effect is the acute neurotoxic effect (e.g. there are no significant variation depending on the duration of the study) which is rapidly reversible and similar across species. The conduct of an additional subchronic dermal toxicity study in the rabbit or in the rat, beside being not in line with the EU animal welfare policy, would not contribute to the understanding of the hazards or add relevant information to the risk assessment. In this study, plasma, RBC, and brain cholinesterase activities were decreased in females at the high dose of 75 mg/kg bw/day. No treatment-related decreases in plasma, RBC, and brain cholinesterase activities were noted in females at dose levels of 50 mg/kg bw/day and below and in males at any dose level (up to 75 mg/kg bw/day). In the second study, no treatment-related effects were noted in males and females at **50 mg/kg bw/day**, which is therefore the **subchronic dermal NOAEL**.

Chronic toxicity and carcinogenicity

Oxamyl did not exhibit evidence of cumulative toxicity in chronic toxicity studies in rats, mice, or dogs. In one chronic toxicity/oncogenicity study in rats, decreased body weights, body weight gains, and food efficiency were noted in males and females at the mid- and high-doses (100 and 150 ppm), together with an increased incidence of several clinical signs of toxicity were recorded in one or both sexes at 100 and 150 ppm including hyperreactivity, swollen legs or paws, sore skin and alopecia. No pathological findings were recorded that corroborated these signs. In addition, plasma cholinesterase activity was inhibited in males at several sampling intervals and in females at one month at the mid- and high-doses, not associated with other treatment-related toxicity. No effects on rat brain or erythrocyte acetylcholinesterase activities were recorded. In another long-term rat study, decreased body weight was noted in males and females at the mid- and high-doses. Blood cholinesterase activity was decreased in males and females at the high-dose level. Oxamyl was not oncogenic in rats in long-term feeding studies. The incidence of adrenal tumours (pheochromocytoma) and pituitary tumours (chromophobe adenoma) in male rats in the long-term study, was not significantly increased in the treated groups compared to controls nor was a dose-response trend evident.

In a mouse oncogenicity study, significant reductions in body weight in 50 ppm males and females and 75 ppm females were recorded associated with decreased food consumption, increased RBC count, and decreased corpuscular haemoglobin concentration levels were observed at the high-dose level. The combined incidences of lymphoma and pulmonary and hepatocellular adenoma / adenocarcinoma did not show a dose-related increase except in females where the incidence of pulmonary adenoma / adenocarcinoma was increased at the low and high doses compared to concurrent controls. However, as no dose- response relationship was evident and the incidence was less than in historical controls, they were considered unrelated to treatment.

Based on the results of chronic feeding studies in rats and mice, oxamyl is not a carcinogen, and the lowest NOAEL of approximately 1.97 mg/kg/day is based on lower body weights and body weight gain as well as plasma cholinesterase inhibition observed with dietary administration of 4.19 mg/kg bw/day and higher in rats.

It is also evident that the duration of the study does not affect the reference value and that differences among species are limited.

Oxamyl showed no evidence of oncogenicity in long-term rat and mouse studies. The overall weight of the evidence suggests that oxamyl does not present a concern for carcinogenicity.

Genetic toxicity

The mutagenic and DNA damaging potential of oxamyl was studied in several *in vitro* test systems using bacterial and mammalian cells. Oxamyl did not show any evidence of gene mutations, chromosome aberrations, or DNA damage and repair. Additionally, an *in vivo* test for chromosome damage in mice produced negative results. Based on these data, oxamyl does not pose a mutagenic or genotoxic concern.

Reproductive and developmental toxicity

A two-generation rat reproduction study conducted with oxamyl did not reveal evidence of reproductive toxicity. Parental toxicity consisted of decreased body weight, body weight gain, and food consumption in males and females of both generations at the mid- and high-dose levels (75 and 150 ppm, respectively). A statistically significant increase in the incidence of alopecia was noted in F1 females at the mid- and high-dose during the premating and gestation periods, and an increased incidence of hyperactivity was noted in F1 males and females (statistically significant in males) at the high dose. The NOEL for parental toxicity was 25 ppm (equivalent to 1.43 mg/kg bw/day). No treatment-related reproductive effects were noted; however, pup toxicity (decreased viability and decreased body weight) occurred in the 150 ppm group at the same dose levels causing parental toxicity. A statistically significant decrease in the mean number of pups (males and females combined) born per litter, born alive per litter and alive on day 4 preculling in the F_1 and F_2 generations was also recorded at 150 ppm. A significant treatment-related reduction in F_1 and F_2 male and female pup mean body weights also occurred at ≥ 75 ppm throughout most of the lactation period. Adverse foetal effects were seen at dose levels (≥ 75 ppm) which also caused significant effects on parental weight gain giving a developmental NOEL of 25 ppm (equivalent to 1.43 mg/kg bw/day).

A three-generation rat reproductive study with oxamyl was conducted as part of a chronic toxicity study, which due to some limitations can only be considered as a supporting study. No treatment-related reproductive effects were noted. Parental toxicity consisted of decreased body weight in males and females at the mid- and high-dose levels of 100 and 150 ppm, respectively, and decreased food consumption and blood cholinesterase activity in males and females at the high dose. Slightly decreased weanling weight was observed at 100 and 150 ppm but was reversible when pups were transferred to control diet. The offspring NOEL was 50 ppm (2.5 mg/kg bw/day) and was based on the reduction in pup body weight at ≥100 ppm. The reproductive NOEL was 50 ppm (2.5 mg/kg bw/day) based on a possibly treatment-related reduction in litter size at ≥100 ppm, higher than the value derived from the key study, described above.

The potential to cause developmental toxicity was investigated in rat and rabbit developmental studies. Oxamyl showed no evidence of developmental toxicity in the rat and rabbit developmental studies. In the rabbit developmental study, maternal toxicity consisted of decreased body weight gain during the treatment period at the mid- and high-dose levels (2 and 4 mg/kg bw/day, respectively). Slightly decreased foetal viability was noted at the high dose, but this finding was not statistically significant.

In the rat developmental study in which oxamyl was administered as a bolus dose by gavage, maternal toxicity consisted of transient tremors and decreased body weight changes and food consumption at the two highest dose levels (0.8 and 1.5 mg/kg bw/day, respectively). Slight but statistically significant decreases in foetal body weight were noted at dose levels of ≥0.5 mg/kg bw/day; the slightly reduced foetal body weight for the 0.5 mg/kg bw/day group was within the laboratory's historical control range, and corresponds to a equally slight but not statistically significant decrease body weight in the dams. It is considered that the appropriate foetal NOAEL for this study is 0.5 mg/kg bw/day, which is the same as the NOAEL for maternal toxicity, also in view of results obtained in another a rat developmental study (only supporting) in which oxamyl was administered by diet. Significantly less maternal toxicity was observed, again primarily consisting of effects on body weight and nutritional parameters at 100, 150, and 300 ppm (8.2, 11.6, and 20.5 mg/kg bw/day, respectively). No effects were observed on the reproductive outcome or in the foetuses. The NOEL in this study is 50 ppm (4.5 mg/kg bw/day), approximately 10-fold greater than the NOEL obtained in the rat developmental study when oxamyl was administered by gavage.

Oxamyl is therefore, not considered to be uniquely toxic to the conceptus and is not considered to have any unique toxicity to the reproductive system.

Neurotoxicity

Oxamyl was negative for delayed neurotoxic effects in a study conducted in hens.

A single oral dose study in human volunteers was conducted with oxamyl. In this study, a statistically significant and biologically relevant increase in saliva secretion was noted at the highest dose level of 0.15 mg/kg bw one hour following dose administration. In addition, statistically significant and biologically relevant decreases in plasma and RBC cholinesterase activities were observed at 0.15 mg/kg bw with the period of maximum depression occurring at 45 and 60 minutes for plasma cholinesterase activity and from 30 to 60 minutes for RBC cholinesterase activity. Plasma and RBC cholinesterase activities returned to baseline values within 4 and 3 hours, respectively, following administration of oxamyl. No adverse treatment-related effects were noted on ECG, vital signs, haematology and clinical chemistry parameters (including plasma and RBC cholinesterase), urinalysis, or clinical signs at dose levels of **0.09 mg/kg bw (NOAEL in this study for acute neurotoxicity in human after oral administration)** and lower.

Acute and subchronic neurotoxicity studies in rats were conducted with oxamyl. In the acute neurotoxicity study, treatment-related clinical signs related to cholinesterase inhibition were noted in male and females at the mid- and high-dose levels (1 and 2 mg/kg bw for males and 0.75 and 1.5 mg/kg bw for females, respectively). Decreased body weight gain was noted in males at the mid- and high-dose and in females at the high dose. Plasma, RBC, and brain cholinesterase inhibition was noted in males and females at the mid- and high-dose levels on Day 1. No treatment-related neuropathological findings were observed. The acute neurotoxicity NOAEL in rats was derived as **0.1 mg/kg bw, indicating no significant species difference when compared to the NOAEL obtained in humans.**

In the subchronic neurotoxicity rat study, treatment-related clinical signs of toxicity related to cholinesterase inhibition were noted in males and females at ≥100 ppm. Decreased body weight, body weight gain, food consumption, and food efficiency were also noted at doses ≥100 ppm. Plasma, RBC, and brain cholinesterase inhibition was noted in males and females at 250 ppm. No treatment-related neuropathological findings were noted. The subchronic neurotoxicity NOAEL in rats was **derived as 1.6 mg/kg bw, indicating that the most relevant effect is associated with acute exposure with no cumulative effects regarding neurotoxicity.**

A reversibility study was conducted with oxamyl. The objective of this study was to determine the length of time needed for recovery from inhibition of cholinesterase activity following an acute oral exposure to oxamyl at a concentration of 1.0 mg/kg bw. Clinical signs (predominantly tremors) were noted, and plasma, RBC, and brain cholinesterase activities were decreased within 30 minutes of dosing with **recovery occurring within 2 hours post dosing**. In a subacute study in which rats were administered oxamyl at a concentration of 2.4 mg/kg bw/day five times per week for two weeks, mild fasciculations, slight pallor, salivation, and body weight loss were noted.

A 4-hour inhalation study was conducted in which marginal effects on RBC and brain cholinesterase activities were induced at 0.0049 mg/L, the lowest concentration evaluated.

Finally, a **comparison of the sensitivity to acetylcholinesterase inhibition in adult rats and pre-weanling rats** was conducted for oxamyl. For RBC cholinesterase inhibition (the more affected parameter), effects were essentially the same in preweanling pups and adults based on results at the dose in common (0.15 mg/kg) and linear trends across the range of doses tested. For brain cholinesterase (the less affected parameter), preweanling pups appeared to be slightly more sensitive than adults based on results at the common dose. At lower doses (0.075, 0.1, and 0.125 mg/kg), however, **the range of responses for brain cholinesterase activity in pups tended to overlap with the linear trend line in dose response for adults**. Therefore, the sensitivity of the pre-weanling rats was not enhanced compared to the adult rats.

In conclusion, acute acetylcholinesterase inhibition is the most sensitive effect, but is rapidly reversible as it has been observed across species that metabolism is quite rapid and complete within a few hours. Moreover, a bolus acute dose represents the most serious and relevant dosing regimen for development of a risk assessment benchmark due to the fact that in rodent studies, small amounts of the test material are consumed over a 24 hr period and therefore, metabolism and recover occurs during dosing. The point of departure for the risk assessment should be the acute neurotoxicity study conducted in the rat by gavage where the NOAEL was 0.1 mg/kg.

Summary of studies with metabolites

The principal metabolites of oxamyl found in plants, soil, water, and/or sediment are IN-A2213, IN-N0079, IN–D2708, IN-T2921, and IN-L2953. These metabolites, with the exception of IN-T2921, have been observed

in their free or conjugated forms in metabolism studies performed in rats and mice (AMR 1226-88; O/ME 33, summarised in Point CA 5.1.1). IN-T2921 was not detected in either the rat or the mouse; however, this metabolite is a proposed intermediate in the formation of IN-D2708 from IN-N0079 and was identified in goat rumen fluid (, DuPont Report O/ME 38 [AMR 09-80], in the Oxamyl EU Renewal Dossier, Document M-CA, Section 6, DuPont-40933 EU).

IN-A2213 was investigated in an acute oral study in which the approximate lethal dose (acute) was the maximum dose tested, 11000 mg/kg bw. Mortality was also recorded at 2200 mg/kg bw/day in a subacute study as well as histopathological changes. These were reversed at the lower dose of 1000 mg/kg bw/day. Clinical signs of toxicity and body weight loss were recorded at both doses levels.

The acute oral LD_{50} for **IN-L2953** was 6675 mg/kg bw with clinical signs of toxicity including tremors and body weight loss recorded at lower doses.

The toxicity of **IN-N0079** was investigated in acute, 10-dose and 90-day oral studies. The approximate lethal dose (acute) was 450 mg/kg bw and was associated with clinical signs of toxicity such as abnormal posture, salivation, hyper-responsiveness to noise and body weight loss. The latter finding was also recorded in the subacute and subchronic studies. Other findings in these studies include organ weight perturbations, reversible spleen, thymus and bone marrow atrophy, and cytoplasmic de-vacuolation of centrilobular hepatocytes (subacute) and clinical signs of toxicity, decreased body weight gain, haematology, clinical chemistry and urinalysis effects, and decreased F1 pup body weight (subchronic). **IN-N0079** was not mutagenic when investigated using the Ames test with and without metabolic activation.

The acute oral LD_{50} for **IN-D2708** was 3540 mg/kg bw with clinical signs of toxicity including irregular respiration, abnormal posture and weakness, and body weight loss recorded at lower doses.

The principal metabolites of oxamyl found in plants, soil, water, and/or sediment are IN-A2213, IN-N0079, IN-D2708, IN-L2953, and on IN-T2921. The % of excretion in the urine or faeces for IN-A2213 (I); IN-L2953 (II); IN-D2708 (III); IN-KP532 (IV) indicate that they are in the range of 27% to 46%. Therefore they are covered by the parent studies and new studies (both for toxicity and genotoxicity) were not requested.The available studies although have only been accepted as supporting are sufficient to indicate that they are less toxic than the parent**. However, data are missing for IN-T2921, which was never detected in metabolism studies with rodents. This is identified as a data gap**.

Summary of endocrine studies with oxamyl

Oxamyl was not found to be endocrine active in any of the studies conducted to fulfil the Tier 1 Endocrine Disruption Screening Program. Therefore, oxamyl is concluded not to have any endocrine disrupting potential.

Acceptable Daily Intake (ADI), Acute Reference Dose (ARfD), and Acceptable Operator Exposure Level (AOEL)

Oxamyl-induced inhibition of cholinesterase activity occurs shortly after exposure and is readily reversible. In the subchronic and chronic toxicity studies in rodents and in dogs there was a delay of at least 1 week between test substance administration and cholinesterase evaluation. It is possible that, in these studies, cholinergic effects were manifested after the first administration of Oxamyl and would explain together with the administration regimen (bolus dose vs dietary) why the NOAEL derived from the acute neurotoxicity study is lower (**NOAEL= 0.1 mg/kg bw).** In that acute neurotoxicity study in rats, plasma, erythrocyte and brain (all regions) cholinesterase inhibition on day 1 were recorded in males and females at 1 mg/kg bw (in the same range in which subchronic and chronic NOAEL were set). This finding was repeated in the acute reversibility gavage study in rats where inhibition of plasma, erythrocyte and brain cholinesterase activity, accompanied by clinical signs of cholinesterase inhibition, was recorded at 1 mg/kg bw, 30 minutes after dosing. Although the route of administration was by gavage, the magnitude of the response and the scope of the effects observed (brain and erythrocyte acetylcholinesterase as well as plasma cholinesterase) suggest that, at the very least, plasma cholinesterase could be perturbed after the initial Oxamyl administration in the subchronic and chronic

studies. In the acute neurotoxicity study in rats, a decrease in plasma, erythrocyte and brain cholinesterase activity was recorded at 0.75 mg/kg bw (LOAEL). No intermediate doses were investigated.

On this basis, using the NOAEL from the acute neurotoxicity study in rats as PoD and an assessment factor of 100 (a 10 for interspecies differences and a 10 for intraspecies differences) was applied (EFSA, 2005), the EU Commission has established the **ADI, AOEL, and ARfD for oxamyl at 0.001 mg/kg bw/day**.

The values set by the WHO for JMPR (using the NOAEL=0.09 mg/kg bw derived from the human study as the PoD, to which an assessment factor of 10 was used for intraspecies differences) differ from those set by the EU since EU policy was to not accept for ethical reason data from studies in humans as the basis of setting health-based guidance values for plant protection products. There is new guidance that indicates human data should at least be considered in the development of assessment factors for the derivation of reference values (COMMISSION REGULATION [EU] No 283/2013). However, too few subjects were enrolled in the McFarlane (1999) neurotoxicity study in humans (5/doses, 10 placebos), in a very limited range of age (19-39 y old) to allow to use the data as the PoD, and the study can be used only as supporting.

On the other hand, studies summarised in this RAR indicate that acute acetylcholinesterase inhibition is the most sensitive effect, but is rapidly reversible as it has been observed across species that metabolism is quite rapid and complete within a few hours. Moreover, a bolus acute dose represents the most serious and relevant dosing regimen for development of a risk assessment benchmark due to the fact that in rodent studies, small amounts of the test material are consumed over a 24 hr period and therefore, metabolism and recover occurs during dosing. It is also evident that the duration of the study does not affect the reference value and that differences among species are limited.

Indeed, the most appropriate PoD for the risk assessment is the acute neurotoxicity study conducted in the rat by gavage where the NOAEL was 0.1 mg/kg. However, due to the limited species differences (corroborated also by human data in which a NOAEL is determined as low as the one detected in the rat), it can be considered the possibility to lower the assessment factor waiving the interspecies difference contribution.

Concluding, interspecies assessment factors help increase the confidence in safety assessment by providing a means to account for uncertainties. The most sensitive endpoint in all species is acute neurotoxicity observed due to inhibition of acetylcholinesterase. Therefore, an assessment factor of 10× is sufficient for this risk assessment due to the fact that human data exist and corroborate the most sensitive endpoint in the rat.

Endpoint Basis: rat acute neurotox 0.1 mg/kg bw (current)

Inter/intra species assessment factor 10×

ARfD/ADI/AOEL = 0.01 mg/kg bw

Table 142 Species comparison of lowest NOAELs in toxicity studies conducted with oxamyl

Acute oral toxicity—bolus dose (capsule or gavage)	Human: 0.09 mg/kg bw based on cholinesterase inhibition (HLO-1998-01505[a])	Rat: NOEL 0.1 mg/kg bw based on cholinesterase inhibition (HLR 1118-96[a])
Subchronic oral toxicity—diet	Rat: 1.69 mg/kg bw/day based on cholinesterase inhibition (HL-1998-00708[a])	Dog: 1.36 mg/kg bw/day based on cholinesterase inhibition (HLO 555-90[b])
Developmental toxicity—gavage	Rat: 0.5 mg/kg bw/day based on decreased maternal and foetal weights and maternal signs of toxicity (HLR 473-88[c])	Rabbit: 1 mg/kg bw/day based on decreased maternal weight (HLO 801-80[c])

[a] Summarised in Point CA 5.7.1 in this document.
[b] Summarised in Point CA 5.3.2 in this document.
[c] Summarised in Point CA 5.6.2 in this document.

Parametric value for drinking water

The WHO approach to the Maximum Allowable Concentration (MAC) in water is to allocate one tenth of the ADI to water, and this allocation is converted to a concentration in water (*i.e.*, MAC) as follows:

$$\frac{\text{ADI} \times \text{body weight} \times \text{P}}{2\text{L}}$$

Where:
Body weight = body weight (60 kg) for adults
P = fraction of the ADI allocated to drinking water (*i.e.*, 1/10)
C = daily consumption of water (2 litres)

$$\frac{0.01\ \text{mg} \times 60 \times 0.1}{2\text{L}}$$

Therefore the MAC oxamyl of 0.03 mg/L in drinking water is proposed.

However, in the EU the limit for drinking water is fixed at 0.1 µg/L for all the active substances.

B.6.11 References relied on

List of information, tests and studies which are considered as relied upon by the RMS for the evaluation with a view to the approval of the active substance.

Studies marked in yellow are submitted for the first time.

Sorted by Annex Point

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.1.1/01	Han, J.C.-Y, Harvey, J.	1977	Metabolism of oxamyl and selected metabolites in the rat DuPont Experimental Station O/ME 33 J. Agric. Food Chem. 26:902-910 1978 Previously submitted at the EU level for Annex I inclusion Published: Yes	Y	N		Authors
B.6.1.1/02	[redacted]	1990	Biokinetics and metabolism of ^{14}C-oxamyl in rats [redacted] AMR 1226-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.1.2/01	Mingoia, R.T.	2014a	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in human skin DuPont Haskell Laboratory DuPont-39017 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.1.2/02	Mingoia, R.T.	2014b	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in rabbit skin DuPont Haskell Laboratory DuPont-39524 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.2.1/01	[redacted]	2009	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral toxicity study in rats - up-and-down procedure [redacted] DuPont-26931 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.2.2/01	[redacted]	1988	Acute dermal toxicity study of IN D1410-196 in rabbits [redacted] HLR 114-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.3/01	[redacted]	2001	Oxamyl (DPX-D1410) Technical (98% w/w): inhalation median lethal concentration (LC_{50}) study in rats [redacted] DuPont-6331 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.6/01	[redacted]	1999	Oxamyl Technical: Evaluation of the potential dermal sensitization in the guinea pig (Magnusson-Kligman maximization and Buehler tests) [redacted] DuPont-3021 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.2.6/02	[redacted]	1988	Closed-patch repeated insult dermal sensitization (Buehler method) with IN D1410-304 in guinea pigs [redacted] HLR 179-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.7/01	Markell, L.K.	2015	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* 3T3 NRU phototoxicity test DuPont Haskell Laboratory DuPont-42100 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.3.2/01	[redacted]	1969	Ninety-day feeding study in rats with 1–(dimethylcarbamoyl)-N-(methylcarbamoyloxy)thioformimidic acid, methyl ester (IND-1410) [redacted] HLR 308-69 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.3.2/02	[redacted]	1990	Chronic toxicity study with oxamyl (IN D1410-196) one-year feeding study in dogs (2 volumes) [redacted] HLR 381-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.3.2/03	[redacted]	[redacted]	52-week dietary toxicity study with IND-1410 (oxamyl) in male dogs [redacted] HLO 555-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.3.3/01	[redacted]	1999	Oxamyl technical: 21-day repeated dose dermal toxicity study in rabbits [redacted] DuPont-1599 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.4.1/01	Gladnick, N.L.	1999	Oxamyl technical: Bacterial reverse mutation test in *Salmonella typhimurium* and *Escherichia coli* DuPont Haskell Laboratory DuPont-3084 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.4.1/02	Gudi, R., Schadly, E.H.	2000	Oxamyl technical: *In vitro* mammalian chromosome aberration test BioReliance DuPont-2936 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.4.1/03	San, R.H., Clarke, J.J.	2000	Oxamyl technical: *In vitro* mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay BioReliance DuPont-2937 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.4.1/04	Vincent, D.R.	1987	Assessment of IN D1410-196 in the *in vitro* unscheduled DNA synthesis assay in rat primary hepatocytes (revision 1) DuPont Haskell Laboratory HLR 719-82, Revision No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.4.2/01	[redacted]	2002	Oxamyl (DPX-D1410) technical (98% w/w): Mouse bone marrow micronucleus assay [redacted] DuPont-10618 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.5.1/01	[redacted]	1991	Combined chronic toxicity/oncogenicity study with oxamyl (IN D1410-196) long-term feeding study in rats [redacted] HLR 278-91 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.5.2/01	[redacted]	1981	Long term feeding study in mice with oxamyl [redacted] HLO 252-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.5.2/02	[redacted]	1990	Long term feeding study in mice with oxamyl [redacted] HLO 252-81, Amendment No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.6.1/01	[redacted]	1990	Reproductive and fertility effects with oxamyl (IN D1410) multi-generation reproduction study in rats [redacted] HLR 423-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.6.2/01	[redacted]	1988	Teratogenicity study of IN D1410-196 in the rat [redacted] HLR 473-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.6.2/02	Munley, S.M.	1998	DuPont's position on foetal weight changes in rats following developmental toxicity testing with oxamyl DuPont Haskell Laboratory DuPont-1954 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.6.2/03	[redacted]	1980	Teratology study in rabbits - Oxamyl [redacted] HLO 801-80 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/01	[redacted]	1997	Acute oral neurotoxicity study of oxamyl technical in rats [redacted] HLR 1118-96 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.1/02	[redacted]	1998	Oxamyl technical: subchronic oral neurotoxicity study in rats. [redacted] HL-1998-00708 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/03	[redacted]	1999	A randomised double blind ascending oral dose study with oxamyl [redacted] HLO-1998-01505 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/04	[redacted]	1997	Reversibility study with carbamate insecticides in rats [redacted] HL-1997-00641 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/05	[redacted]	2001	Cholinesterase inhibition determination in rats exposed to inhalation atmospheres of oxamyl technical (96.9%) [redacted] DuPont-4383, Revision No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.1/06	[redacted]	2005	Oxamyl (DPX-D1410) technical (98% w/w): Relative sensitivity of preweanling rat pups and adult rats to inhibition and recovery of cholinesterase activity [redacted] DuPont-16755 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.7.1/07	Moser, V.C., McDaniel, K.L., Phillips, P.M., Lowitt, A.B.	2010	Time-course, dose-response, and age comparative sensitivity of N-methyl carbamates in rats Toxicological Sciences 114(1), 113-123 (2010) Not applicable GLP: No Published: Yes	Y	N		Authors
B.6.7.1/08	McDaniel, K.L., Padilla, S., Marshall, R.S., Phillips, P.M., Podhorniak, L., Qian, Y., Moser, V.C.	2007	Comparison of acute neurobehavioural and cholinesterase inhibitory effects of N-methyl carbamates in rats Toxicological Sciences 98(2), 552-560 (2007) Not applicable GLP: No Published: Yes	Y	N		Authors
B.6.7.1/09	Padilla, S., Marshall, R.S., Hunter, D.L., Lowit, A.	2007	Time course of cholinesterase inhibition in adult rats treated acutely with carboryl, carbofuran, formetanate, methomyl, methiocarb, oxamyl or propoxur Toxicology and Appliced Pharmacology 219, 202-209 (2007) Not applicable GLP: No Published: Yes	Y	N		Authors

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.2/01	[redacted]	1970	Oral ALD and delayed paralysis test (white leghorn chickens) [redacted] HLR 234-70 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/01	[redacted]	1968	Acute oral test (CHR-CD rats) [redacted] HLR 300-68 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/02	[redacted]	1971	Ten-dose subacute oral tests [redacted] HLR 228-71 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/03	[redacted]	1973	Oral LD_{50} Test [redacted] HLR 126-73 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/04	[redacted]	1974	Acute oral test - ALD [redacted] HLR 585-74 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.1/05	[redacted]	1976	10 day subacute test [redacted] HLR 390-76 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/06	[redacted]	1976	Ninety day feeding study in rats with 1 cyano-N,N-dimethylformamide (INN-79) metabolite of Vydate [redacted] HLR 630-76 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/07	Sippel, M.E.	1978	Mutagenic activity of formamide, 1-cyano-N,N-dimethyl- in the salmonella/microsome assay DuPont Haskell Laboratory HLR 284-78 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.8.1/08	[redacted]	1972	Oral LD_{50} test [redacted] HLR 399-72 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.2/01	[redacted]	1968	Ten dose subacute oral test [redacted] HLR 150-68 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.2/02	Green, J.W.	2003	Statistical analysis of HLO-1998-01505: Assessment of study design and power DuPont Haskell Laboratory DuPont-12251 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.8.3/01	Bentley, K.S.	2010	Oxamyl: Additional information in support of comparative cholinesterase study DuPont–16755 (MRID 46615301) - pilot data DuPont Haskell Laboratory DuPont-30415 GLP: No Published: No	N	N		DuPont
B.6.8.3/02	[redacted]	2012a	Pubertal development and thyroid function in intact juvenile/peripubertal male rats following oral administration of oxamyl (DPX-D1410) technical (98% w/w) [redacted] DuPont-33933 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.3/03	[redacted]	2012b	Pubertal development and thyroid function in intact juvenile/peripubertal female rats following oral administration of oxamyl (DPX–D1410) technical (98% w/w) [redacted] DuPont-33934 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/04	Snajdr, S.I.	2012a	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* aromatase inhibition using human recombinant microsomes DuPont Haskell Laboratory DuPont-32072 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/05	Willoughby, J.A.	2012	Oxamyl (DPX-D1410) technical: Estrogen receptor transcriptional activation (human cell line (HeLa-9903)) CeeTox, Inc. DuPont-32073, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/06	Snajdr, S.I.	2012b	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* estrogen receptor binding assay using rat uterine cytosol (ER-RUC) DuPont Haskell Laboratory DuPont-32074 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.3/07	[redacted]	2011a	Oxamyl (DPX-D1410) technical (98% w/w/): 3-Day uterotrophic assay for detecting estrogenic activity [redacted] DuPont-32075 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/08	[redacted]	2011b	Oxamyl (DPX-D1410) technical (98% w/w): 10-Day Hershberger bioassay for detecting androgenic activity [redacted] DuPont-32076 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/09	Nabb, D.L.	2012	Oxamyl (DPX-D1410) technical (98% w/w): H295R steroidogenesis assay DuPont Haskell Laboratory DuPont-32077 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/10	Snajdr, S.I.	2012c	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* androgen receptor binding assay using rat prostate cytosol DuPont-32153 GLP: Yes Published: No	Y	N	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Sorted by Author

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.5.2/01	[redacted]	1981	Long term feeding study in mice with oxamyl [redacted] HLO 252-81 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/04	[redacted]	1974	Acute oral test - ALD [redacted] HLR 585-74 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/08	[redacted]	1972	Oral LD_{50} test [redacted] HLR 399-72 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.3/01	Bentley, K.S.	2010	Oxamyl: Additional information in support of comparative cholinesterase study DuPont–16755 (MRID 46615301) - pilot data DuPont Haskell Laboratory DuPont-30415 GLP: No Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.2.2/01	[redacted]	1988	Acute dermal toxicity study of IN D1410-196 in rabbits [redacted] HLR 114-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.6/02	[redacted]	1988	Closed-patch repeated insult dermal sensitization (Buehler method) with IN D1410-304 in guinea pigs [redacted] HLR 179-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.1/01	[redacted]	2009	Oxamyl (DPX-D1410) technical (98% w/w): Acute oral toxicity study in rats - up-and-down procedure [redacted] DuPont-26931 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.1/03	[redacted]	1973	Oral LD_{50} Test [redacted] HLR 126-73 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.1/05	[redacted]	1976	10 day subacute test [redacted] HLR 390-76 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.3.2/03	[redacted]	1991	52-week dietary toxicity study with IND-1410 (oxamyl) in male dogs [redacted] HLO 555-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.4.2/01	[redacted]	2002	Oxamyl (DPX-D1410) technical (98% w/w): Mouse bone marrow micronucleus assay [redacted] DuPont-10618 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.1/01	[redacted]	1968	Acute oral test (CHR-CD rats) [redacted] HLR 300-68 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.2/01	[redacted]	1968	Ten dose subacute oral test [redacted] HLR 150-68 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.4.1/01	Gladnick, N.L.	1999	Oxamyl technical: Bacterial reverse mutation test in *Salmonella typhimurium* and *Escherichia coli* DuPont Haskell Laboratory DuPont-3084 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.8.2/02	Green, J.W.	2003	Statistical analysis of HLO-1998-01505: Assessment of study design and power DuPont Haskell Laboratory DuPont-12251 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.4.1/02	Gudi, R., Schadly, E.H.	2000	Oxamyl technical: *In vitro* mammalian chromosome aberration test BioReliance DuPont-2936 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.1.1/01	Han, J.C.-Y, Harvey, J.	1977	Metabolism of oxamyl and selected metabolites in the rat DuPont Experimental Station O/ME 33 J. Agric. Food Chem. 26:902-910 1978 Previously submitted at the EU level for Annex I inclusion Published: Yes	Y	N		Authors
B.6.1.1/02	[redacted]	1990	Biokinetics and metabolism of ^{14}C-oxamyl in rats [redacted] AMR 1226-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.6.2/03	[redacted]	1980	Teratology study in rabbits - Oxamyl [redacted] HLO 801-80 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.6.1/01	[redacted]	1990	Reproductive and fertility effects with oxamyl (IN D1410) multi-generation reproduction study in rats [redacted] HLR 423-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.1/06	[redacted]	1976	Ninety day feeding study in rats with 1 cyano-N,N-dimethylformamide (INN-79) metabolite of Vydate [redacted] HLR 630-76 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.3/01	[redacted]	2001	Oxamyl (DPX-D1410) Technical (98% w/w): inhalation median lethal concentration (LC_{50}) study in rats [redacted] DuPont-6331 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.2.6/01	[redacted]	1999	Oxamyl Technical: Evaluation of the potential dermal sensitization in the guinea pig (Magnusson-Kligman maximization and Buehler tests) [redacted] DuPont-3021 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.2/01	[redacted]	1970	Oral ALD and delayed paralysis test (white leghorn chickens) [redacted] HLR 234-70 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.3.3/01	[redacted]	1999	Oxamyl technical: 21-day repeated dose dermal toxicity study in rabbits [redacted] DuPont-1599 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.5.1/01	[redacted]	1991	Combined chronic toxicity/oncogenicity study with oxamyl (IN D1410-196) long-term feeding study in rats [redacted] HLR 278-91 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/01	[redacted]	1997	Acute oral neurotoxicity study of oxamyl technical in rats [redacted] HLR 1118-96 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.1/02	[redacted]	1998	Oxamyl technical: subchronic oral neurotoxicity study in rats. [redacted] HL-1998-00708 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/04	[redacted]	1997	Reversibility study with carbamate insecticides in rats [redacted] HL-1997-00641 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/06	[redacted]	2005	Oxamyl (DPX-D1410) technical (98% w/w): Relative sensitivity of preweanling rat pups and adult rats to inhibition and recovery of cholinesterase activity [redacted] DuPont-16755 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.2.7/01	Markell, L.K.	2015	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* 3T3 NRU phototoxicity test DuPont Haskell Laboratory DuPont-42100 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.1/08	McDaniel, K.L., Padilla, S., Marshall, R.S., Phillips, P.M., Podhorniak, L., Qian, Y., Moser, V.C.	2007	Comparison of acute neurobehavioural and cholinesterase inhibitory effects of N-methyl carbamates in rats Toxicological Sciences 98(2), 552-560 (2007) Not applicable GLP: No Published: Yes	Y	N		Authors
B.6.7.1/03	[redacted]	1999	A randomised double blind ascending oral dose study with oxamyl [redacted] HLO-1998-01505 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.3.2/02	[redacted]	1990	Chronic toxicity study with oxamyl (IN D1410-196) one-year feeding study in dogs (2 volumes) [redacted] HLR 381-90 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.1.2/01	Mingoia, R.T.	2014a	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in human skin DuPont Haskell Laboratory DuPont-39017 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.1.2/02	Mingoia, R.T.	2014b	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* percutaneous absorption of oxamyl in rabbit skin DuPont Haskell Laboratory DuPont-39524 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.7.1/07	Moser, V.C., McDaniel, K.L., Phillips, P.M., Lowitt, A.B.	2010	Time-course, dose-response, and age comparative sensitivity of N-methyl carbamates in rats Toxicological Sciences 114(1), 113-123 (2010) Not applicable GLP: No Published: Yes	Y	N		Authors
B.6.6.2/02	Munley, S.M.	1998	DuPont's position on foetal weight changes in rats following developmental toxicity testing with oxamyl DuPont Haskell Laboratory DuPont-1954 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.3/09	Nabb, D.L.	2012	Oxamyl (DPX-D1410) technical (98% w/w): H295R steroidogenesis assay DuPont Haskell Laboratory DuPont-32077 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.7.1/05	[redacted]	2001	Cholinesterase inhibition determination in rats exposed to inhalation atmospheres of oxamyl technical (96.9%) [redacted] DuPont-4383, Revision No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.7.1/09	Padilla, S., Marshall, R.S., Hunter, D.L., Lowit, A.	2007	Time course of cholinesterase inhibition in adult rats treated acutely with carboryl, carbofuran, formetanate, methomyl, methiocarb, oxamyl or propoxur Toxicology and Appliced Pharmacology 219, 202-209 (2007) Not applicable GLP: No Published: Yes	Y	N		Authors
B.6.6.2/01	[redacted]	1988	Teratogenicity study of IN D1410-196 in the rat [redacted] HLR 473-88 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.4.1/03	San, R.H., Clarke, J.J.	2000	Oxamyl technical: *In vitro* mammalian cell gene mutation (CHO/HGPRT) test with an independent repeat assay BioReliance DuPont-2937 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.8.1/07	Sippel, M.E.	1978	Mutagenic activity of formamide, 1-cyano-N,N-dimethyl- in the salmonella/microsome assay DuPont Haskell Laboratory HLR 284-78 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont
B.6.8.3/04	Snajdr, S.I.	2012a	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* aromatase inhibition using human recombinant microsomes DuPont Haskell Laboratory DuPont-32072 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/06	Snajdr, S.I.	2012b	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* estrogen receptor binding assay using rat uterine cytosol (ER-RUC) DuPont Haskell Laboratory DuPont-32074 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.3/07	[redacted]	2011a	Oxamyl (DPX-D1410) technical (98% w/w/): 3-Day uterotrophic assay for detecting estrogenic activity [redacted] DuPont-32075 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/08	[redacted]	2011b	Oxamyl (DPX-D1410) technical (98% w/w): 10-Day Hershberger bioassay for detecting androgenic activity [redacted] DuPont-32076 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/10	Snajdr, S.I.	2012c	Oxamyl (DPX-D1410) technical (98% w/w): *In vitro* androgen receptor binding assay using rat prostate cytosol DuPont-32153 GLP: Yes Published: No	Y	N	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.3.2/01	[redacted]	1969	Ninety-day feeding study in rats with 1–(dimethylcarbamoyl)-N-(methylcarbamoyloxy)thioformimidic acid, methyl ester (IND-1410) [redacted] HLR 308-69 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.5.2/02	[redacted]	1990	Long term feeding study in mice with oxamyl [redacted] HLO 252-81, Amendment No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.3/02	[redacted]	2012a	Pubertal development and thyroid function in intact juvenile/peripubertal male rats following oral administration of oxamyl (DPX-D1410) technical (98% w/w) [redacted] DuPont-33933 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.8.3/03	[redacted]	2012b	Pubertal development and thyroid function in intact juvenile/peripubertal female rats following oral administration of oxamyl (DPX–D1410) technical (98% w/w) [redacted] DuPont-33934 GLP: Yes Published: No	Y	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont
B.6.4.1/04	Vincent, D.R.	1987	Assessment of IN D1410-196 in the *in vitro* unscheduled DNA synthesis assay in rat primary hepatocytes (revision 1) DuPont Haskell Laboratory HLR 719-82, Revision No. 1 Previously submitted at the EU level for Annex I inclusion Published: No	N	N		DuPont

Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	Vertebrate study Y/N	Data Protection Y/N	Justification if data protection is claimed	Owner
B.6.8.1/02	[redacted]	1971	Ten-dose subacute oral tests [redacted] HLR 228-71 Previously submitted at the EU level for Annex I inclusion Published: No	Y	N		DuPont
B.6.8.3/05	Willoughby, J.A.	2012	Oxamyl (DPX-D1410) technical: Estrogen receptor transcriptional activation (human cell line (HeLa-9903)) CeeTox, Inc. DuPont-32073, Revision No. 1 GLP: Yes Published: No	N	Y	The study is necessary for the regulatory decision, conducted according to GLP and has not previously been protected or submitted	DuPont

APPENDIX 1
SUMMARIES OF PUBLISHED LITERATURE DETERMINED TO BE RELEVANT OR POTENTIALLY RELEVANT TO THE OXAMYL RENEWAL SUBMISSION

The studies in this appendix are relevant to the oxamyl submission; however, they are not relevant to the risk assessment. Therefore, they are considered supplemental.

CA 5.1.2

Title: Estimation of tissue: blood partition coefficients of carbamates and organophosphorous (OP) pesticides using relative lipid content and Log P as the mechanistic determinants

Authors: Knaak, James B.; Chang, Daniel; Dary, Curtis C.; Tornero-Velez, Rogelio; Okino, Miles; Power, Fred

Source: Abstracts of Papers, 230th ACS National Meeting, Washington, DC, United States, Aug. 28-Sept. 1, 2005, pp. AGRO-132

Summary:

PBPK/PD models were developed for carbamate and OP pesticides to aid in human risk assessments. Tissue:plasma (Pt:p) partition coeffs. (PCs) are important pesticide specific input parameters. The exptl. determination of Pt:p requires steady-state conditions and may be confounded by metabolism in plasma. Hence there has been considerable interest in predicting Pt:p based on tissue composition and measures of lipophilicity, such as the n-octanol:water PC (Ko:w) or the oil:water PC (Kov:w). We employed the tissue-composition based algorithm of Haddad (2000) and Ko:w or Kov:w to estimate adipose tissue:blood PC (PCat:b) for 10 carbamates and two OP pesticides. The Ko:w and Kov:w were determined for the pesticides of interest using SMILES notations and Accord for Excel- 1;PCo:w x Fneat3; + Fweat PCarb= 1;PCo:w x Fneb3; + Fweb where neat, neb are neutral lipid equivalent and weat and web are water-equivalent. In evaluating OPs and carbamates, the predictions ranged from being nearly identical for oxamyl (0.233 vs. 0.52) to over 10-fold different for propoxur (2.33 vs. 30.7). This work suggests that constituitive measures of lipophilicity may describe chemical partitioning in more complex matrixes, however, the divergent results require further investigation. "Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy."

Being an abstract the reliability of data could not be check; therefore the relevance is strongly limited.

CA 5.4.1 and CA 5.8.2

Title: Modulation of the genotoxicity of pesticides reacted with redox-modified smectite clay

Authors: Sorensen, Kara C.; Stucki, Joseph W.; Warner, Richard E.; Wagner, Elizabeth D.; Plewa, Michael J.

Source: Environmental and Molecular Mutagenesis, (2005) Vol. 46, No. 3, pp. 174-181

Summary:

Pesticides are toxic agents intentionally released into the environment; their use raises public health and environmental concerns. In recent years there has been much attention to the biotic degradation of pesticides. Abiotic mechanisms in the soil can contribute to pesticide degradation yet the toxicological impact of such degradation is unclear. This study combines for the first time an investigation into abiotic mechanisms of degradation coupled with toxicological endpoints in mammalian cells. The genotoxicity of three commonly used agricultural pesticides was assessed before and after exposure to redox-modified clay minerals. The objectives of the study were to determine the genotoxicity of 2,4-dichlorophenoxy acetic acid (2,4-D), dicamba, and **oxamyl**, using single cell gel electrophoresis with Chinese hamster ovary (CHO) cells, and to determine the effect of the iron oxidation state in clay minerals (ferruginous smectite SWa-l) on the genotoxic potency of the pesticides. 2,4-D alone or following reaction with redox-modified clays did not induce DNA damage in CHO cells. Oxamyl alone induced a concentration-dependent increase in genomic DNA damage; however, its genotoxicity declined after reaction with reduced clay minerals. Dicamba was not genotoxic when directly analyzed. When dicamba was reacted with reduced clay, a concentration-dependent increase in genomic DNA damage was observed. This is the first reported case of a pesticide being converted into a genotoxin after exposure to redox-modified smectites. These data introduce a new paradigm on the interaction between redox-modified clays and pesticide-related environmental genotoxicity.

CA 5.5.1

Title: A topological substructural approach applied to the computational prediction of rodent carcinogenicity

Authors: Helguera, Aliuska Morales; Cabrera Perez, Miguel Angel; Gonzalez, Maykel Perez; Ruiz, Reinaldo Molina; Gonzalez Diaz, Humberto

Source: Bioorganic & Medicinal Chemistry, (2005) Vol. 13, No. 7, pp. 2477-2488

Summary:

The carcinogenic activity has been investigated by using a topological substructural molecular design approach (TOPS-MODE). A discriminant model was developed to predict the carcinogenic and noncarcinogenic activity on a data set of 189 compounds. The percentage of correct classification was 76.32%. The predictive power of the model was validated by three tests: an external test set (compounds not used in the develop of the model, with a 72.97% of good classification), a leave-group-out cross-validation procedure (4-fold full cross-validation, removing 20% of compounds in each cycle, with a good prediction of 76.31%) and two external prediction sets (the first and second exercises of the National Toxicology Program). This methodology evidenced that the hydrophobicity increase the carcinogenic activity and the dipole moment of the mol. decrease it; suggesting the capacity of the TOPS−MODE descriptors to estimate this property for new drug candidates. Finally, the positive and negative fragment contributions to the carcinogenic activity were identified (structural alerts) and their potentialities in the lead generation process and in the design of 'safer' chemicals were evaluated.

CA 5.5.1

Title: Human toxicological effect and damage factors of carcinogenic and noncarcinogenic chemicals for life cycle impact assessment

Authors: Huijbregts, Mark A. J.; Rombouts, Linda J. A.; Ragas, Ad M. J.; van de Meent, Dik

Source: Integrated Environmental Assessment and Management, (2005) Vol. 1, No. 3, pp. 181-244

Summary:

Chemical fate, effect, and damage should be accounted for in the analysis of human health impacts by toxic chemicals in life-cycle assessment (LCA). The goal of this article is to present a new method to derive human damage and effect factors of toxic pollutants, starting from a lognormal dose-response function. Human damage factors are expressed as disability-adjusted life-years (DALYs). Human effect factors contain a disease-specific and a substance-specific component. The disease-specific component depends on the probability of disease occurrence and the distribution of sensitivities in the human population. The substance-specific component, equal to the inverse of the ED50, represents the toxic potency of a substance. The new method has been applied to calculate combined human damage and effect factors for 1192 substances. The total range of 7 to 9 orders of magnitude between the substances is dominated by the range in toxic potencies. For the combined factors, the typical uncertainty, represented by the square root of the ratio of the 97.5th and 2.5th percentiles, is a factor of 25 for carcinogenic effects and a factor of 125 for noncarcinogenic effects. The interspecies conversion factor, the (non)cancer effect conversion factor, and the average noncancer damage factor dominate the overall uncertainty.

CA 5.6

Title: Effect of agropesticides use on male reproductive function: A study on farmers in Djutitsa (Cameroon)

Authors: Manfo, Faustin Pascal Tsague; Moundipa, Paul Fewou; Dechaud, Henri; Tchana, Ang'le Nkouatchoua; Nantia, Edouard Akono; Zabot, Marie-Therese; Pugeat, Michel

Source: Environmental Toxicology, (2012) Vol. 27, No. 7, pp. 423-432

Summary:

This study aimed at investigating the effect of agropesticides on male reproductive function in farmers in Djutitsa (West Cameroon). To this end, 47 farmers in Djutitsa were asked questions on their health status and pesticide use in agriculture. Thereafter, their blood samples were collected for assessment of sex hormones including serum LH (LH), FSH (FSH), androstenedione, testosterone, as well as sex hormone binding globulin

(SHBG). Their serum triiodothyronine (T3) and thyroxine (T4) levels were also measured. Thirty seven men not exposed to agropesticides were recruited as control group. Fifty six pesticides containing 25 active substances were currently used by farmers enrolled in our study, and most of their symptoms were related to spread/use of these chemicals. Compared to the control group, there was no significant difference in FSH, LH, SHBG, estradiol, and thyroid hormones (T3 and T4) levels. Farmers had significantly lower serum testosterone (20.93 ± 1.03 nM vs. 24.32 ± 1.32 nM; P <0.05) and higher androstenedione level (3.83 ± 0.20 nM vs. 2.80 ± 0.15 nM; P <0.001). Their serum free testosterone as well as bioavailable testosterone were unchanged, while estradiol/testosterone and androstenedione/testosterone ratios were significantly increased (0.45 ± 0.03% vs. 0.33 ± 0.02%; P <0.01 and 12.26 ± 3.64 vs 19.31 ± 6.82; P <0.001, respectively). Our results suggest that male farmers of Djutitsa (West Cameroon) are exposed to agropesticides due to improper protective tool, and this exposure may impair their reproductive function through inhibition of testosterone synthesis; probably by inhibition of testicular 17β- hydroxysteroid dehydrogenase (17HSD3) and induction of aromatase (CYP19).

CA 5.6.1

Title: A retrospective analysis of the two-generation study: What is the added value of the second generation?

Authors: Janer, Gemma; Hakkert, Betty C.; Slob, Wout; Vermeire, Theo; Piersma, Aldert H.

Source: Reproductive Toxicology, (2007) Vol. 24, No. 1, pp. 97-102

Summary:

Increasing pressure is exerted by some stakeholders to reduce the two-generation study to a one-generation study, a measure that would considerably reduce the number of animals and other costs involved in these lengthy studies. The present study retrospectively evaluates 176 multi-generation studies to assess potential differences between the first and the second generation, both in terms of the types of effects observed and in terms of the effective doses. All substances classified as reproductive toxicants by the Directive 92/32/EEC or considered as toxic to fertility by the California EPA for which we found a multi-generation study were included (n = 58 studies). The second generation in the two-generation studies considered affected neither the overall NOAEL nor the critical effect. Therefore, it had no impact on the ensuing risk assessment, nor on classification and labeling. However, several substances did show an increased sensitivity of the F1 adults in comparison to the P0. These results support the proposal of replacing the current two-generation study by a one-generation study with a more extensive assessment of parameters at F1 adulthood.

CA 5.6.2

Title: Predictive models of prenatal developmental toxicity from ToxCast High-Throughput Screening data

Authors: Sipes, Nisha S.; Martin, Matthew T.; Reif, David M.; Kleinstreuer, Nicole C.; Judson, Richard S.; Singh, Amar V.; Chandler, Kelly J.; Dix, David J.; Kavlock, Robert J.; Knudsen, Thomas B.

Source: Toxicological Sciences, (2011) Vol. 124, No. 1, pp. 109-127

Summary:

Environmental Protection Agency's ToxCast project is profiling the *in vitro* bioactivity of chemicals to assess pathway-level and cell-based signatures that correlate with observed *in vivo* toxicity. The authors hypothesized that developmental toxicity in guideline animal studies captured in the ToxRefDB database would correlate with cell-based and cell-free *in vitro* high-throughput screening (HTS) data to reveal meaningful mechanistic relationships and provide models identifying chemicals with the potential to cause developmental toxicity. To test this hypothesis, the authors built statistical associations based on HTS and *in vivo* developmental toxicity data from ToxRefDB. Univariate associations were used to filter HTS assays based on statistical correlation with distinct *in vivo* endpoint. This revealed 423 total associations with distinctly different patterns for rat (301 associations) and rabbit (122 associations) across multiple HTS assay platforms. From these associations, linear discriminant analysis with cross-validation was used to build the models. Species-specific models of predicted developmental toxicity revealed strong balanced accuracy (>70%) and unique correlations between assay targets such as transforming growth factor beta, retinoic acid receptor, and G-protein-coupled receptor signaling in the rat and inflammatory signals, such as interleukins (IL) (IL1a and IL8) and chemokines (CCL2), in the rabbit. Species-specific toxicity endpoints were associated with one another through common Gene Ontology biological processes, such as cleft palate to urogenital defects through placenta and embryonic

development. This work indicates the utility of HTS assays for developing pathway-level models predictive of developmental toxicity.

CA 5.7.1

Title: Impact of chemical proportions on the acute neurotoxicity of a mixture of seven carbamates in preweanling and adult rats

Authors: Moser, Virginia C.; Padilla, Stephanie; Simmons, Jane Ellen; Haber, Lynne T.; Hertzberg, Richard C.

Source: Toxicological Sciences, (2012) Vol. 129, No. 1, pp. 126-134

Summary:

Statistical design and environmental relevance are important aspects of studies of chemical mixtures, such as pesticides. We used a dose-additivity model to test experimentally the default assumptions of dose additivity for two mixtures of seven N-methylcarbamates (carbaryl, carbofuran, formetanate, methomyl, methiocarb, oxamyl, and propoxur). The best-fitting models were selected for the single-chemical dose-response data and used to develop a combined prediction model, which was then compared with the experimental mixture data. We evaluated behavioral (motor activity) and cholinesterase (ChE)-inhibitory (brain, red blood cells) outcomes at the time of peak acute effects following oral gavage in adult and preweanling (17 days old) Long-Evans male rats. The mixtures varied only in their mixing ratios. In the relative potency mixture, proportions of each carbamate were set at equitoxic component doses. A California environmental mixture was based on the 2005 sales of each carbamate in California. In adult rats, the relative potency mixture showed dose additivity for red blood cell ChE and motor activity, and brain ChE inhibition showed a modest greater-than additive (synergistic) response, but only at a middle dose. In rat pups, the relative potency mixture was either dose-additive (brain ChE inhibition, motor activity) or slightly less-than additive (red blood cell ChE inhibition). On the other hand, at both ages, the environmental mixture showed greater-than additive responses on all three endpoints, with significant deviations from predicted at most to all doses tested. Thus, we observed different interactive properties for different mixing ratios of these chemicals. These approaches for studying pesticide mixtures can improve evaluations of potential toxicity under varying experimental conditions that may mimic human exposures.

CA 5.7.1

Title: Esterase metabolism of cholinesterase inhibitors using rat liver *in vitro*

Authors: Moser, V.C.; Padilla, S.

Source: Toxicology 281 (2011) 56-62

Summary:

A variety of chemicals, such as organophosphate (OP) and carbamate pesticides, nerve agents, and industrial chemicals, inhibit acetylcholinesterase (AChE) leading to overstimulation of the cholinergic nervous system. The resultant neurotoxicity is similar across mammalian species; however, the relative potencies of the chemicals across and within species depend in part on chemical-specific metabolic and detoxification processes. Carboxylesterases and A-esterases (paraoxonases, PON) are two enzymatic detoxification pathways that have been widely studied. We used an *in vitro* system to measure esterase-dependent detoxification of 15 AChE inhibitors. The target enzyme AChE served as a bioassay of inhibitor concentration following incubation with detoxifying tissue. Concentration-inhibition curves were determined for the inhibitor in the presence of buffer (no liver), rat liver plus calcium (to stimulate PONs and thereby measure both PON and carboxylesterase), and rat liver plus EGTA (to inhibit calcium-dependent PONs, measuring carboxylesterase activity). Point estimates (concentrations calculated to produce 20, 50, and 80% inhibition) were compared across conditions and served as a measure of esterase-mediated detoxification. Results with well-known inhibitors (chlorpyrifos oxon, paraoxon, methyl paraoxon, malaoxon) were in agreement with the literature, serving to support the use of this assay. Only a few other inhibitors showed slight or a trend towards detoxification *via* carboxylesterases or PONs (mevinphos, aldicarb, oxamyl). There was no apparent PON- or carboxylesterase-mediated detoxification of the remaining inhibitors (carbofuran, chlorfenvinphos, dicrotophos, fenamiphos, methamidophos, methomyl, monocrotophos, phosphamidon), suggesting that the influence of esterases on these chemicals is minimal. Thus, generalizations regarding these metabolic pathways may not be appropriate. As with other aspects of AChE inhibitors, their metabolic patterns appear to be chemical-specific.

CA 5.7.1

Title: A method to determine precise benchmark doses for carbamate anticholinesterases

Authors: Lassiter, T. Leon; Brimijoin, Stephen

Source: Toxicological Sciences 96 (1), 154-161 (2007)

Summary:

In determining benchmark doses for risk assessment and regulation of carbamate anticholinesterase pesticides like formetanate, oxamyl, and methomyl, one needs to quantitate low levels of cholinesterase inhibition. For improved accuracy while using fewer subjects, we developed an assay based on the recognized ability of carbamates to protect cholinesterase from irreversible inactivation. This assay measures enzyme that survives diisopropylfluorophosphate exposure *in vitro* and then reactivates by decarbamylation after small molecules are removed with size-exclusion centrifugation. The 99% silencing of unprotected cholinesterase yields a low background. Comparisons of recovered activity with initial activity (representing carbamate-free enzyme) use each sample as its own control. As a result, carbamate-protection assays can demonstrate a statistically significant 2–3% inhibition of brain cholinesterase in a single experimental group of modest size. When applied to brain samples from formetanate-treated rats, such an assay predicted a benchmark dose of 0.19 mg/kg for 10% inhibition (BMD10), with a lower 95% confidence limit of 0.15 mg/kg (BMDL10). Protection assays should enable precise determinations of benchmark doses for other carbamates, as well as accurate assessment of *in vivo* inhibition half-lives under low-dose scenarios.

CA 5.8.1

Title: QSAR study on acute toxicity of carboxylic acids and their derivatives

Authors: Cui, Yi; Jiang, Jun-cheng; Pan, Yong; Cao, Hong-yin; Wang, Rui

Source: Huanjing Kexue Yu Jishu, (2010) Vol. 33, No. 4, pp. 29-34

Summary:

This QSAR study relates to the structure of 27 sorts of carboxylic acids and their derivatives, in which a set of five descriptors are chosen by using the variable selection method of genetic algorithm (GA). The molecular size and steric effects are found to influence LC50 of carboxylic acids and their derivatives. Models of multiple linear regression (MLR) and support vector machine (SVM), tested by internal and external validations, have been employed to simulate the possible quantitative relationship between these selected descriptors and LC50 respectively, showing good robustness and high predictive ability as well as generalization. By comparing SVM and MLR in terms of the predictive mean absolute errors obtained for both training set and prediction set, the result of SVM appears to be better than that of MLR.

CA 5.8.1

Title: Oxalic acid as a uremic toxin

Authors: Mydlik, M.; Derzsiova, K.

Source: Journal of Renal Nutrition (2008), Volume 18, Number 1, pp. 33-39

Summary:

Objective: Oxalic acid (OA) is thought to be a uremic toxin that participates in the pathogenesis of uremic syndrome. The objectives of this study were to: (1) evaluate the plasma levels of OA in patients with chronic renal disease with various levels of glomerular filtration rate and after renal transplantation; (2) investigate the salivary secretion of OA and ascorbic acid in healthy subjects and in patients with chronic renal failure (CRF); (3) examine the influence of water and sodium diuresis and furosemide administration on the urinary excretion of OA and ascorbic acid in healthy subjects and in CRF patients without dialysis treatment; and (4) evaluate the influence of renal replacement therapy (RRT) on secondary hyperoxalemia in hemodialysis patients.

Design and Setting: This study was conducted at the Nephrological Department of P.J. Safarik University. Sixty-one patients with chronic renal disease, 64 CRF patients, 32 continuous ambulatory peritoneal dialysis (CAPD) patients, 15 hemodialysis patients, 21 patients after renal transplantation, and 15 healthy subjects were examined. Maximal water diuresis, diets with low (2 g/day) and high (15 g/day) sodium intake, administration

of intravenous furosemide (20 mg), and renal replacement therapy (CAPD, hemodialysis, hemofiltration, and postdilution hemodiafiltration) were utilized in the study.

Results: In patients with chronic renal disease and those after renal transplantation, direct relationships between plasma OA and serum creatinine were found (r = 0.904 and 0.9431, respectively, P <.01). Despite a high level of plasma OA in uremic patients (23.1 ± 10 μmol/L), there was no significant difference in salivary OA between control subjects (128 ± 19 μmol/L) and CRF patients (135 ± 24 μmol/L). The urinary excretion of OA during maximal water diuresis (from 37.5 to 110.3 μmol/4 hours) and after intravenous furosemide (from 34.5 to 66.7 μmol/3 hours) increased significantly, but was not affected by high intake of NaCl. The most significant decrease of plasma OA was observed during postdilution hemodiafiltration (63.3%).

Conclusion: Our study indicates that renal replacement therapy is not effective for a permanent reduction of elevated plasma levels of OA.

CA 5.8.1

Title: Correspondence Re: "C. Guo, K.E. McMartin, The cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation, Toxicology 208 (3) (2005) 347-355"

Authors: Lewin-Smith Michael R; Kalasinsky Victor F; Mullick Florabel G

Source: Toxicology, (2006) Vol. 222, No. 1-2, pp. 160-1; author reply 162. Electronic Publication Date: 6 Mar 2006.

Summary:

We read with interest the article published by Chungang Guo and Kenneth E. McMartin in the March 2005 issue of Toxicology cited above (Guo and McMartin, 2005). Our attention was drawn to the statement in the abstract that "Oxalate has two forms *in vivo*: oxalate ions and calcium oxalate monohydrate (COM) crystals that readily form in the presence of calcium." We believe that without further qualification this statement is an oversimplification, which was not further clarified in the text. It is widely known that at least one other form of oxalate exists *in vivo*, namely calcium oxalate dihydrate (COD) (weddelite) which is a frequent finding in the chemical analysis of urinary calculi and is often present in combination with COM. The presence *in vivo* of forms of oxalate other than COM may eventually be demonstrated to have little impact on the overall toxicity associated with ethylene glycol poisoning. However, we believe it would be a service to your readers to point out that other forms of oxalate exist *in vivo*, and in some instances (*e.g.* in the thyroid), appear to have little relationship to histopathologic changes. The evidence for the specific toxicity of COM can be more fully appreciated given this additional contextual information.

CA 5.8.1

Title: Oxalate upregulates expression of IL-2Rβ and activates IL-2R signaling in HK-2 cells, a line of human renal epithelial cells

Authors: Koul, Sweaty; Khandrika, Lakshmipathi; Pshak, Thomas J.; Iguchi, Naoko; Pal, Mintu; Steffan, Joshua J.; Koul, Hari K.

Source: *Am J Physiol Renal Physiol* 306: F1039-1046, 2014

Summary:

The role of inflammation in oxalate-induced nephrolithiasis is debated. Our gene expression study indicated an increase in interleukin-2 receptor β (IL-2Rβ) mRNA in response to oxalate (Koul S, Khandrika L, Meacham RB, Koul HK. PLoS ONE 7: e43886, 2012). Herein, we evaluated IL-2Rβ expression and its downstream signaling pathway in HK-2 cells in an effort to understand the mechanisms of oxalate nephrotoxicity. HK-2 cells were exposed to oxalate for various time points in the presence or absence of SB203580, a specific p38 MAPK inhibitor. Gene expression data were analyzed by Ingenuity Pathway Analysis software. mRNA expression was quantitated *via* real-time PCR, and changes in protein expression/kinase activation were analyzed by Western blotting. Exposure of HK-2 cells to oxalate resulted in increased transcription of IL-2Rβ mRNA and increased protein levels. Oxalate treatment also activated the IL-2Rβ signaling pathway (JAK1/STAT5 phosphorylation). Moreover, the increase in IL-2Rβ protein was dependent upon p38 MAPK activity. These results suggest that oxalate-induced activation of the IL-2Rβ pathway may lead to a plethora of cellular changes, the most common

of which is the induction of inflammation. These results suggest a central role for the p38 MAPK pathway in mediating the effects of oxalate in renal cells, and additional studies may provide the key to unlocking novel biochemical targets in stone disease.

CA 5.8.1

Title: Oxalate induced expression of monocyte chemoattractant protein-1 (MCP-1) in HK-2 cells involves reactive oxygen species

Authors: Habibzadegah-Tari, Pouran; Byer, Karen; Khan, Saeed R.

Source: Urological research, (2005) Vol. 33, No. 6, pp. 440-7. Electronic Publication Date: 24 Nov 2005.

Summary:

Oxalate is a toxic end product of metabolism largely because of its propensity to crystallize and form calcium oxalate, which is insoluble at physiologic pH and often deposits at very unfortunate sites, notably the kidneys. In the current study, we investigated the oxalate-induced injury and up-regulation of monocyte-chemoattractant protein-1 (MCP-1) in HK-2 cells, a proximal tubular epithelial cell line derived from normal human kidney. The cells were exposed to oxalate ions for different lengths of time. The culture media was tested for LDH release, a cell injury marker. mRNA was isolated from the cells and subjected to reverse transcriptase–polymerase chain reaction. The data showed that oxalate exposure resulted in cell injury in a time and concentration dependent manner. The MCP-1 mRNA increased following exposure to oxalate and was reduced upon treatment with free radical scavengers, catalase and superoxide dismutase. These data support the importance of reactive oxygen species in the induction of expression of MCP-1 in renal epithelial cells. To our knowledge, this is the first report of MCP-1 expression and its upregulation by oxalate exposure in HK-2 cells.

CA 5.8.2

Title: Consideration of dosimetry in evaluation of ToxCast™ data

Authors: Aylward, Lesa L.; Hays, Sean M.

Source: Journal of Applied Toxicology, (2011) Vol. 31, No. 8, pp. 741-751

Summary:

The US Environmental Protection Agency (US EPA) Toxcast™ program has the stated goal of predicting hazard, characterizing toxicity pathways and prioritizing the toxicity testing of environmental chemicals through the use of *in vitro* high-throughput screening (HTS) assays. This analysis integrates data from biomonitoring and from *in vivo* toxicity and pharmacokinetic studies to examine the physiological relevance of the tested and responding *in vitro* concentrations for five case study chemicals: triclosan, 2,4-dichlorophenoxyacetic acid, perfluorooctanoic acid, monobutyl phthalate and mono-2(ethylhexyl)phthalate. This analysis also examines the ToxCast™ phase 1 data set for approx. 50 chemicals belonging to four 'common mechanism groups' which have been the subject of cumulative risk assessments by the US EPA for both the pattern of key responses and the relative potencies of included chemicals compared with the *in vivo* relative potencies. Responding concentrations *in vitro* were generally in the range of serum or plasma concentrations associated with no-observed to lowest-observed effect levels for the case study chemicals, while available biomonitoring data demonstrating actual exposures were generally lower. ToxCast™ assay endpoints related to acetylcholinesterase (AChE) inhibition had low sensitivity for detecting organophosphate pesticides but good sensitivity for detecting N-methyl carbamates. However, *in vitro* relative potencies did not correlate with *in vivo* potency. Both qualitative and quantitative predictive power is probably affected by the lack of comprehensive metabolic activity in most current *in vitro* systems explored in the ToxCast™ program, and this remains a fundamental challenge for high-throughput toxicity screening efforts.

CA 5.8.2

Title: Activity profiles of 309 ToxCast™ chemicals evaluated across 292 biochemical targets

Authors: Knudsen, Thomas B.; Houck, Keith A.; Sipes, Nisha S.; Singh, Amar V.; Judson, Richard S.; Martin, Matthew T.; Weissman, Arthur; Kleinstreuer, Nicole C.; Mortensen, Holly M.; *et al.*

Source: Toxicology, (2011) Vol. 282, No. 1-2, pp. 1-15

Summary:

Understanding the potential health risks posed by environmental chemicals is a significant challenge elevated by the large number of diverse chemicals with generally uncharacterized exposures, mechanisms, and toxicities. The present study is a performance evaluation and critical analysis of assay results for an array of 292 high−throughput cell-free assays aimed at preliminary toxicity evaluation of 320 environmental chemicals in EPA's ToxCast™ project (Phase I). The chemicals (309 unique, 11 replicates) were mainly precursors or the active agent of commercial pesticides, for which a wealth of *in vivo* toxicity data is available. Biochemical HTS (high throughput screening) profiled cell and tissue extracts using semi-automated biochemical and pharmacological methodologies to evaluate a subset of G-protein coupled receptors (GPCRs), CYP450 enzymes (CYPs), kinases, phosphatases, proteases, HDACs, nuclear receptors, ion channels, and transporters. The primary screen tested all chemicals at a relatively high concentration 25 μM concentration (or 10 μM for CYP assays), and a secondary screen retested 9132 chemical-assay pairs in 8−point concentration series from 0.023 to 50 μM (or 0.009-20 μM for CYPs). Mapping relations across 93,440 chemical-assay pairs based on half−maximal activity concentration (AC50) revealed both known and novel targets in signaling and metabolic pathways. The primary dataset, summary data, and details on quality control checks are available for download at http://www.epa.gov/ncct/toxcast/.

CA 5.8.2

Title: Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by Toxcast Chemicals

Authors: Rotroff, Daniel M.; Beam, Andrew L.; Dix, David J.; Farmer, Adam; Freeman, Kimberly M.; Houck, Keith A.; Judson, Richard S.; LeCluyse, Edward L.; Martin, Matthew T.; *et al.*

Source: Journal of Toxicology and Environmental Health, Part B: Critical Reviews, (2010) Vol. 13, No. 2-4, pp. 329-346

Summary:

Primary human hepatocyte cultures are useful *in vitro* model systems of human liver because when cultured under appropriate conditions the hepatocytes retain liver-like functionality such as metabolism, transport, and cell signaling. This model system was used to characterize the concentration- and time-response of the 320 ToxCast chemicals for changes in expression of genes regulated by nuclear receptors. Fourteen gene targets were monitored in quantitative nuclease protection assays: six representative cytochromes P-450, four hepatic transporters, three Phase II conjugating enzymes, and one endogenous metabolism gene involved in cholesterol synthesis. These gene targets are sentinels of five major signaling pathways: AhR, CAR, PXR, FXR, and PPARα. Besides gene expression, the relative potency and efficacy for these chemicals to modulate cellular health and enzymic activity were assessed. Results demonstrated that the culture system was an effective model of chemical-induced responses by prototypical inducers such as phenobarbital and rifampicin. Gene expression results identified various ToxCast chemicals that were potent or efficacious inducers of one or more of the 14 genes, and by inference the 5 nuclear receptor signaling pathways. Significant relative risk associations with rodent *in vivo* chronic toxicity effects are reported for the five major receptor pathways. These gene expression data are being incorporated into the larger ToxCast predictive modeling effort.

5.8.2

Title: *In vitro* screening of environmental chemicals for targeted testing prioritization: The ToxCast Project

Authors: Judson, Richard S.; Houck, Keith A.; Kavlock, Robert J.; Knudsen, Thomas B.; Martin, Matthew T.; Mortensen, Holly M.; Reif, David M.; Rotroff, Daniel M.; Shah, Imran; *et al.*

Source: Environmental Health Perspectives, (2010) Vol. 118, No. 4, pp. 485-492

Summary:

Background: Chemical toxicity testing is being transformed by advances in biology and computer modeling, concerns over animal use, and the thousands of environmental chemicals lacking toxicity data. The U.S. Environmental Protection Agency's ToxCast program aims to address these concerns by screening and prioritizing chemicals for potential human toxicity using *in vitro* assays and *in silico* approaches.

Objectives: This project aims to evaluate the use of *in vitro* assays for understanding the types of molecular and pathway perturbations caused by environmental chemicals and to build initial prioritization models of *in vivo* toxicity.

Methods: We tested 309 mostly pesticide active chemicals in 467 assays across nine technologies, including high-throughput cell-free assays and cell-based assays, in multiple human primary cells and cell lines plus rat primary hepatocytes. Both individual and composite scores for effects on genes and pathways were analyzed.

Results: Chemicals displayed a broad spectrum of activity at the molecular and pathway levels. We saw many expected interactions, including endocrine and xenobiotic metabolism enzyme activity. Chemicals ranged in promiscuity across pathways, from no activity to affecting dozens of pathways. We found a statistically significant inverse association between the number of pathways perturbed by a chemical at low *in vitro* concentrations and the lowest *in vivo* dose at which a chemical causes toxicity. We also found associations between a small set of *in vitro* assays and rodent liver lesion formation.

Conclusions: This approach promises to provide meaningful data on the thousands of untested environmental chemicals and to guide targeted testing of environmental contaminants.

CA 5.8.2

Title: Consensus QSAR models: Do the benefits outweigh the complexity?

Authors: Hewitt, Mark; Cronin, Mark T. D.; Madden, Judith C.; Rowe, Philip H.; Johnson, Clara; Obi, Anrdrea; Enoch, Steven J.

Source: Journal of Chemical Information and Modeling, (2007) Vol. 47, No. 4, pp. 1460-1468

Summary:

This study has assessed the use of consensus regression, as compared to single multiple linear regression, models for the development of quantitative structure-activity relationships (QSARs). To provide a comparison, four data sets of varying size and complexity were analyzed: silastic membrane flux, toxicity of phenols to *Tetrahymena pyriformis*, acute toxicity to the fathead minnow and flash point. For each data set, a genetic algorithm was used to develop a model population and the performance of consensus models was compared to that of the best single model. Two consensus models were developed, one using the top 10 models, and the other using a subset of models chosen to provide maximal coverage of model space. The results highlight the ability of the genetic algorithm to develop predictive models from a large descriptor pool. However, the consensus models were shown to offer no significant improvements over single regression models, which are as statistically robust as the equivalent consensus models. Consensus models developed from a selection of the best QSARs were shown not to be superior to a selection of diverse in "model space" QSARs. For the data sets analyzed in this study, and in light of the Organization for Economic Cooperation and Development principles for the validation of QSARs, the increase in model complexity when using consensus models does not seem warranted given the minimal improvement in model statistics.

CA 5.8.2

Title: True prediction of lowest observed adverse effect levels

Authors: Garcia-Domenech, R.; Julian-Ortiz, J. V.; Besalu, E.

Source: Molecular Diversity, (2006) Vol. 10, No. 2, pp. 159-168

Summary:

A database of structurally heterogeneous chemical structures with their experimental values of Lowest Observed Adverse Effect Levels (LOAELs) was modeled using graph theoretical descriptors. Variable selection for multiple linear regression (MLR) and linear discriminant analysis (LDA) was accomplished by the Internal Test Set (ITS) method in order to achieve true predicted LOAEL values. The results obtained can be considered good taking into account the structural diversity of the training set.

CA 5.8.2

Title: Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signaling assays

Authors: Sipes, Nisha S.; Martin, Matthew T.; Kothiya, Parth; Reif, David M.; Judson, Richard S.; Richard, Ann M.; Houck, Keith A.; Dix, David J.; Kavlock, Robert J.; Knudsen, Thomas B.

Source: Chemical Research in Toxicology, (2013) Vol. 26, No. 6, pp. 878-895

Summary:

Understanding potential health risks is a significant challenge due to the largenumbers of diverse chemicals with poorly characterized exposures and mechanisms of toxicities. The present study analyzes 976 chemicals (including failed pharmaceuticals, alternative plasticizers, food additives, and pesticides) in Phases I and II of the U.S. EPA's ToxCast project across 331 cell-free enzymatic and ligand-binding high-throughput screening (HTS) assays. Half-maximal activity concentrations (AC50) were identified for 729 chemicals in 256 assays (7135 chemical-assay pairs). Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). Heavy metals, surfactants, and dithiocarbamate fungicides showed promiscuous but distinctly different patterns of activity, whereas many of the pharmaceutical compounds showed promiscuous activity across GPCRs. Literature analysis confirmed >50% of the activities for the most potent chemical-assay pairs (54) but also revealed 10 missed interactions. Twenty-two chemicals with known estrogenic activity were correctly identified for the majority (77%), missing only the weaker interactions. In many cases, novel findings for previously unreported chemical-target combinations clustered with known chemical-target interactions. Results from this large inventory of chemical-biological interactions can inform read−across methods as well as link potential targets to molecular initiating events in adverse outcome pathways for diverse toxicities.

CA 5.8.3

Title: Endocrine profiling and prioritization of environmental chemicals using ToxCast data

Authors: Reif, David M.; Martin, Matthew T.; Tan, Shirlee W.; Houck, Keith A.; Judson, Richard S.; Richard, Ann M.; Knudsen, Thomas B.; Dix, David J.; Kavlock, Robert J.

Source: Environmental Health Perspectives, (2010) Vol. 118, No. 12, pp. 1714-1720

Summary:

Background: The prioritization of chemicals for toxicity testing is a primary goal of the U.S. Environmental Protection Agency (EPA) ToxCast™ program. Phase I of ToxCast used a battery of 467 *in vitro*, high−throughput screening assays to assess 309 environmental chemicals. One important mode of action leading to toxicity is endocrine disruption, and the U.S. EPA's Endocrine Disruptor Screening Program (EDSP) has been charged with screening pesticide chemicals and environmental contaminants for their potential to affect the endocrine systems of humans and wildlife.

Objective: The goal of this study was to develop a flexible method to facilitate the rational prioritization of chemicals for further evaluation and demonstrate its application as a candidate decision-support tool for EDSP.

Methods: Focusing on estrogen, androgen, and thyroid pathways, we defined putative endocrine profiles and derived a relative rank or score for the entire ToxCast library of 309 unique chemicals. Effects on other nuclear receptors and xenobiotic metabolizing enzymes were also considered, as were pertinent chemical descriptors and pathways relevant to endocrine-mediated signaling.

Results: Combining multiple data sources into an overall, weight-of-evidence Toxicological Priority Index (ToxPi) score for prioritizing further chemical testing resulted in more robust conclusions than any single data source taken alone.

Conclusions: Incorporating data from *in vitro* assays, chemical descriptors, and biological pathways in this prioritization schema provided a flexible, comprehensive visualization and ranking of each chemical's potential endocrine activity. Importantly, ToxPi profiles provide a transparent visualization of the relative contribution of all information sources to an overall priority ranking. The method developed here is readily adaptable to diverse chemical prioritization tasks.

CA 5.8.3

Title: The use and acceptance of Other Scientifically Relevant Information (OSRI) in the U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program

Authors: Bishop, Patricia L.; Willett, Catherine E.

Source: Birth Defects Research, Part B: Developmental and Reproductive Toxicology, (2014) Vol. 101, No. 1, pp. 3-22

Summary:

The U.S. Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program (EDSP) currently relies on an initial screening battery (Tier 1) consisting of five *in vitro* and six *in vivo* assays to evaluate a chemical's potential to interact with the endocrine system. Chemical companies may request test waivers based on Other Scientifically Relevant Information (OSRI) that is functionally equivalent to data gathered in the screening battery or that provides information on a potential endocrine effect. Respondents for 47 of the first 67 chemicals evaluated in the EDSP submitted OSRI *in lieu* of some or all Tier 1 tests, seeking 412 waivers, of which EPA granted only 93. For 20 of the 47 chemicals, EPA denied all OSRI and required the entire Tier 1 battery. Often, the OSRI accepted was either identical to data generated by the Tier 1 assay or indicated a positive result. Although identified as potential sources of OSRI in EPA guidance, Part 158 guideline studies for pesticide registration were seldom accepted by EPA. The 93 waivers reduced animal use by at least 3325 animals. We estimate 27,731 animals were used in the actual Tier 1 tests, with additional animals being used in preparation for testing. Even with EPA's shift toward applying 21st-century toxicology tools to screening of endocrine disruptors in the future, acceptance of OSRI will remain a primary means for avoiding duplicative testing and reducing use of animals in the EDSP. Therefore, it is essential that EPA develop a consistent and transparent basis for accepting OSRI.

CA 5.9.4

Title: Dietary exposure to pesticide residues from commodities alleged to contain the highest contamination levels

Authors: Winter, Carl K.; Katz, Josh M.

Source: Journal of Toxicology, (2011) Article ID 589674, 7 pp.

Summary:

Probabilistic techniques were used to characterize dietary exposure of consumers to pesticides found in twelve commodities implicated as having the greatest potential for pesticide residue contamination by a United States-based environmental advocacy group. Estimates of exposures were derived for the ten most frequently detected pesticide residues on each of the twelve commodities based upon residue findings from the United States Department of Agriculture's Pesticide Data Program. All pesticide exposure estimates were well below established chronic reference doses (RfDs). Only one of the 120 exposure estimates exceeded 1% of the RfD (methamidophos on bell peppers at 2% of the RfD), and only seven exposure estimates (5.8%) exceeded 0.1% of the RfD. Three quarters of the pesticide/commodity combinations demonstrated exposure estimates below 0.01% of the RfD (corresponding to exposures one million times below chronic No Observable Adverse Effect Levels from animal toxicology studies), and 40.8% had exposure estimates below 0.001% of the RfD. It is concluded that (1) exposures to the most commonly detected pesticides on the twelve commodities pose negligible risks to consumers, (2) substitution of organic forms of the twelve commodities for conventional forms does not result in any appreciable reduction of consumer risks, and (3) the methodology used by the environmental advocacy group to rank commodities with respect to pesticide risks lacks scientific credibility.

CA 5.9.4

Title: Hematological, biochemical effects, and self-reported symptoms in pesticide retailers

Authors: Rojas-Garcia, Aurora Elizabeth; Medina-Diaz, Irma Martha; Robledo-Marenco, Maria de Lourdes; Barron-Vivanco, Briscia Socorro; Giron-Perez, Manuel Ivan; Velazquez-Fernandez, Jesus Bernardino; Gonzalez-Arias, Cyndia Azucena; Albores-Medina, Arnulfo; Quintanilla-Vega, Betzabet; *et al.*

Source: Journal of Occupational and Environmental Medicine, (2011) Vol. 53, No. 5, pp. 517-521

Summary:

Objective: This study evaluates the effects of exposure to pesticides on the health of pesticide retailers. Methods: The study population comprised 83-male pesticide retailers and 98 controls. Serum butyrylcholinesterase levels and complete blood analysis were performed in a certified laboratory and each subject completed a structured questionnaire. Results: Butyrylcholinesterase activity and hematological parameters such as Hb and hematocrit were significantly lower in pesticide retailers than in control subjects. In contrast, platelet count as well as hepatic parameters such as glutamic-pyruvate transaminase and gamma−glutamyl transpeptidase activities was higher in pesticide retailers. Furthermore, pesticide retailers experienced burning sensations in the skin more frequently than controls. Conclusions: These preliminary results suggest the importance of evaluating further toxicological biomarkers in these populations.

CA 5.9.4

Title: Screening of pesticides in blood with liquid chromatography-linear ion trap mass spectrometry

Authors: Dulaurent, Sylvain; Moesch, Christian; Marquet, Pierre; Gaulier, Jean-Michel; Lachatre, Gerard

Source: Analytical and Bioanalytical Chemistry, (2010) Vol. 396, No. 6, pp. 2235-2249

Summary:

In clinical or forensic toxicology, general unknown screening procedures are used to identify as many xenobiotics as possible, belonging to numerous chemical classes. We present here a general unknown screening procedure based on liquid chromatography coupled with use of a single linear ion trap mass spectrometer, and focus on the identification of pesticides and/or metabolites in whole blood. After solid-phase extraction (SPE), the compounds of interest were separated using a reversed-phase column and identified by the mass spectrometer operated first in the full-scan mass spectrometry (MS) mode, in the positive and negative polarities, followed by MS2 and MS3 scanning of ions selected in data-dependent acquisition. The total scan time was 2.45 s. Two mass spectral libraries (MS2 and MS3), each of 450 spectra, were created for the 320 pesticides and metabolites detected after injection of pure solutions. Robustness of the spectra and matrix effects were studied and were satisfactory for the present application. Detection limits for the 320 compounds were studied by extracting 1 mL spiked blood at concentrations between 10 μg/L and 10 mg/L. If necessary, it was possible to decrease the detection limits of some compounds by 10-100-fold by scanning MS2 in only one polarity, owing to a shorter total scan time. However, at the same time, the detection specificity decreased as no confirmation could be recorded in the following MS3 scan and no information could be registered in the other polarity. Thus, in these rare cases, confirmation by another method was required.

CA 5.9.4

Title: Assessment of pesticide exposure in the agricultural population of Costa Rica

Authors: Monge, Patricia; Partanen, Timo; Wesseling, Catharina; Bravo, Viria; Ruepert, Clemens; Burstyn, Igor

Source: Annals of Occupational Hygiene, (2005) Vol. 49, No. 5, pp. 375-384

Summary:

We describe a model for the retrospective assessment of parental exposure to 26 pesticides, selected by toxicity−based prioritization, in a population-based case-control study of childhood leukemia in Costa Rica (301 cases, 582 controls). The model was applied to a subset of 227 parents who had been employed or self−employed in agriculture or livestock breeding. It combines external data on pesticide use for 14 crops, 21 calendar years and 14 regions, and individual interview data on determinants (task and technology, personal protective equipment, field reentry, storing of pesticides, personal hygiene) of exposure. Recall was enhanced by use of checklists of pesticides in the interview. An external database provided information on the application rate (proxy for intensity of potential exposure) for each pesticide. The calendar time was individually converted to five time windows (year before conception, first, second and third trimester, and first year of the child). Time-windowed individual data on determinants of exposure and their expert-based general weights and their category-specific hazard values jointly provided an individual determinant score. This score was multiplied by the application rate to obtain an individual index of exposure intensity during application. Finally, average exposure intensity during entire time windows was estimated by incorporating in the model the individual time fraction of exposure during application. Estimates of exposure intensities were proxies assumed to be proportional to dermal exposure intensity, which represents the major pathway of occupational exposure to pesticides. A simulated sensitivity analysis resulted in a correlation coefficient of 0.91 between two sets of

10000 values of individual exposure indexes, based on two different but realistic sets expert-assigned weights. Lack of measurement data on concurrent exposures in comparable circumstances precluded direct validation of the model.