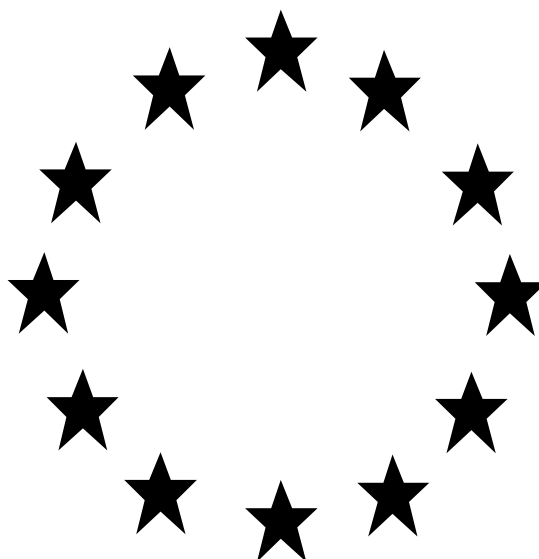




Additional Report to the DAR

- public version -

**Risk assessment provided by the rapporteur Member State
Italy for the existing active substance**

CHLOROPICRIN

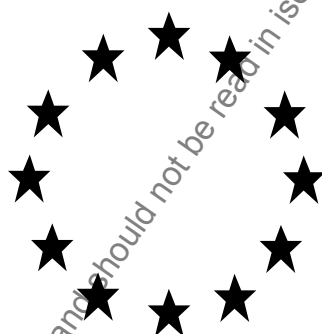
**upon resubmission in the framework of the accelerated procedure in
accordance with Commission Regulation (EC) No 33/2008**

**Volume 3
Annex B6**

March 2010

**Ministry of Health
Rome
Italy**

**Addendum to Draft Assessment Report
regarding resubmission of Chloropicrin**



Chloropicrin

Volume 3

Annex B.6: Toxicology and metabolism

March 2010

B.6 TOXICOLOGY AND METABOLISM

B.6.1 Absorption, distribution, excretion and metabolism

Kidd, G.G., Davidson, J. [¹⁴C]-Chloropicrin: Absorption, Distribution, Metabolism and Excretion in the Rat. [REDACTED] Unpublished report No. 24991. Owner European Chloropicrin Group. 2005. (IIA 5.1/02)

GLP: Yes

Methods:

The rate and extent of absorption, distribution, metabolism and elimination of Chloropicrin was evaluated in rats following oral administration of [¹⁴C]-Chloropicrin (Kidd and Davidson, 2005).

The study was compliant with GLP and with EU test guideline B36 (Directive 87/302/EEC). The method used differs from the recommended guideline in that the structures of the metabolites were not fully identified and an appropriate metabolic pathway was not proposed. However, these deviations were not considered to have affected the scientific validity and interpretation of the results and details of the metabolites and metabolic pathway are presented in IIA 5.1/03 and IIA 5.1/04, which follow.

In a preliminary study, two Sprague Dawley rats/sex received a single oral administration of [¹⁴C]-Chloropicrin (Perkin-Elmer, Batch No. 3541-179, radiochemical purity 99.18%, specific activity 52 mCi/mmol) at a concentration of 1 mg/kg. An excretion kinetics study was conducted using 4 rats/sex/group as follows: a single low-dose (at 1 mg/kg), a single high-dose (at 100 mg/kg) and a repeated low-dose (at 1 mg/kg for 14 consecutive days with non-radiolabelled Chloropicrin (Niklor, Batch No. 306022) followed by the administration of one dose of [¹⁴C]-Chloropicrin of 100 mg/kg on day 15). In the preliminary and the excretion studies, samples of urine, faeces, expired air and selected tissues were collected to determine levels of total radioactivity. A blood kinetics study (using 4 rats/sex) and a tissue distribution study (using 8 rats/sex) were conducted using the same dosing regime described for the excretion study, to determine the total residues present in selected tissues at maximum and half maximum blood concentration levels.

Results:

A. PILOT STUDY

The main route of excretion was via the expired air, with means of 37.74% and 42.94% of the recovered dose for males and females, respectively, mainly trapped as ¹⁴CO₂. Levels of total radioactivity in all the tissues were low following sacrifice for both males and females, with the highest concentrations measured in the stomach. No single tissue accounted for more than 1% of the administered dose, however, the residual carcasses accounted for between 6 and 7% for both male and female rats.

B. MAIN STUDY

The overall recovery of radioactivity at low dose ranged between 100.1% and 107.9%. The recovery of radioactivity at high dose in male rats was slightly lower with 87.72% and 87.3% of dose recovered. The recovery at high dose in females was 100.4% and 102.9% of dose. The recoveries for the repeat dosed animals ranged between 103.7% and 108.4% of dose recovered.

The main route of elimination was *via* the expired air in both males and females, with means ranging from 41.23% to 63.03% of the recovered dose. Elimination *via* urine was a significant route and accounted for approximately 20% of the administered dose in the single and repeated low dose groups. Approximately 30% of the dose was eliminated *via* urine in the single high oral dose group. Elimination *via* faeces was similar in the single and repeated low dose groups, with approximately 15% of the administered dose. Significant lower levels were observed in the single high dose group (10%). Results are summarised in Table B.6.1-1.

The blood kinetics were similar in the single and repeated low dose groups. Maximum levels of radioactivity were observed between 6 and 24 hours after dosing. In the high dose group, the maximum levels of radioactivity were reached at 24 hours post dose. Results are summarised in Table B.6.1-1.

Table B.6.1-1: Mean Chloropicrin at each dose level in the metabolism study

	Matrix	Males	Females
Excretion kinetics: Single low oral dose (1 mg/kg)	Cumulative expired air Cumulative urine Cumulative faeces Whole blood Lung Liver Stomach Kidney	57.56% 18.14% 15.70% 0.083 ppm 0.097 ppm 0.188 ppm 0.224 ppm 0.110 ppm	60.38% 18.61% 14.83% 0.127 ppm 0.123 ppm 0.247 ppm 0.488 ppm 0.134 ppm
Blood kinetics: Single low oral dose (1 mg/kg)	Blood C _{max} Blood ½ C _{max}	0.417 ppm at 6 h 0.221 ppm at 48 h	0.443 ppm at 24 h 0.201 ppm at 72 h
Excretion kinetics: Single high oral dose (100 mg/kg)	Cumulative expired air Cumulative urine Cumulative faeces Whole blood Lung Liver Stomach Kidney	41.23% 29.50% 11.52% 9.86 ppm 13.27 ppm 14.60 ppm 30.80 ppm 11.79 ppm	49.19% 33.78% 9.34% 10.75 ppm 13.50 ppm 14.92 ppm 34.81 ppm 12.84 ppm
Blood kinetics: Single high oral dose (100 mg/kg)	Blood C _{max} Blood ½ C _{max}	50.225 ppm at 24 h 23.85 ppm at 48 h	52.505 ppm at 24 h 27.94 ppm at 48 h
Excretion kinetics: Repeated low oral dose (1 mg/kg)	Cumulative expired air Cumulative urine Cumulative faeces Whole blood Lung Liver Stomach Kidney	63.03% 17.40% 14.27% 0.120 ppm 0.128 ppm 0.264 ppm 0.453 ppm 0.155 ppm	59.22% 20.02% 14.80% 0.106 ppm 0.115 ppm 0.248 ppm 0.397 ppm 0.143 ppm
Blood kinetics: Repeated low oral dose (1 mg/kg)	Blood C _{max} Blood ½ C _{max}	0.437 ppm at 8 h 0.259 ppm at 48 h	0.502 ppm at 8 h 0.258 ppm at 48 h

$C_{\max}$: maximum concentration

$\frac{1}{2} C_{\max}$: approximately half the maximum concentration

The levels of radioactivity in tissues and body fluids, at maximum and half maximum blood concentration levels ($C_{\max}$ and $\frac{1}{2} C_{\max}$), were similar in all dosing regimes. Therefore, it was considered that the distribution and elimination were not affected by increasing blood concentrations or prolonged exposure. The highest concentrations of total radioactivity were observed in stomach (dosing site). Only the highly vascular tissues (kidneys, liver, lungs and spleen) had residue levels similar or greater than those measured in the whole blood. The residual carcasses accounted for between 5 and 8% of the administered dose following the collection period. Results are summarised in Table B.6.1-2. Cumulative and $T_{\max}$ tissue levels were proportional to blood concentration and consistent with increasing dose or duration of exposure. $T_{\max}$ represents the time (6, 8, 24, 48 hours post exposure) when maximum blood levels were observed during the phase of blood collection.

Table B.6.1-2: Concentration of total radioactivity in tissues collected at sacrifice at $T_{\max}$ (6 and 24 hours respectively for males and females rats) following single or repeated oral dosing

Tissue	Tissue distribution study					
	Single Oral Dose (1 mg/kg)		Single Oral Dose (100 mg/kg)		Repeated Oral Dose (1 mg/kg)	
	Males (ppm)	Females (ppm)	Males (ppm)	Females (ppm)	Males (ppm)	Females (ppm)
Blood	0.359	0.338	42.026	42.921	0.445	0.481
Lung	0.273	0.339	40.569	39.424	0.351	0.384
Stomach	3.959	1.983	163.379	102.645	4.525	5.529
Marrow	0.554	1.313	48.832	47.238	0.655	0.540
Kidneys	0.480	0.497	55.293	57.144	0.626	0.641
Spleen	0.301	0.301	34.886	28.843	0.305	0.336
Intestine	0.709	0.411	62.616	53.119	1.091	0.970
Liver	1.669	1.006	87.258	77.636	1.800	1.530

Analysis of urine samples showed that Chloropicrin was extensively transformed into several polar metabolites. Details of the retention times of each metabolite and their proportion of the initial dose are provided in the following Table B.6.1-3.

Table B.6.1-3: Percentage of the dose represented by each metabolite in males and females

Excretion	Male Urinary Metabolites		Female Urinary Metabolites	
	Retention Time (min)	% Dose	Retention Time (min)	% Dose
Low dose	4.7	2.72	37.0	3.59
	10.4	2.19	12.8	2.78
	5.4	2.22	19.6	2.35
	13.5	1.39	29.6	1.54
	16.5	1.77	-	-
	37.3	2.33	-	-
High dose	11.5	16.92	11.2	19.05
	19.4	3.65	21.1	3.42
	41.2	2.93	41.3	4.51
Repeat dose	41.3	8.00	40.39	4.02
	20.7	6.19	10.3	2.86
	-	-	5.4	1.69
	-	-	12.2	1.42
	-	-	19.1	2.36
	-	-	26.9	1.37

Conclusions:

In conclusion, Chloropicrin is mainly excreted via expired air as $^{14}\text{CO}_2$ (approximately 60% of the low dose and 40-50% of the high dose). Elimination via urine accounted for 20% (low dose) to 30% (high dose) and via faeces accounted for 10 to 15% (both doses). The maximum levels of total radioactivity in blood samples were measured between 6 and 24 hours following dosing. Small amount of total radioactivity were found in tissues, (< 1% in each one), with the highest values measured in the stomach and liver. The residual carcasses accounted for between 5 and 8% of the administered dose following the collection period. No significant differences were reported between single and repeated dose treatment and between genders.

The results showed that Chloropicrin is rapidly (C_{max} : 6-24 h) and extensively absorbed (>85%), (based on data on radioactivity recovered in expired air, urine and carcass) and widely distributed to different tissues. The presence of $^{14}\text{CO}_2$ in expired air and of polar metabolites in urine, indicated that the test substance is extensively metabolized and converted into several polar metabolites which have not been identified. This toxicokinetic study provides sufficient information on the absorption, distribution, metabolism and excretion and is therefore considered acceptable. The metabolites and pathways of Chloropicrin biotransformation in the mammalian body are described in more detail in IIA 5.1/03 and IIA 5.1/04 which follow.

The study is considered acceptable.

Sparks, S.E., Quistad, G.B., Casida, J.E. Chloropicrin: Reactions with Biological Thiols and Metabolism in Mice. University of California, Berkeley. Chem. Res. Toxicol., 10, 1001-1007, 1997. (IIA 5.1/03)

GLP: No

Methods:

In order to study the metabolism of Chloropicrin, male albino Swiss-Webster mice were administered with [^{14}C]-Chloropicrin ($^{14}\text{CCl}_3\text{NO}_2$, 1.5 mCi/mmol, > 98% radiochemical purity) intraperitoneally and orally at 1-3 mg/kg using triethylene glycol monomethyl ether as a carrier vehicle. Urine, faeces and volatile compounds were collected for analysis. Animals

were sacrificed at either 1 or 48 hours for tissues collection. Chloropicrin was also tested in different *in vitro* experiments so that reactions with thiols and non-thiol amino acids, GSH, proteins, liver microsomes and cytosol could be studied.

Results:

Radiocarbon recoveries showed that the urine was the major route for excretion of metabolites, with 51-55% excreted within 48 hours after i.p. or oral administration and about 9% in the feces. Radioactivity present in expired air accounted for 18 and 8% after i.p. and oral dosing, respectively. However, it has to be noted that the total radioactivity recovery was quite low, namely, 81 and 65% with the two route of administration and therefore it is likely that a higher recovery could be attained by increasing the number of traps for volatile compounds or improving their trapping efficiency.

Tissue levels of metabolites established two relationships: the radiocarbon recoveries decreased greatly between 1 and 48 hours, and the tissue levels at 48 hours from oral administration were similar to the levels measured from the intraperitoneal administration. 1 hour after treatment, 6% of the administered radiocarbon compounds were detected in the liver. However, $^{14}\text{CCl}_3\text{NO}_2$ (^{14}C -Chloropicrin), $^{14}\text{CHCl}_2\text{NO}_2$ and $^{14}\text{CH}_2\text{ClNO}_2$ were not found. Only 57% of the radiocarbon in the liver was extractable and consisted of polar compounds. $^{14}\text{CHCl}_2\text{NO}_2$ was not found in blood. The urinary metabolites at 24 hours were polar and non-volatile. ^{14}C -Chloropicrin is expected to react with N-acetylcysteine and cysteine in buffer either alone or with GST enhancement to form products excreted in urine. One half of the radioactivity in feces is methanol extractable and characterized by high polarity.

Conclusions:

The principal initial reactions of Chloropicrin in the cell involve rapid dechlorination to CHCl_2NO_2 and conversion of glutathione (GSH) to glutathione disulfide (GSSG) plus possible adduct formation with thiol proteins (Figure B6.1-1). GSH reacts with a molar equivalent of Chloropicrin to give both CHCl_2NO_2 and GSSG. The initial adduct with Chloropicrin and GSH is apparently unstable, since attempts to isolate such product were unsuccessful.

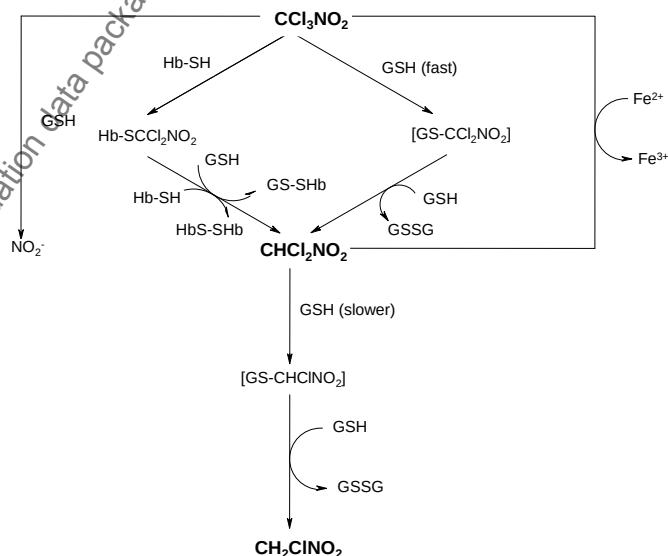


Figure B.6.1-1: Proposed pathways for reaction of Chloropicrin with GSH and Hb-SH as a model thiol protein

Chloropicrin oxidises protein thiols with formation of disulfide bonds. [¹⁴C]-Chloropicrin reacts with Hb, used as a model thiol protein, to give a labelled adduct with the globin component. The Hb adduct formation presumably involves derivatisation of a cysteine in the β-chain. When GSH and protein thiols are present in similar molar concentrations, as expected in liver, there is a preferential reaction with GSH; thus, treatment of equimolar GSH and Hb with a molar equivalent of Chloropicrin produced 73% CHCl₂NO₂ and 4% Hb adduct. Chloropicrin is also rapidly degraded into CHCl₂NO₂ and CH₂ClNO₂ *in vitro* by liver microsomes and liver cytosol, in the presence of NADPH.

The study is considered acceptable although not conducted to GLP principles.

Sparks, S.E., Quistad, G.B., Li, W., Casida, J.E. Chloropicrin Dechlorination in Relation to Toxic Action. University of California, Berkeley. J. Biochem. Molecular Toxicology, 14, 1: 26-32. 2000 (IIA 5.1/04)

GLP: No

Methods

To identify metabolites, urine was collected from 4 Swiss-Webster mice treated intraperitoneally with Chloropicrin (CCl₃NO₂) at 0.03 mmol/kg. Captan and dimethyl sulfoxide were used as positive and solvent controls respectively (Sparks *et al.*, 2000).

Results

Metabolism of Chloropicrin gave raphanusamic acid in the urine from each of four treated mice in an amount greater than 20-fold above the background that was determined in the solvent control mice. Raphanusamic acid was detected at a 2- to 48-fold higher level in urine from mice given captan at the same molar equivalent dose, as positive control.

A proposed metabolic pathway for Chloropicrin involves conversion to raphanusamic acid possibly via CSCI₂ formed from GSH and cysteine adducts as intermediates and β-lyase action (Figure B6.1-2). It is proposed that the initially formed GS-CCl₂NO₂ metabolite either reacts further with GSH forming CHCl₂NO₂ and CH₂ClNO₂ or is cleaved by cysteine β-lyase, leading to raphanusamic acid.

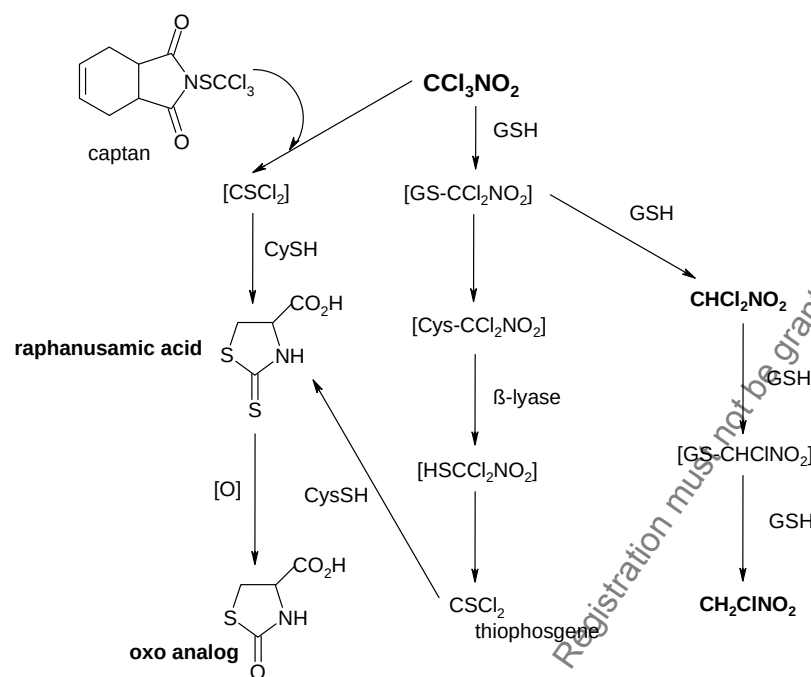


Figure B.6.1-2: Metabolism of Chloropicrin in mice by dechlorination and conversion to raphanusamic acid via thiophosgene

Conclusion:

Chloropicrin is rapidly dechlorinated into CHCl_2NO_2 with conversion of GSH to GSSG. It is also proposed that the GS-adduct intermediate could cleave, leading to raphanusamic acid. A minor pathway has been identified involving adduct formation with thiol proteins such as Hb-adduct. Chloropicrin is also metabolised into CHCl_2NO_2 and CH_2ClNO_2 by liver microsomes and liver cytosol.

The study is considered acceptable although not conducted to GLP principles.

Overall conclusion of ADME studies (IIA 5.1):

Chloropicrin is mainly excreted via expired air as $^{14}\text{CO}_2$ (approximately 60% of the low dose and 40-50% of the high dose). Elimination via urine accounted for 20% (low dose) to 30% (high dose) and via faeces accounted for 10-15% (both doses). The maximum levels of total radioactivity in blood samples were measured 6 to 24 hours after dosing. Small amounts of total radioactivity were found in tissues (< 1% in each one), with the highest values measured in the stomach and liver. The residual carcass accounted for 5-8% of the administered dose following the collection period. No significant differences were reported between single and repeated dose treatment and between genders.

The results showed that Chloropicrin is rapidly (C_{max} : 6-24 h) and extensively absorbed (>85%), based on data on radioactivity recovered in expired air, urine and carcass and widely distributed to different tissues. The presence of $^{14}\text{CO}_2$ in expired air and of polar metabolites in urine, indicated that the test substance is extensively metabolised and converted into several polar metabolites. Chloropicrin is rapidly dechlorinated to CHCl_2NO_2 with conversion of GSH to GSSG. A minor pathway has been identified involving adduct formation with thiol proteins such as Hb-adduct. These processes could be involved in Chloropicrin toxicity. On the basis of these results both Chloropicrin and its metabolites are considered toxicologically relevant compounds.

B.6.2 Acute toxicity including irritancy and skin sensitisation (IIA 5.2)

B.6.2.6 Skin sensitisation studies (IIA 5.2.6)

No study is available to address this point. Undiluted Chloropicrin is severely and immediately irritating to the upper respiratory tract, eyes and skin following direct contact. Moreover, due to its high volatility, the topical application of Chloropicrin is not technically conceivable. For these reasons dermal sensitization testing with Chloropicrin has not been undertaken taking into account animal welfare as well as methodological concerns. Furthermore, it is recognised that methods for assessing dermal sensitization are subject to false positive results if the test material is known to be a strong skin irritant and considering the high volatility of Chloropicrin results would be difficult to interpret in the case of loss of the test item from the skin surface.

On the basis of these assumptions and taking into account the animal welfare issue, the RMS agrees with the Notifier, considering inappropriate and unnecessary to carry out such a study.

Chloropicrin is not classified as a skin sensitizer according to the Council Directive 67/7548/EEC. It has not been reported to be a sensitizer in the literature (TOXLINE and MEDLINE). Moreover, the aggressive action of Chloropicrin on skin is a major deterrent to allowing skin contact, a fact which is recognised in label warning statements and recommendations for personal protective equipment for those using Chloropicrin. During application and tarp-removal for Chloropicrin, the operator/worker wears full PPE including a half face mask, coveralls and gloves and therefore would not come into contact with Chloropicrin at any stage. This eliminates the risk of a sensitising effect. Furthermore, Chloropicrin is applied by a closed system and operator exposure to liquid Chloropicrin is not expected.

Therefore, it is concluded that Chloropicrin does not require classification as a skin sensitizer.

B 6.2.7 Summary of acute toxicity, including irritancy and sensitisation, studies (IIA 5.2)

Chloropicrin is harmful following ingestion and is irritant to the skin and eyes. It is unlikely to be a skin sensitizer.

The 4-hour whole body inhalation LC_{50} for Chloropicrin vapour in rats ranged from 11.9ppm (0.08mg/l) to 18.9 ppm (0.127 mg/l) for both sexes. Studies carried out to compare the effects on male rats following administration by whole-body and nose-only inhalation exposure for 4 hours indicate that highest acute inhalation toxicity was in the nose-only exposure experiment with a LC_{50} of 6.6 ppm (equivalent to 0.04 mg/l). Therefore Chloropicrin resulted highly toxic after inhalation.

The potential for sensory irritation in mice inhaling Chloropicrin vapour for 30 minutes (head only exposure) was also evaluated in a standard RD_{50} study. The RD_{50} value for mice was 2.34 ppm (0.016 mg/l). No adverse effects were observed in mice at the RD_{50} concentration of Chloropicrin vapour other than transient respiratory rate changes, characteristic of sensory irritation.

B.6.3 Short-term toxicity (IIA 5.3)

B.6.3.1 Oral 28-day toxicity (IIA 5.3.1)

B.6.3.2.3 Oral 1 year toxicity in dogs (IIA 5.3.4 – EU 5.3.2)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made

Wisler, J. A. Evaluation of Chloropicrin in a One Year Oral (Capsule) Toxicity Study in Dogs. [REDACTED] Unpublished report-No 656-005. Owner European Chloropicrin Group. 1994. IIA, 5.3.4/01 (EU-5.3.2)

GLP: Yes

Methods:

A one year oral (capsule) toxicity study with Chloropicrin (purity, 99.6%; Lot 920130-1, Niklor Chemical Corporation) in dogs was performed. This work was completed in 1994 and followed EPA Pesticide Assessment Guidelines for a non-rodent carcinogenicity and chronic toxicity study as well as the EPA Good Laboratory Practices Regulations. Sixteen male and 16 female 4-month old Beagle dogs were tested with 0, 0.1, 1.0 or 5.0 mg/kg/day Chloropicrin administered orally each day for one year in a gelatine capsule at approximate volumes of 0.1ml/kg. The dosing vehicle and control material was corn oil. Dose suspension stability was confirmed prior to test initiation and dose concentrations were confirmed at 16 intervals during the study. The animal body weight range at study initiation was 10.7-14.8 Kg (males) and 9-12.0 Kg (females). Test and control materials were administered daily for one year. The observational parameters included survival, morbidity and clinical signs twice daily throughout study and a detailed clinical examination weekly throughout study. Individual body weight and food consumption were determined prior to study initiation, weekly during weeks 1-14 and once every 4 weeks beyond week 14 until study termination. Ophthalmoscopic examination (cornea, conjunctiva, sclera, iris, and fundus) was conducted at study initiation and study months 3, 6, 9 and 12. Physical examination by veterinarian was completed at study initiation and study months 3, 6, 9 and 12 and clinical chemistry, haematology and urinalysis were performed at initiation and study months 3, 6, 9 and 12. Post-mortem observations included gross necropsy and organ weights (5 organs) on all animals. Tissue for microscopic analysis was collected (50 tissues plus lesions) from all animals.

Results:

Chloropicrin doses were stable throughout the administration period and were 89-114% of the intended dose. All animals survived to study termination. There was a dose-related incidence of emesis affecting all animals at 1 mg/kg/day and above. This did not interfere with test material administration. Soft stool/diarrhea, ptalysism and discoloured faeces (during the last 13 weeks of the study) occurred in the high-dose animals. Body weight in high-dose males was decreased relative to controls. Body weights are presented in Tables B.6.3.2.2-1 and B.6.3.2.2-2. Physical examination, ophthalmoscopy and urinalysis did not reveal treatment-related effects. Haemoglobin levels were depressed in high-dose male animals. MCV and MCH were also decreased in high-dose animals of both sexes. Serum calcium concentrations were depressed and serum aspartate aminotransferase (AST), total protein, albumin and globulin levels were significantly decreased in the high-dose group relative to controls. No gross anatomic changes neither hystopathological alterations were noted in treated animals. There were no statistically-significant differences in absolute and relative organ weights among any of the treated animals.

Table B.6.3.2.2-1: Summary of Male Body Weight Values

Week of Study	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
0	12.8 ± 0.80	13.1 ± 1.46	12.6 ± 1.22	12.6 ± 1.21
1	12.7 ± 0.62	13.1 ± 1.42	12.5 ± 0.93	12.5 ± 1.26
2	13.1 ± 0.87	13.8 ± 1.70	13.3 ± 1.35	13.0 ± 1.33
3	13.4 ± 1.07	13.7 ± 1.74	13.2 ± 0.87	13.2 ± 1.42
4	13.7 ± 1.19	14.6 ± 1.76	13.6 ± 1.09	13.4 ± 1.57
5	13.8 ± 1.34	14.8 ± 1.92	13.9 ± 1.30	13.4 ± 1.35
6	14.0 ± 1.41	15.1 ± 2.16	14.1 ± 1.51	13.5 ± 1.40
7	14.2 ± 1.31	15.5 ± 2.28	14.1 ± 1.53	13.6 ± 1.39
8	14.3 ± 1.50	15.5 ± 2.40	14.5 ± 1.63	13.6 ± 1.45
9	14.5 ± 1.45	15.7 ± 2.46	14.7 ± 2.04	13.6 ± 1.50
10	14.6 ± 1.63	15.7 ± 2.62	15.0 ± 2.20	13.6 ± 1.51
11	14.7 ± 1.69	15.6 ± 2.30	14.9 ± 2.22	13.6 ± 1.36
12	14.8 ± 1.70	15.8 ± 2.45	14.9 ± 2.22	13.8 ± 1.50
13	14.1 ± 1.85	15.1 ± 2.59	14.4 ± 2.49	13.3 ± 1.39
14	14.8 ± 1.72	15.9 ± 2.47	15.4 ± 2.34	14.0 ± 1.40
18	15.1 ± 1.59	16.0 ± 2.95	15.7 ± 2.56	14.5 ± 1.29
22	15.5 ± 1.79	16.5 ± 2.95	15.8 ± 2.70	14.3 ± 1.44
26	15.5 ± 1.40	16.7 ± 3.02	16.4 ± 2.54	14.7 ± 1.50
30	15.6 ± 1.39	17.2 ± 3.08	16.5 ± 2.73	14.9 ± 1.19
34	15.9 ± 1.66	17.6 ± 3.23	16.5 ± 2.99	14.8 ± 1.10
38	16.2 ± 1.60	17.6 ± 3.19	17.1 ± 3.09	14.8 ± 1.16
42	16.5 ± 1.41	17.8 ± 3.05	17.6 ± 3.69	15.2 ± 1.52
46	16.4 ± 1.73	17.6 ± 3.27	17.4 ± 3.56	15.3 ± 1.22
50	16.5 ± 1.81	17.5 ± 3.63	17.9 ± 3.63	15.3 ± 1.54
52	16.6 ± 1.70	18.1 ± 3.53	17.8 ± 4.02	15.0 ± 1.41

Table B.6.3.2.2-1: Summary of Female Body Weight Values

Week Study	0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
0	9.2 ± 0.67	9.5 ± 0.93	9.8 ± 1.55	9.2 ± 0.95
1	9.3 ± 0.69	9.6 ± 0.93	9.8 ± 1.48	9.2 ± 0.92
2	9.6 ± 0.77	9.8 ± 1.19	10.4 ± 1.52	9.7 ± 0.92
3	9.7 ± 0.85	10.1 ± 1.08	10.6 ± 1.64	9.8 ± 1.04
4	9.7 ± 0.80	10.2 ± 1.20	10.7 ± 1.69	9.9 ± 0.98
5	9.9 ± 0.90	10.3 ± 1.22	10.9 ± 1.69	10.0 ± 1.10
6	10.1 ± 0.87	10.7 ± 1.37	11.1 ± 1.78	10.3 ± 1.04
7	10.1 ± 0.76	10.7 ± 1.26	11.1 ± 1.80	10.3 ± 0.93
8	10.1 ± 0.87	10.8 ± 1.44	11.2 ± 1.90	10.6 ± 0.96
9	10.2 ± 0.90	11.0 ± 1.45	11.5 ± 2.00	10.4 ± 0.90
10	10.3 ± 0.96	11.0 ± 1.50	11.5 ± 1.90	10.7 ± 0.79
11	10.2 ± 0.85	11.0 ± 1.51	11.6 ± 1.97	10.6 ± 0.88
12	10.3 ± 0.78	11.3 ± 1.73	11.7 ± 1.71	10.8 ± 1.00
13	10.0 ± 0.81	10.8 ± 1.87	11.3 ± 1.59	10.3 ± 1.00
14	10.5 ± 0.86	11.2 ± 1.59	11.6 ± 1.76	11.0 ± 0.90
18	10.6 ± 0.61	11.7 ± 1.72	12.3 ± 1.91	11.1 ± 0.95
22	10.7 ± 0.75	11.6 ± 1.93	12.3 ± 2.07	11.0 ± 1.08
26	10.9 ± 1.34	12.1 ± 2.19	12.6 ± 2.54	11.4 ± 1.33
30	11.0 ± 1.42	12.5 ± 2.43	13.1 ± 2.73	11.5 ± 1.30
34	11.1 ± 0.98	12.7 ± 2.64	13.3 ± 3.31	11.8 ± 1.01
38	11.2 ± 0.63	13.1 ± 3.06	13.8 ± 3.85	11.9 ± 0.97
42	11.4 ± 0.61	13.0 ± 2.91	14.2 ± 3.75	12.2 ± 1.23
46	11.9 ± 0.57	13.5 ± 3.24	14.2 ± 3.51	12.1 ± 0.95
50	11.7 ± 0.48	13.6 ± 3.24	14.4 ± 3.43	12.1 ± 1.46
52	11.6 ± 0.43	13.5 ± 3.33	14.2 ± 3.39	11.6 ± 1.73

The RMS considered emesis, as an adverse effect due to the irritant properties of Chloropicrin. The emesis was recorded usually as either frothy emesis or food-like emesis with varying degrees of severity described as slight, moderate or marked. Although emesis was present also in some animals both in the control group and at 0.1 mg/kg/day, some significant differences, in terms of incidence and severity (moderate to marked), with respect to the control group were evident at doses ≥ 1 mg/kg/day.

The incidence of emesis (frothy and food-like) is summarised in Table B.6.3.2.2-2.

Table B.6.3.2.2-2: Incidence of emesis

Sex	Weeks		Emesis incidence (% dogs affected)			
			0 mg/kg/day	0.1 mg/kg/day	1.0 mg/kg/day	5.0 mg/kg/day
Males	1-13	Food-like	0	50	100	100
		Frothy	50	0	25	100
	14-26	Food-like	25	75	75	100
		Frothy	25	50	75	100
	27-39	Food-like	25	75	25	100
		Frothy	25	50	75	100
	40-52	Food-like	25	75	25	100
		Frothy	50	50	75	100
Females	1-13	Food-like	25	0	50	100
		Frothy	25	25	25	100
	14-26	Food-like	25	0	50	100
		Frothy	75	25	25	100
	27-39	Food-like	0	0	75	100
		Frothy	50	25	50	100
	40-52	Food-like	25	0	75	100
		Frothy	0	25	75	100

Although greater inter-individual variation especially in females, was recorded, in both sexes the incidence and severity of emesis was significantly increased in the higher dose groups, and so the incidence of emesis is considered dose-related, and therefore of toxicological significance.

Conclusions:

Based on the results obtained in this study the incidence of emesis is considered as treatment-related, and therefore of toxicological significance. Moreover, although no damages to tissue architecture were noted, emesis has to be considered as an adverse effect due to the irritant properties of chloropicrin. In conclusions, emesis affected animals at 1 mg/kg and above and therefore a **NOAEL of 0.1 mg/kg bw/d is established**.

The study is considered acceptable.

B.6.3.3 Oral 90-day toxicity (IIA 5.3.6)

For all of the repeat dose inhalation toxicity studies the rat systemic absorbed doses were calculated using the following equation:

Internal daily dose (mg/kg bw/day) = Inhalation exposure (mg/l) x 45 l/kg bw/h (rat respiration rate) x duration of exposure per day (hours). Absorption was assumed to be 100%.

The same equation was used for studies in the mouse except that a default respiration rate of 80 l/kg bw/h was used.

B.6.3.3.1 90-day inhalation toxicity in rats and mice (IIA, 5.3.6)

Chun, J.S., Kintigh, W.J. Chloropicrin: Ninety-Day Inhalation Toxicology Study In Rats And Mice.

Unpublished report-No 91N0098. Owner European Chloropicrin Group.
1993. IIA, 5.3.6/01 (EU- 5.3.3)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

GLP: Yes

Methods:

Ten male and ten female CD rats and CD-1 mice were exposed in whole body inhalation chambers to Chloropicrin vapour at concentrations of 0, 0.3, 1.0 or 3.0 ppm for 6 hours per day, 5 days per week for 13 weeks in 900L stainless steel and glass dynamic chambers. Chloropicrin was 99.6% pure (Lot numbers 31291-A and 920130-2 from Niklos Chemical Company). At initiation of exposures, the body weight range for rats was 252-349 g for males and 169-223 g for females. The weight range for mice at study initiation was 27-38 g and 21-27 g for males and females, respectively. The animals were observed for standard toxicological parameters during the in-life phase of the investigation and were subjected to necropsy and extensive histopathological examination following termination. Ocular examination, organ-body weight ratios were performed and special attention was given to respiratory tissues and all lesions.

Results:

Treatment-related mortality occurred in 3 of 10 male rats in the high-dose group during the exposures. Clinical signs of exposure in the high-dose group included discoloration in rats and dehydration in mice. During the first week of exposures, decreases in body weight and body weight gain was evident in rats of each sex in the high-dose groups and in male of the 1 ppm group. Body weight and/or body weight gain were decreased in male mice from the 1 and 3 ppm groups and females from the high-dose group during the entire study period. These effects diminished with continued exposure but ultimately weight gain suppression was seen in male mice of each treatment group, in female mice of the mid- and high-exposure groups and rats of each sex in the mid-and high-dose groups. At the end of the study male mean bodyweight of the 0.3 ppm group was 94% of the male mean control bodyweight. Decreases in food consumption paralleled the decrease in weight gain. Increases in haemoglobin concentration, haematocrit and total erythrocyte count were observed for male mice in all exposure groups. An increase in haemoglobin concentration was significant also in male rats from the high-dose group. Female mice showed a dose-related decrease in monocyte count. Total serum protein, albumin and calcium were increased in male mice from the 3 ppm group. Increased relative and absolute lung weight was seen in rats and mice in the mid- and high-dose groups. A slight increased lung weight relative to brain weight, but not in absolute and relative organ weight, were observed in the male rats from the 0.3 ppm group. Accompanying these increased weights were microscopic lesions in the lungs and airways consistent with acute and chronic inflammation and non-specific irritation. These changes included hyperplasia of the respiratory epithelium of the nasal cavities, goblet cell hyperplasia, bronchial/bronchiolar epithelial hyperplasia and peribronchial fibrosis. The respiratory tissue changes, seen in rats and mice of each sex, were evidenced in animals from the mid- and high-exposure groups. Two female mice from the low exposure group (0.3 ppm) had evidence of hyalin inclusion in the respiratory tissue. Increased incidence of goblet cell hyperplasia in the nasal cavities was evidenced, also in female rats at 0.3 ppm. No treatment related changes were seen in organs or tissues, in the eyes or in haematological, biochemical or urine parameters other than those specified above.

Conclusions:

Systemic effects in male and female animals from both species and mortality in male rats were seen at the highest exposure level (3 ppm). Respiratory system effects, including inflammatory changes in the nasal cavities and lungs, were seen in rats and mice exposed to 1 ppm. Body weight gain decrements were also seen in mice of each sex and male rats exposed at this level. Exposure to 0.3 ppm produced slight decreases in body weight and food

consumption in male mice as well as minimal microscopic changes in the nasal cavities, of female rats and mice. The dose-dependent weight loss, together with adverse effects indicative of respiratory inflammation, in mice and female rats, evidenced at 0.3 ppm (equivalent to 0.54 mg/kg/day for rats and 0.96 mg/kg/day for mice) and above, lead RMS to consider that a NOAEL could not be derived. Effects of exposure to chloropicrin occur in rodents at concentrations of 0.3 ppm or greater. These effects, inflammatory lesions, can be seen under microscopic examination in the respiratory tract.

The study is considered acceptable, although it does not provide any NOAEL.

Yoshida, M., Ikeda, T., Iwasaki, M., Ikeda, M., Harada, T., Ebino, K., Tsuda, S., Shirasu, Y. Subchronic Inhalation Toxicity of Chloropicrin Vapor in Rats. Mitsukaido Laboratories, Institute of Environmental Toxicology, Uchimoriya-cho, Mitsukaido 302-02, Japan. Published: J.Pesticide Sci. 12. 673-681. 1987. IIA, 5.3.6/02. (EU – 5.3.3)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

GLP: No

Methods:

Yoshida, *et al.*, exposed groups of 12 male Fischer-344 rats to the Chloropicrin vapour actual concentrations of 0, 0.37, 0.67, 1.58 or 2.93 ppm for 6 hours/day, 5 days/week, for 13 consecutive weeks. These exposure levels were selected on the basis of the results of the 2-week pilot study. Chloropicrin test material was 99.7% pure and was obtained from a commercial chemical supplier (Wako Pure Chemical Industries, Inc.). Male F-344 rats were 8 weeks old at study initiation. The animals were observed for standard toxicological parameters during the in-life phase of the investigation and were subjected to necropsy and extensive histopathological examination following termination. Ocular examination and organ-body weight ratios were also reported. Chamber concentrations of Chloropicrin were measured twice per day using a gas chromatographic method.

Results:

Exposures over the 13 weeks ranged from 106 to 126% of nominal. All animals survived exposures. Eyelid closure and decreased spontaneous motor activity were reported for 2.93 and 1.58 ppm Chloropicrin groups in the study. These behavioural changes were observed also in the 0.67 and 0.37 exposed groups during the later half of the treatment period. All groups showed dose-dependent growth suppression, reaching a statistically significance in the two higher dose groups.

Some alterations in haematology and serum biochemistry, including increases in hematocrit, haemoglobin, RBC, BUN and AIP were reported in 2.93 ppm group. The rats exposed to 1.58 and 0.67 ppm Chloropicrin had a significant increase of RBC and haemoglobin, respectively. There were no treatment-related gross lesions reported. Absolute and relative lung weights were reported for the high-dose group. The rats from the 1.58 ppm group showed a significant increase in the relative lung weight. Histopathological changes were found in the respiratory system and occurred in the two highest dose levels. These consisted of inflammation of the nasal mucosa, hypertrophy of the laryngeal and tracheal epithelium, bronchus and bronchiolar hypertrophy and degeneration including necrosis.

Conclusions:

On the basis of the results obtained from this study, decrease in mean body weights, changes in haematological and serum biochemical parameters, changes in organ weights and

histopathological lesions in the respiratory tract, were evidenced at 0.67 ppm (equivalent to 1.21 mg/kg bw/day) and above. However, some behavioural effects and slight weight loss were observed also in the low dose group (0.37 ppm).

The study is considered acceptable, although not conducted according to GLP principles. However, it has been considered by RMS only as a supportive study, since it does not provide a clear NOAEL and the description gives very limited information on single and raw data, as expected being published on a scientific Journal.

B 6.3.4 Summary of short term toxicity

The short-term oral toxicity studies on Chloropicrin were performed at 90-day in rats and at 8 weeks and 1 year in dogs. The NOAEL for Chloropicrin administered in rats by oral gavage for 90 days, has been established equal to 8 mg/kg, based on effects measured at the higher dose (mainly the histopathological changes in the forestomach).

In a 1-year dog study, based on emesis observed at 1 mg/kg and above, a NOAEL of 0.1 mg/kg bw/d was established.

From the 90-day inhalation toxicity studies performed on rats and mice it has been established that respiratory tissue is the major target of toxic effects due to exposure to Chloropicrin vapour. Due to adverse effects occurring in the upper respiratory tissue of animals inhaling chloropicrin vapour at the lowest concentrations tested, that is 0.3 ppm (equivalent to 0.54 mg/kg/day for rats and 0.96 mg/kg/day for mice) a reliable NOAEL could not be determined from the key study.

Table B.6.3.1 Summary of short term toxicity studies with Chloropicrin

Type of study	Species	Dose range tested	NOEL/NOAEL	Reference
Oral 90 d	Rat	0-30 mg/kg bw/day	NOAEL: 10 mg/kg bw/day	Hilaski (1996) (rep-No 656-002)
Oral 90 d	Rat	0-32 mg/kg bw/day	NOAEL: 8 mg/kg bw/day	Condie (1994) Drug and Chemical Toxicology, 17 (2). 125-137
8 weeks oral	Dog	0-30 mg/kg bw/day	NOEL/NOAEL not established	Hilaski (1996) (rep-No 656-004)
Oral, 1-year	Dog	0-5 mg/kg bw/day	NOAEL: 0.1 mg/kg bw/day	Wisler (1994) (rep-No 656-005)
Inhalation 90 d-key study	Rat, Mouse	0-3.0 ppm/6 hrs/day	LOAEL: 0.54 mg/kg bw/day (rat) 0.96 mg/kg bw/day (mice)	Chun (1993) (rep-No 91N0098)
Inhalation 90 d-supportive study	Rat	0-2.93 ppm/6 hrs/day	NOAEL: not established	Yoshida (1987) J.Pesticide Sci. 12. 673-681.

B 6.4 Genotoxicity (IIA 5.4)

No new data or modification of evaluation.

B6.4.3 Summary of genotoxicity

The results of mutagenicity tests in vitro indicate that pure (>99%) Chloropicrin is weakly mutagenic in bacteria in the presence of exogenous metabolic activation, and clastogenic in cultured mammalian cells, only in the absence of metabolic activation and at doses producing significant toxicity. Negative results were obtained in a mammalian cell gene mutation assay. In vivo, negative results were obtained in an adequately performed mouse bone marrow micronucleus test, with evidence of toxicity to target cells, and in the rat liver UDS. Based on these findings, it is concluded that the genotoxic potential observed in vitro is not expressed in detectable way in vivo, and therefore Chloropicrin can be evaluated as **not genotoxic** in vivo.

B.6.5 Long-term toxicity and carcinogenicity (IIA 5.5)

B.6.5.2 Inhalation study in rats

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

Burleigh-Flayer, H.D., Benson, C.L. Chloropicrin: Vapor Inhalation Oncogenicity Study in CD Rats.

Unpublished report-No 92N1106. Owner European Chloropicrin Group. 1995. IIA, 5.5.2/01 (EU- 5.5)

GLP: Yes

Methods:

The study was performed in accordance with EPA Pesticide Assessment Guidelines for chronic/oncogenicity toxicity studies and in compliance with EPA Good Laboratory Practices Regulations. Groups of 50 male and 50 female CD rats were exposed in whole body inhalation chambers to 0, 0.1, 0.5 or 1.0 ppm Chloropicrin vapour for 6 hours per day, 5 days per week for 107 or 78 weeks, respectively. The test material was 99.6% pure Chloropicrin (Lot numbers 31291-A and 920130-2 from Niklor Chemical Company). Mean Chloropicrin concentrations in the exposure chambers were 0.100 ppm (± 0.0061), 0.499 ppm (± 0.0219) and 0.985 ppm (± 0.0443). Each chamber atmosphere was analysed by GC/EC approximately six times during each exposure day. The distribution of Chloropicrin vapour within exposure chambers was monitored and found to be uniform. The high-dose chamber was monitored for the presence of phosgene, which was not detected, since phosgene is a possible thermal degradation product of Chloropicrin at high temperature. Study observations included: exposure chamber atmosphere characterization, animal survival, daily observations for pharmacotoxic signs during exposure and during non-exposure periods, weekly recording of food consumption and body weight, ophthalmoscopic examination, haematology and clinical chemistry determinations. At gross necropsy, relative and absolute organ weights were taken, and histopathological analysis was conducted of over 60 representative tissues from high-dose and control animals as well as lesions and target tissue from all animals on study.

Results:

Mean Chloropicrin analytical concentrations of 0.100 (+/- 0.0061), 0.499 (+/- 0.0219), and 0.995 (+/- 0.0443) ppm were measured. A concentration-dependent increase in mortality rate was observed for mid- (0.5 ppm) and high-dose (1 ppm) male rats. However, the number of survivors at study termination was adequate for evaluation of carcinogenic potential. There were no differences in the mortality rate or mean survival times for female animals from the Chloropicrin exposure groups compared to control group (Tables B.6.5-1 and B.6.5-2). Clinical signs were observed during exposures to 1.0 ppm, which included hypoactivity and a

decreased startle reflex. During non-exposure periods no clinical signs related to test material exposure were reported for any group.

Decreased body weight gain was reported for males in the 0.5 and 1.0 ppm groups at the end of Study Weeks 1 and 2. A concentration-related decrease in body weight gain was observed for female rats from the 0.5 and 1.0 ppm exposure groups at the end of the first and second week of exposure. No effects in food consumption were reported for both sexes in any exposure group during the study. No exposure-related changes in haematological parameters were observed for male or female rats in any treated animals at any time during the study. Prior to study termination, no exposure-related ophthalmic lesions were observed in male or female rats.

A slight increase in lung weight (absolute and relative as a percentage of body and brain weight) was noted for both male and female animals in the 1.0 ppm group. There were no gross lesions at necropsy for either sex. The only non-neoplastic lesion observed microscopically was rhinitis within the anterior nasal cavities of male rats in the 1.0 ppm group. The incidence of this lesion was increased in all male rats in the high dose group animals surviving until the end of the study (87% incidence). No exposure-related differences in microscopic frequencies of non-neoplastic lesions were observed for female rats. There were no increased frequencies of neoplastic lesions in either sex that were believed to be related to Chloropicrin exposure. No differences were noted in the relative frequencies of the causes of death in any of the groups of Chloropicrin exposed male or female rats, as compared with controls.

The no-observed-effect level (NOEL) for non-neoplastic effects was 0.1 ppm

Table B.6.5.2 -1: Summary of Mortality (Males)

	Concentration			
	0 ppm	0.1 ppm	0.5 ppm	1.0 ppm
Total Number of Animals	50	50	50	50
Number Sacrificed	29	21	17	15
Number Found Dead	12	19	21	19
Number Sacrificed Moribund	9	10	12	16
Number Accidental Death	0	0	0	0
Mean Survival Time (Days)	696	669	672*	647**
Mortality Rate (%)	42	58	66	70

^a Statistics were performed for mean survival time only using product-limit survival analysis.

*Significantly different from control $p < 0.05$.

**Significantly different from control $p < 0.01$.

Table B.6.5.2 -2: Summary of Mortality (Females)

	Concentration			
	0 ppm	0.1 ppm	0.5 ppm	1.0 ppm
Total Number of Animals	50	50	50	50
Number Sacrificed	25	18	21	22
Number Found Dead	14	15	11	12
Number Sacrificed Moribund	10	17	17	16
Number Accidental Death	1	0	1	0
Mean Survival Time (Days) ^a	697	673	670	661
Mortality Rate (%)	48	64	56	56

^a Statistics were performed for mean survival time only using product-limit survival analysis.

*Significantly different from control $p < 0.05$.

**Significantly different from control $p < 0.01$.

A very low incidence of malignant leukocyte neoplasms was reported. 5 high dose male animals had either lymphosarcomas or granulocytic leukaemias compared with one control animal. There was also one female high dose animal with lymphosarcoma. In most cases these neoplasms were in multiple organs or tissues reflecting their origins in the circulatory system. None of the incidences was statistically significant using Fischer's Exact Test (one-tailed) since probability values exceeded 5%.

Historical control data from the testing facility were not included in the report and as declared by the Notifier would not be possible to obtain. However, in the absence of this information a compilation of spontaneous neoplasms in the breeder of the [REDACTED] rats, published by [REDACTED] (Giknis and Clifford, 2004), has been provided by the Notifier. Information on spontaneous neoplasms was obtained from up to 31 studies conducted between 1989 and 2002 in testing facilities in Europe, the USA, Canada and Japan. The present study was reported in 1995 and therefore, the timescale is considered relevant. The data show that these neoplasms are not rare in rats although they occur at a low incidence. The incidence of large granulocytic or granulocytic leukaemia in high dose males slightly exceeds the spontaneous historical control incidence, however, it is still very low. The incidence of lymphosarcoma in male and female high dose animals is well within the historical control range.

The data from the present study as well as spontaneous neoplastic data from studies in which specific neoplasms were diagnosed are summarised in Table B.6.5.2-3.

Table B.6.5.2-3: Incidence of leukocytic neoplasms (%)

Neoplasm	Male		Female	
	Control	1 ppm	Control	1 ppm
Lymphosarcoma	1/50 (2%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
Large granulocytic or granulocytic leukaemia	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
HC lymphoma malignant ¹	0.91 – 6.00% (15 studies)	-	1.11 – 10.0% (14 studies)	-
HC granulocytic leukaemia	0.56 – 2.86% (8 studies)	-	1.43 – 2.73% (3 studies)	-

HC: Historic control data (Giknis and Clifford, 2004)

¹ Equivalent to lymphosarcoma reported in present study

Conclusions:

Based on findings of mortality and the effects on the body weight gain in the 0.5 ppm and 1.0 ppm groups, the **NOEL for non-neoplastic effects in rats was 0.1 ppm (0.18 mg/kg bw per day)**.

Considering that the incidence of rare neoplasms was not treatment related, the NOEL for carcinogenic effects was determined to be greater than 1.0 ppm (> 1.84 mg/kg bw).

The study is considered acceptable.

B.6.5.3 Inhalation study in mice IIA 5.5.3 (EU – 5.5)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

Burleigh-Flayer, H.D., Kintigh, W.J., Benson, C.L. Chloropicrin: Vapor Inhalation Oncogenicity Study in CD Mice.

Unpublished report-No 92N1105. Owner European Chloropicrin Group.1995. IIA, 5.5.3/01 (EU- 5.5)

GLP: Yes

Methods:

Fifty male and fifty female CD-1 mice were tested in a study of identical design as the rats study described above (IIA. 5.5.2/01). The exposure levels for Chloropicrin were 0, 0.1, 0.5 and 1.0 ppm. The test material was 99.6% pure Chloropicrin (Lot numbers 31291-A and 920130-2 from Niklor Chemical Company). Mean Chloropicrin concentrations in the exposure chambers were 0.100 ppm (± 0.0064), 0.498 ppm (± 0.0223) and 0.988 ppm (± 0.0434). Each chamber atmosphere was analysed by GC/EC approximately 6 six times during each exposure day. The distribution of Chloropicrin vapour within exposure chambers was monitored and found to be uniform. The high-dose chamber was monitored for the presence of phosgene since phosgene is a possible thermal degradation product of Chloropicrin at high temperature. No phosgene was detected.

Results:

No differences in the mortality rate or mean survival times were noted for any male or female animal in the Chloropicrin exposure group, when compared to the control (Tables B.6.5-3 and B.6.5-4). The few clinical signs, reported during exposure in one animal from each group, were not clearly exposure-related. No clinical signs were noted during the non-exposure periods. Concentration-related decreases in body weight and body weight gain were observed for male and female mice in the 0.5 and 1.0 ppm groups. Decreases in food consumption generally corresponded with changes in body weight, with decreases being noted for male mice in the 1.0 ppm group and for female mice from the 0.5 and 1.0 ppm groups. No exposure-related changes in haematological parameters were observed in male or female mice from any treated group after approximately 12 months of the exposure or at study termination.

Increases in absolute and/or relative lung weight were observed for male and female mice in the 0.5 and 1.0 ppm group at the sacrifice. A decrease in absolute brain weight, not corresponding with any microscopic alteration, was observed in females at 1 ppm exposure. No gross lesions at necropsy in animals from any group could be attributed to Chloropicrin exposure. Microscopic evaluation yielded evidence of an increased incidence of several non-neoplastic lesions in the respiratory tracts of both sexes of mice in the 0.5 and 1.0 ppm groups. An increased incidence of serous exudate and rhinitis of the nasal cavities were

observed for both male and female mice in the 0.5 and 1.0 ppm groups. Other lesions noted in the nasal cavities included olfactory epithelium atrophy and hyaline inclusions in the epithelium for female mice in the 0.5 ppm group as well as male and female mice in the 1.0 ppm group at sacrifice. Non-neoplastic lung lesions primarily affected the large airways and included bronchiectasis, bronchial submucosal fibrosis, and peribronchial lymphocytic infiltrates in males and females in the 0.5 and 1.0 ppm groups at the sacrifice. A slightly increased incidence of peribronchial lymphocytic infiltrates was noted for males in the 0.1 ppm group only at study termination. Additional lesions in the lung included an increased incidence of alveolar histiocytosis (males and females), alveolar protein deposits (females only), and bronchoalveolar cell hyperplasia (females only) for animals at the sacrifice, as well as for all animals on study in the 1.0 ppm group.

The incidence of the main treatment related histopathological changes in the lungs of animals killed at termination (week 82) is summarised in the following tables.

Table B.6.5.3-1: Incidence of selected non-neoplastic lung findings - males

Lung histopathology	Control (36 examined)	0.1 ppm (27 examined)	0.5 ppm (30 examined)	1 ppm (41 examined)
Peribronchial lymphocyte infiltrates	1	6*	8**	12**
Bronchiectasis	0	3	22**	35**
Bronchial submucosal fibrosis	0	0	11**	16**

Table B.6.5.3-2: Incidence of selected non-neoplastic lung findings – females

Lung histopathology	Control (34 examined)	0.1 ppm (34 examined)	0.5 ppm (31 examined)	1 ppm (32 examined)
Peribronchial lymphocyte infiltrates	4	9	14**	23**
Bronchiectasis	0	4	19**	30**
Bronchial submucosal fibrosis	0	0	10**	17**

* Significantly different from control group ($p < 0.05$)

** Significantly different from control group ($p < 0.01$)

The increased incidence of peribronchial lymphocyte infiltrates was only observed in animals killed at termination and not in unscheduled deaths. The incidence of bronchiectasis and submucosal fibrosis was significantly increased at 0.5 ppm and 1 ppm with an associated significant increase in peribronchial lymphocyte infiltrates. Peribronchial lymphocytic infiltrates is a nonspecific inflammatory lesion and it is attributed to chemical exposure when it is found in association with other forms of airway disease, such as bronchiectasis and submucosal fibrosis. Since there was no treatment related increase in airway disease at 0.1 ppm, the low increase in the incidence of peribronchial lymphocyte infiltrates also at this dose level is considered not to be of toxicological significance. An independent pathology peer review substantiated this interpretation by concluding that the marginally increased incidence of peribronchial lymphoid infiltrates in male mice at 0.1 ppm is unlikely to be a test article effect as slightly elevated incidences are common in laboratory maintained mice (Kovatch, 1996).

The only exposure-related non-neoplastic lesion observed outside the respiratory tract was an increased incidence of corneal mineralization and corneal vascularization in the eyes of female animals from the 1.0 ppm group that were sacrificed at Study Week 82.

The only notable neoplastic condition observed was a slightly increased incidence of lung adenomas in all male and female mice of the 0.5 and 1.0 ppm groups on study; however, none of the increases was statistically significant. There were no other increased frequencies of neoplastic lesions noted for male or female mice from any exposure group. There were no differences noted in the relative frequencies of the cause of death for any group of Chloropicrin-exposed male or female mice.

Table B.6.5.3-3: Summary of Mortality (Males)

	Concentration			
	0 ppm	0.1 ppm	0.5 ppm	1.0 ppm
Total Number of Animals	50	50	50	50
Number Sacrificed	36	27	30	41
Number Found Dead	12	19	17	6
Number Sacrificed Moribund	2	3	1	1
Number Procedural Death	0	0	0	1
Number Accidental Death	0	1	2	1
Mean Survival Time (Days) ^a	525	516	515	552
Mortality Rate (%) ^b	28	44	36	14

^a Statistics were performed for mean survival time only. No statistical difference was observed for either sex of animals.

^b Mortality rates include animals that were sacrificed moribund but not procedural/accidental deaths.

Table B.6.5.3 -4: Summary of Mortality (Females)

	Concentration			
	0 ppm	0.1 ppm	0.5 ppm	1.0 ppm
Total Number of Animals	50	50	50	50
Number Sacrificed	34	34	31	32
Number Found Dead	6	11	10	14
Number Sacrificed Moribund	8	4	6	2
Number Procedural Death	0	0	0	0
Number Accidental Death	2	1	3	2
Mean Survival Time (Days) ^a	530	526	550	523
Mortality Rate (%) ^b	28	30	32	32

^a Statistics were performed for mean survival time only. No statistical difference was observed for either sex of animals.

^b Mortality rates include animals that were sacrificed moribund but not procedural/accidental deaths.

A low incidence of mice with lung carcinomas was reported. The incidences of lung carcinomas as well as spontaneous neoplastic data from studies in which these specific tumours were diagnosed are summarised in Table B.6.5.3 -5 & -6.

Table B.6.5.3 -5: Incidence of lung carcinomas (%) - males

	Concentration			
	Control	0.1 ppm	0.5 ppm	1 ppm
Lung carcinomas	1/50 (2%)	0/50 (0%)	5/50 (10%)	2/50 (4%)
HC lung carcinomas	1.43- 26.0%	-	-	-

Table B.6.5.3 -6: Incidence of lung carcinomas (%) - females

	Concentration			
	Control	0.1 ppm	0.5 ppm	1 ppm
Lung carcinomas	0/50 (0%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
HC lung carcinomas	0.77-18.4%	-	-	-

HC: Historic control data (Giknis and Clifford, 2005)

None of the incidences was statistically significant using Fischer's Exact Test (one-tailed) since probability values exceeded 5%.

Historical control data from the testing facility were not included in the report and as declared by the Notifier would not be possible to obtain. However, in the absence of this information a compilation of spontaneous neoplasms in the breeder of the [REDACTED] mice, published by [REDACTED] (Giknis and Clifford, 2005), has been provided by the Notifier. Information on spontaneous neoplasms was obtained from up to 54 studies of 78 – 104 weeks duration conducted between 1987 and 2000 in 11 testing facilities. The present study was reported in 1995 and is, therefore, relevant to this timescale. The historical control data show that lung carcinomas are not rare and were diagnosed in 45 (males) and 41 studies (females). The incidence of lung carcinomas in both male and female dose groups was well within the historical control range. Although a higher incidence was recorded in males at 0.5 ppm, this was still within the historical range with a clear no dose response relationship. The low incidence of lung tumours in treated groups in the present study is considered not to be treatment related.

Conclusions:

Based on the results obtained from this study, **the no-observed-effect level (NOEL) for non neoplastic toxic effects in mice was 0.1 ppm (0.32 mg/kg bw per day).**

Considering that the incidence of rare lung carcinomas was not treatment related, the no-observed-effect level (NOEL) for carcinogenicity was determined to be greater than 1.0 ppm.

The study is considered acceptable.

B 6.5.4 Summary of Long-term toxicity and carcinogenicity

From the long-term oral toxicity study performed via gavage with CD rats, a NOAEL of 0.1 mg/kg bw per day was established on the basis of the incidence of epithelial hyperplasia and hyperkeratosis in the nonglandular stomach. A NOEL could not be clearly determined. Lifetime exposures via whole body inhalation to CD-1 mice and CD rats have been reported for Chloropicrin. From these studies it has been established that respiratory tissue is the primary target organ for toxic effects after Chloropicrin chronic vapour exposure. The NOEL was 0.1 ppm both for rats (0.18 mg/kg bw per day) and mice (0.32 mg/kg bw per day).

A number of long-term bioassays have been carried out with Chloropicrin to evaluate the potential of this compound to cause chronic and/or carcinogenic effects. The bioassays have employed two strains of rats and one mouse strain. Lifetime exposures to Chloropicrin of CD-1 mice and CD rats by inhalation and oral exposure of CD rats by gavage have been reported. On the basis of these studies, the weight of evidence demonstrates no carcinogenic potential for Chloropicrin.

Table B.6.5.-5 Summary of long-term toxicity and carcinogenicity studies with Chloropicrin

Type of study	Species	Dose range tested	NOEL/NOAEL	Reference
Oral 104 weeks	Rat	0-10 mg/kg bw/day	NOAEL: 0.1 mg/kg bw/day	Slauter (1995) (rep-No 656-003)
Inhalation 107 ♂ or 78 ♀ weeks	Rat	0-1.0 ppm/6hrs/day	NOEL for non-neoplastic effects: 0.18 mg/kg bw per day	Burleigh-Flayer (1995) (rep-No92N1106)
Inhalation 107 ♂ or 78 ♀ weeks	Mouse	0-1.0 ppm/6hrs/day	NOEL for non-neoplastic effects: 0.32 mg/kg bw per day	Burleigh-Flayer (1995) (rep-No92N1105)

B 6.6 Reproductive toxicity (IIA 5.6.1)

B.6.6.1 Multi-generation study in rats (IIA 5.6.1)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

Schardein, J.L., 1984, Two-Generation Inhalation Reproduction/Fertility Study in Rats.
Unpublished report-No 656-011.Owner European Chloropicrin Group.1995. IIA, 5.6.1/01 (EU- 5.6.1)

GLP: Yes

Methods:

Chloropicrin vapour (Chloropicrin purity, 99.6%; Lot 920130-1, Niklor Chemical Corporation) was administered by whole body inhalation for six hours per day, seven days per week, to 26 male and 26 female CD rats at concentrations of 0, 0.5, 1.0 or 1.5 ppm through two generations. Actual exposure concentrations were determined at hourly intervals during exposures. Please refer to Table B.6.6.1-1 and B.6.6.1-2. The chamber atmosphere was

checked for the presence of aerosolised Chloropicrin. Chloropicrin vapour concentrations were measured by GC/ECD. The initial test group, the F₀ parents, began exposures at 43 days of age and continued until approximately 120 days of age. The F₀ parents were mated to produce F₁ litters, and male and female rats were randomly selected to become F₁ parents.

Results:

Exposure Concentration Analysis:

Table B.6.6.1-1: F₀ Generation

Group number	Nominal mean ± S.D. (ppm)	Actual mean ± S.D. (ppm)
2	0.47 ± 0.021	0.45 ± 0.039
3	0.88 ± 0.040	0.97 ± 0.033
4	1.6 ± 0.12	1.5 ± 0.054

S.D.- Standard deviation

Table B.6.6.1-2: F₁ Generation

Group number	Nominal mean ± S.D. (ppm)	Actual mean ± S.D. (ppm)
2	0.47 ± 0.012	0.46 ± 0.011
3	0.91 ± 0.022	0.96 ± 0.021
4	1.6 ± 0.05	1.5 ± 0.00

S.D.- Standard deviation

Body weight:

There were no treatment related changes in bodyweight of F₀ males. An isolated statistically significant reduction in male bodyweight at 1.5 ppm compared with the control group occurred in week 1 of exposure. Thereafter bodyweights were comparable with controls. There were no statistically significant differences in bodyweight of females up to week 10 of the study. Although statistically significant reductions were observed in F₀ females at 1.0 and 1.5 ppm compared with control in weeks 11 and 12, the group sizes were markedly lower because animals were in the process of being paired with males and only those not paired were weighed at this time. The absence of any treatment related effect on females following mating, during the periods of gestation and lactation, support the view that these isolated statistically significant differences at weeks 11 and 12 are not related to Chloropicrin exposure.

There were no treatment related changes in bodyweight of F₁ males. Overall bodyweight gain from week 4 to 24 of age was very similar to the control group, being almost identical at 1.0 ppm and only 3% lower at 1.5 ppm. A trend for slightly lower bodyweights was noted for F₁ females at 1.0 and 1.5 ppm, achieving statistical significance on occasions. However, this was reflected in bodyweight gain differences which were only 4% lower than the control group gain over the period weeks 4 to 17. This marginal effect on bodyweight is considered not to be of toxicological significance.

A summary of body weights values for F₀ and F₁ is reported in Tables B.6.6.1-5-8

Table B.6.6.1-5: Males: Summary of Body Weight values – F₀ Weekly

Parameters Measured	Weeks of Age	0 ppm (Control)			0.5 ppm			1.0 ppm			1.5 ppm		
		Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Body	0	176	6.9	26	180	7.5	26	180	6.1	26	182	7.5	26

Weight (grams)	1	241	13.7	26	241	11.8	26	232	16.1	26	218 ¹	31.3	25
	2	291	21.7	26	295	15.2	26	288	19.3	26	282	21.4	25
	3	336	22.9	26	334	23.2	26	335	24.2	26	328	19.5	25
	4	371	25.8	26	368	20.0	26	372	30.4	26	368	17.9	25
	5	397	28.3	26	389	25.1	26	401	36.3	26	390	25.3	25
	6	422	30.9	26	423	24.0	26	422	41.6	26	417	27.7	25
	7	445	27.6	26	444	27.8	26	445	40.0	26	442	28.5	24
	8	462	34.3	26	465	28.1	26	465	43.5	26	461	28.0	24
	9	449	70.6	26	475	29.9	26	476	44.1	26	478	29.9	24
	10	480	45.5	26	491	32.9	26	494	47.8	26	493	33.8	24
	11	490	43.5	26	482	35.3	26	490	46.8	26	483	32.5	24
	12	497	39.5	26	499	33.6	26	499	45.9	26	487	33.4	24
	13	511	42.9	26	535	36.4	26	525	49.3	26	513	35.9	24
	14	525	42.2	26	530	38.5	26	527	44.9	26	530	34.9	24
	15	529	45.5	26	539	41.1	26	540	53.1	26	533	35.5	24
	16	533	44.3	26	548	40.5	26	551	55.7	26	545	40.1	24
	17	539	47.5	26	554	42.4	26	557	52.6	25	552	40.1	24

S.D. – Standard deviation

N – Number of animals

¹ Significantly different from the control group; p ≤ 0.05

Table B.6.6.1-6: Females: Summary of Body Weight values – F0 Weekly

Parameters Measured	Weeks of Age	0 ppm (Control)			0.5 ppm			1.0 ppm			1.5 ppm		
		Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Body Weight (grams)	0	149	5.9	26	150	5.6	26	148	6.4	26	150	4.3	26
	1	180	9.2	26	182	7.3	26	180	10.1	26	178	8.7	25
	2	203	15.5	26	204	10.5	26	204	12.7	26	204	9.5	25
	3	224	15.6	26	224	14.1	25	219	13.7	26	222	11.6	25
	4	239	16.4	26	240	16.1	25	237	12.4	26	240	13.5	25
	5	252	19.7	26	254	15.6	25	250	14.1	26	255	15.6	25
	6	265	19.4	26	265	19.6	25	261	14.6	26	264	17.2	25
	7	270	18.5	26	272	18.0	25	263	14.4	26	265	15.9	24
	8	280	20.2	26	282	19.0	25	273	14.6	26	277	17.8	24
	9	282	20.3	26	284	20.7	25	283	14.9	26	283	16.5	24
	10	288	21.6	26	291	20.1	25	283	15.1	26	286	16.9	24
	11	311	16.8	7	292	17.7	11	290 ¹	14.0	13	288 ¹	17.6	14
	12	337	4.9	2	320	3.8	3	295 ¹	13.4	5	294 ¹	23.8	9
	13	332	N/A	1	336	15.9	3	331	25.5	4	336	18.6	6
	14	342	N/A	1	419	68.9	3	405	54.4	4	423	36.9	6
	15	348	N/A	1	351	N/A	1	335	N/A	1	N/A	N/A	0
	16	357	N/A	1	461	N/A	1	321	N/A	1	N/A	N/A	0
	17	363	N/A	1	N/A	N/A	0	334	N/A	1	N/A	N/A	0

S.D. – Standard deviation

N – Number of animals

N/A – Not applicable

¹ Significantly different from the control group; p ≤ 0.05

Table B.6.6.1-7: Males: Summary of Body Weight values – F1 Weekly

Parameters Measured	Week of Study	0 ppm (Control)			0.5 ppm			1.0 ppm			1.5 ppm		
		Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Body Weight (grams)	4	99	9.1	26	97	11.9	26	92	12.2	26	96	13.2	26
	5	153	11.9	26	152	16.3	26	142 ¹	16.3	26	143	17.9	26
	6	212	18.3	26	211	18.5	26	195 ²	19.1	26	198 ¹	19.5	26
	7	272	20.9	26	274	23.3	26	260	22.7	26	257 ¹	20.6	26
	8	327	25.9	26	329	25.6	26	313	25.7	26	315	24.1	26
	9	371	31.6	26	373	29.2	26	357	29.2	26	354	28.4	26
	10	409	32.4	26	409	31.4	26	390	31.8	26	392	30.6	26
	11	438	33.9	26	440	33.6	26	421	32.9	26	418	33.4	24
	12	465	36.9	26	465	40.2	26	442	33.7	26	440 ¹	35.8	26
	13	488	40.2	26	485	42.8	26	466	39.9	26	463	41.0	26
	14	500	47.4	26	499	45.7	26	482	40.3	26	480	44.6	26
	15	521	42.8	26	521	44.7	26	495	42.1	26	496	46.5	26
	16	527	43.5	26	531	48.3	26	509	41.6	26	507	51.1	26
	17	541	47.3	26	542	44.7	26	521	43.5	26	518	46.5	26
	18	537	48.7	26	543	44.2	26	528	44.0	26	526	47.0	26
	19	543	49.0	26	554	48.6	26	530	45.9	26	527	45.5	26
	20	558	51.3	26	569	52.2	26	530	48.6	26	532	46.9	26
	21	571	56.5	26	579	52.6	26	550	51.6	26	544	49.1	26
	22	573	61.0	26	585	52.9	26	559	50.7	26	553	48.8	26
	23	579	67.2	24	595	59.7	24	571	46.6	26	564	52.8	24
	24	582	68.4	24	594	55.1	24	574	48.5	26	564	57.3	26

S.D. – Standard deviation
N – Number of animals

¹ Significantly different from the control group; $p \leq 0.05$

² Significantly different from the control group; $p \leq 0.01$

Table B.6.6.1-8: Females: Summary of Body Weight values – F1 Weekly

Parameters Measured	Week of Study	0 ppm (Control)			0.5 ppm			1.0 ppm			1.5 ppm		
		Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N	Mean	S.D.	N
Body Weight (grams)	4	92	8.4	26	87	11.7	26	85	11.6	26	85	7.8	26
	5	133	11.2	26	129	16.8	26	122 ¹	14.7	26	120 ²	13.0	26
	6	167	14.2	26	169	18.9	26	156 ¹	17.9	26	152 ²	11.3	26
	7	196	16.2	26	194	19.2	26	188	18.2	26	184	14.1	26
	8	218	16.6	26	217	20.2	26	211	18.5	26	208	17.8	26
	9	237	18.0	26	237	22.2	26	229	19.7	26	226	19.3	26
	10	252	18.4	26	252	22.5	26	241	22.0	26	238 ¹	18.3	26
	11	267	19.9	26	266	22.2	26	258	20.5	26	256	18.8	24
	12	276	21.6	26	272	22.3	26	268	21.5	26	264	22.4	26
	13	283	21.6	26	280	22.5	26	275	21.8	26	272	23.4	26
	14	290	21.8	26	287	24.0	26	277	20.8	26	277	24.1	26
	15	304	23.0	26	296	25.1	26	278 ²	20.4	26	286 ¹	22.4	26
	16	297	26.3	24	295	27.6	25	284	21.2	26	292	22.0	26
	17	306	21.5	24	298	24.7	25	290 ¹	23.3	26	290 ¹	23.2	26
	18	309	16.6	16	299	24.8	10	295	24.1	8	303	20.7	14
	19	323	31.8	2	272	20.5	2	297	N/A	1	338	43.6	5
	20	303	N/A	1	277	N/A	1	304	N/A	1	352	72.8	2
	21	309	N/A	1	N/A	N/A	0	344	N/A	1	N/A	N/A	0
	22	311	N/A	1	N/A	N/A	0	N/A	N/A	0	N/A	N/A	0
	23	309	N/A	1	N/A	N/A	0	N/A	N/A	0	N/A	N/A	0
	24	307	N/A	1	N/A	N/A	0	N/A	N/A	0	N/A	N/A	0

S.D. – Standard deviation

N – Number of animals

N/A – Not applicable

¹ Significantly different from the control group; $p \leq 0.05$

² Significantly different from the control group; $p \leq 0.01$

Antemortem observations:

Table B.6.6.1-3: Summary of F₀ Male Antemortem Observations

	0 ppm (Control)	0.5 ppm	1.0 ppm	1.5 ppm
	No. (%)	No. (%)	No. (%)	No. (%)
Number of Animals Observed	26 -	26	26	26 -
Antemortem observations				
No visible abnormalities ^a	13 (50)	20 (77)	18 (69)	17 (65)
Died/euthanised <i>in extremis</i>			1 (4)	2 (8)
Emaciated		1 (4)	1 (4)	3 (12)
Impaired limb function				1 (4)
Ulceration				1 (4)
Abrasion	1 (4)			

Scabbed area	3 (12)		1 (4)	1 (4)
Focal swelling				1 (4)
Area round eye red	3 (12)		1 (4)	
Material around nose, eye	7 (27)	1 (4)	2 (8)	
Incisor missing	2 (8)			
Malocclusion	2 (8)	2 (8)	1 (4)	1 (4)
Hair loss	9 (35)	3 (12)	5 (19)	4 (15)

^a Includes animals with no visible abnormalities throughout all recorded intervals
% - Percent based on number of animals at the beginning of observation period
-- Not applicable.

Table B.6.6.1-4: Summary of F₀ Female Antemortem Observations

	0 ppm (Control)	0.5 ppm	1.0 ppm	1.5 ppm
	No. (%)	No. (%)	No. (%)	No %
Number of Animals Observed	26 -	26 -	26 -	26 -
Antemortem Observations				
No visible abnormalities ^a	16 (62)	16 (62)	19 (73)	23 (88)
Died	1 (4)	1 (4)	1 (4)	2 (8)
Subcutaneous mass			1 (4)	
Prostrate	1 (4)			
Loss of righting reflex	1 (4)			
Laboured breathing	1 (4)			
Impaired limb function	1 (4)			
Portion of tail missing			2 (8)	
Scabbed area	1 (4)			
Area around eye red		1 (4)	1 (4)	1 (4)
Anogenital staining	1 (4)			
Material around nose, eye			1 (4)	1 (4)
Incisor missing			1	

			(4)	
Malocclusion	1 (4)	1 (4)	1 (4)	1 (4)
Hair loss	9 (35)	8 (31)	3 (12)	1 (4)

^a Includes animals with no visible abnormalities throughout all recorded intervals

% - Percent based on number of animals at the beginning of observation period

-- Not Applicable.

Exposure to Chloropicrin induced systemic toxicity to F0 female rats exposed to 1.5 ppm. This was manifested by an increased incidence and severity of pulmonary inflammation observed macroscopically and microscopically. No such effects were observed in male rats of either generation nor in F1 female rats at 1.5 ppm nor in rats of either gender at exposure levels of 0.5 and 1.0 ppm.

Uterine examination observations:

Table B.6.6.1-9: Summary of F0 Uterine Observations on Lactation Day 21

Group	Number of Implantation Sites		Number of Offspring Delivered		Number of Remaining Sites ^a	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
0 ppm	15.1 ± 2.58	15	13.8 ± 2.98	15	1.3 ± 1.62	15
0.5 ppm	15.0 ± 2.89	21	13.3 ± 4.09	21	1.7 ± 2.27	21
1.0 ppm	15.2 ± 1.75	23	14.3 ± 1.94	23	0.9 ± 1.00	23
1.5 ppm	13.9 ± 3.51	17	12.9 ± 3.33	17	1.1 ± 1.25	17

^a Implantation sites not corresponding to delivered offspring; values represent post implantation loss and/or complete cannibalization

N – Number of animals

Table B.6.6.1-10: Summary of F1 Uterine Observations on Lactation Day 21

Group	Number of Implantation Sites		Number of Offspring Delivered		Number of Remaining Sites ^a	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
0 ppm	14.2 ± 2.74	19	13.4 ± 2.75	19	0.8 ± 0.83	19
0.5 ppm	15.0 ± 2.66	18	14.3 ± 3.36	18	0.8 ± 1.48	18
1.0 ppm	15.4 ± 2.40	20	13.9 ± 2.59	20	1.2 ± 1.01	20
1.5 ppm	14.7 ± 1.98	20	13.5 ± 1.88	20	1.2 ± 1.14	20

^a Implantation sites not corresponding to delivered offspring; values represent post implantation loss and/or complete cannibalization

N – Number of animals

For both generations the number of implantation sites and number of offspring delivered were comparable between the treated groups and the control group. Minor differences in group mean values were insignificant when viewed in the context of the degree of within group variability (S.D. values).

There were no adverse effects on F1 or F2 offspring viability or growth observed at any exposure concentration in this study. There were no consistent antemortem findings among

F1 pups of the exposure treated groups when compared to the control group. For the antemortem observations of the F2 pups none were considered to be the result of exposure to Chloropicrin.

Conclusion:

In conclusion there were no treatment related effects on parental bodyweight of F0 animals. Although slightly reduced bodyweights were observed for F1 females at 1.0 and 1.5 ppm, the differences were considered not to be of toxicological significance. Exposure to Chloropicrin induced systemic toxicity to F0 female rats exposed to 1.5 ppm. Therefore, the NOAEL of Chloropicrin when administered by whole-body inhalation to male and female rats was **1.0 ppm (equivalent to 1.80 mg/kg bw)** with respect to **systemic toxicity (F0 females)**.

No treatment related effect on the number of implantation sites or number of offspring delivered was evidenced. Therefore, a NOEL greater than **1.5 ppm (equivalent to > 2.71 mg/kg bw)** was established for **development toxicity and reproductive performance**.

There was no systemic toxicity in the offspring of either generation. Therefore, a NOEL greater than **1.5 ppm (equivalent to > 2.71 mg/kg bw)** was established for the offsprings.

The study is considered acceptable.

B.6.6.2 Developmental toxicity study in rats (IIA 5.6.10)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

Schardein, J.L., 1993, Inhalation Developmental Toxicity Study in Rats [REDACTED]
[REDACTED] Unpublished report-No 656-007. Owner
European Chloropicrin Group.1995. IIA 5.6.10/01 (EU- 5.6.2)

GLP: Yes

Methods:

Thirty sexually mature virgin female Sprague-Dawley rats per group were impregnated and exposed by whole body inhalation to Chloropicrin vapour (Chloropicrin purity, 99.6%; Lot 920130-1, Niklor Chemical Corporation) for 6 hours per day in 16 m³ stainless steel and glass chamber for days 6-15 of gestation. Exposure levels were 0.4, 1.2 and 3.5 ppm. Please refer to Table B.6.6.2-1 for further details. The actual exposure concentrations were determined at hourly intervals during exposures. The chamber atmosphere was checked for the presence of aerosolised Chloropicrin. Chloropicrin vapour concentrations were measured by GC/ECD.

Table B.6.6.2-1: Actual chamber concentrations

Nominal Concentration	Actual Concentration (Mean)
0.4 ppm	0.4 ppm S.D. 0.02
1.2 ppm	1.2 ppm S.D. 0.06
3.5 ppm	3.5 ppm S.D. 0.08

The animals were observed for survival, appearance, behaviour, body weight, food consumption and pregnancy status. Gravid uterine weights, location of viable and nonviable foetuses (on uterine horns), number of resorption sites, number of implantations and corpora lutea, and gross morphologic changes in internal organs were recorded. Foetal observations

included individual foetal sex and weight. The presence of external malformation or variation was performed, which included measurement of crown-rump length, examination of soft-tissue (half of the foetuses) and skeletal examination (one half of the foetuses). Please refer to Table B.6.6.2-3 for details on foetal development observations.

Results:

The pregnancy rate among the test and control animals was 90%. Maternal mortality appeared to be treatment-related, with 4 deaths occurring in the high-dose group. Clinical signs were dose-related in incidence and severity but almost totally limited to the respiratory system. Statistically significant weight gain suppression and food consumption was seen in a dose-related fashion during exposure intervals in the high and mid-dose groups. These effects extended into the full gestational period for the high-dose group. Reduced foetal body weight was observed in the high-dose group only and was considered to be treatment-related. There were no exposure-related differences among test and control groups in uterine contents or condition.

Table B.6.6.2 -2: Summary of Antemortem and Necropsy Observations

Animals observed	0 ppm (Control) No. %	0.4 ppm No. %	1.2 ppm No. %	3.5 ppm No. %
Number of animals observed	30 -	30 -	30 -	30 -
Antemortem Observations				
No visible abnormalities ^a	27 (90)	28 (93)	27 (90)	16 (53)
Died				4 (13)
Cold to touch				2 (7)
Laboured breathing				4 (13)
Emaciated				7 (23)
Decreased activity				2 (7)
Body surface stained				5 (17)
Staining around mouth				2 (7)
Portion of digit missing			1 (3)	
Impaired limb function			2 (7)	
Scabbed area	1 (3)	1 (3)		1 (3)
Material around nose				5 (17)
Malocclusion				1 (3)
Hair loss	2 (7)	2 (7)	1 (3)	3 (10)
Necropsy Observations				

Animals observed	0 ppm (Control) No. %	0.4 ppm No. %	1.2 ppm No. %	3.5 ppm No. %
No gross lesions	29 (97)	29 (97)	30 (100)	23 (77)
Lung -discoloured				4 (13)
- consolidated				1 (3)
Adrenal gland - enlarged				1 (3)
Kidney - hydronephrosis		1 (3)		
Ovary - fluid	1 (3)			1 (3)
Vagina - fluid				1 (3)
Cervix - fluid				1 (3)

^a Includes animals with no visible abnormalities throughout all recorded intervals
% - Percent based on the number of animals at the beginning of the observation period
- - Not applicable

Table B.6.6.2-3: Foetal Developmental Variation Data

	0 ppm (Control)	0.4 ppm	1.2 ppm	3.5 ppm
Number of litters examined	28	27	30	25
Number of fetuses examined externally	397	343	400	338
Number of fetuses examined viscera	194	172	196	171
Number of fetuses examined skeletally	203	171	203	167
Developmental variations observed	No. of fetuses (No. of litters)			
Renal papillae not developed	6 (4)	2 (2)	3 (2)	1 (1)
Distended ureter(s)	5 (5)	5 (4)	7 (4)	4 (4)
Skull bone(s) reduced in ossification: frontals, supraoccipital, jugal(s), parietal(s), interparietal, squamosal(s) and/or hyoid	1 (1)	3 (3)	5 (5)	9 (3)
Vertebral arches reduced in ossification: C3 – C7, L5 – L6, and/or S1 – Cx2	5 (3)	2 (2)	6 (3)	1 (1)
Vertebral centra reduced in ossification: S1 – Cx1	1 (1)			
25 presacral vertebrae		2	4 (2)	3 (2)
27 presacral vertebrae			2 (2)	
Greater than 13 pairs of full ribs				
Less than 13 pairs of full ribs	7 (5)	4 (4)	14 (6)	12 (7)
14 th rudimentary rib(s)	1 (1)	1 (1)	3 (3)	3 (3)
7 th cervical rib	3 (3)	2 (1)	1 (1)	1 (1)
Rib reduced in ossification: T1				1 (1)
Bent rib(s)	1 (1)	2 (2)	2 (2)	4 (2)
Entire sternum unossified	1 (1)			
Sternebra(e) # 5 or # 6 unossified	29 (13)	33 (14)	56 (23)	41 (18)
Other sternebrae unossified: # 2 or # 4		2 (2)	1 (1)	
Misaligned sternebra: # 3 or # 4	1 (1)	2 (2)	3 (3)	1 (1)
Ischium(a) reduced in ossification	7 (3)	2 (2)	7 (4)	5 (2)
Limb bones reduced in ossification	1 (1)			
Total fetuses (litters) with developmental variations	49 (19)	47 (17)	75 (26)	65 (25)²

² Significantly different from the control group; p ≤ 0.01

The incidence of skeletal variations is summarised in the table above. Reduced or retarded ossification may be an indication of delayed development associated with foetotoxicity, which itself is often associated with maternal toxicity. It has been recognized that skeletal anomalies considered to represent delayed or incomplete ossification are considered as variations and not malformations. Moreover, it is considered as a transient phenomenon and for this reason it is not normally included in compilations of historical control data (Lang, 1993).

There is a slight increase in the incidence of litters with skull bones reduced in ossification in the treated groups compared with the control group. However, in the control low and mid dose groups only one foetus is affected in each litter. This is in contrast to the high dose group where 9 foetuses were affected among 3 litters. There was clear evidence of foetotoxicity (reduced foetal body weight) in the presence of significant maternal toxicity in the high dose group.

A slightly higher incidence of foetuses at 1.2 and 3.5 ppm were affected compared with the control. The litter incidence is the more appropriate unit for comparison. Examination of the historical control data presented in the study report shows that this skeletal variation is commonly observed among control litters. The historical control data also show that this is a variable parameter in this strain of rat. For example, a control incidence of this finding in 19 litters out of 25 litters examined was recorded in one study and incidences of 11 to 15 litters out of approximately 25 litters examined were noted in several studies.

The incidence of developmental variations in all dose groups was increased over that of the concurrent control group, reaching a statistically significance only in the high-dose group (3.5 ppm).

Conclusion:

There is clear evidence of foetotoxicity at 3.5 ppm characterised by reduced foetal bodyweight and an increased incidence of skull bones reduced in ossification, which is indicative of delayed development. This was seen in the presence of significant maternal toxicity. However, although value of total foetuses (litters) with developmental variations reaches a statistically significance only at 3.5 ppm, a dose-response relationship is also evident at the lower dose (1.2 ppm). Therefore, a **developmental NOAEL** for Chloropicrin when administered by whole-body inhalation exposure to mated female rats was judged to be **0.4 ppm (equivalent to 0.74 mg/kg bw)**.

The study is considered acceptable.

B.6.6.3 Developmental toxicity study in rabbits (IIA 5.6.11)

No new data have been submitted by the Notifier, but clarifications on this endpoint have been added. The following part is reported since some amendments have been made.

York, R.G., 1993, Inhalation Development Toxicity Study in New Zealand White Rabbits. [REDACTED] Unpublished report-No 656-009. Owner European Chloropicrin Group.1995. IIA 5.6.11/02 (EU- 5.6.2)

GLP: Yes

Methods:

The developmental toxicity of Chloropicrin has also been evaluated in a non-rodent species. Twenty sexually mature virgin female New Zealand White SPF rabbits per group, were impregnated and exposed by whole body inhalation to Chloropicrin vapour for 6 hours per day in 16 m³ stainless steel and glass chamber for days 7-20 of gestation. Exposure levels were 0.4, 1.2 and 2.0 ppm. The test material was Chloropicrin (Purity, 99.6%; Lot 920130-1, Niklor Chemical Corporation). Please refer to Table B.6.6.3-1 for further details. The actual exposure concentrations were determined at hourly intervals during exposures. The chamber atmosphere was checked for the presence of aerosolised Chloropicrin. Chloropicrin vapour concentrations were measured by GC/ECD.

Table B.6.6.3-1: Actual chamber concentrations

Nominal Concentration	Actual Concentration (Mean)
0.4 ppm	0.4 ppm S.D. 0.005
1.2 ppm	1.2 ppm S.D. 0.00
2.0 ppm	2.0 ppm S.D. 0.05

The animals were observed for maternal survival, appearance, behaviour, body weight, food consumption and pregnancy status. The parameters evaluated were gravid uterine weight, location of viable and nonviable foetuses (on uterine horns), number of resorption sites, number of implantations and corpora lutea and gross morphologic changes in internal organs. The foetal observations included individual foetal sex and weight, and presence of external malformation or variation, which included cranium and heart dissection, soft-tissue examination and skeletal examination.

Results:

Maternal mortality appeared to be dose-related with 2 deaths occurring in the mid-dose group and 10 in the high-dose group. Clinical signs were dose-related in incidence and severity and involved mainly the respiratory system, including pulmonary oedema in the high-dose animals not surviving to study termination. Clinical signs in the low-exposure group and necropsy findings of all animals that survived to study termination were generally comparable to control animals. Statistically significant weight gain suppression and food consumption was seen in a dose-related fashion during the entire gestational interval for the high exposure group and during exposure for the mid-dose group.

Please refer to Table B.6.6.3-2 for a summary of clinical findings for female rabbits.

Table B.6.6.3-2: Summary of Clinical Findings - Survival, Appearance and Behaviour (females)

Animals observed	0 ppm (Control) No. %	0.4 ppm No. %	1.2 ppm No. %	2.0 ppm No. %
Appearance and Condition No visible abnormalities for entire interval Found dead Tissue in refuse pan Aborted	8 (40.0%) 0 0 0	9 (45.0%) 0 0 0	1 (5.0%) 2 (10.0%) 1 (5.0%) 1 (5.0%)	1 (5.0%) 10 (50.0%) 1 (5.0%) 2 (10.0%)
Excretion Decreased defecation Soft stool No stool	9 (45.0%) 6 (30.0%) 0	8 (40.0%) 4 (20.0%) 0	8 (40.0%) 5 (25.0%) 0	8 (40.0%) 3 (15.0%) 1 (5.0%)
Body Surface Body surface stained Hair loss Swelling	0 3 (15.0%) 0	1 (5.0%) 5 (25.0%) 0	1 (5.0%) 5 (25.0%) 0	1 (5.0%) 2 (10.0%) 1 (5.0%)
Respiration Gasping Laboured breathing	0 0	0 0	0 1 (5.0%)	2 (10.0%) 3 (15.0%)
Anogenital Anogenital staining	2 (10.0%)	2 (10.0%)	2 (10.0%)	5 (25.0%)
Oral /nasal Increased salivation Malocclusion Material around nose Staining around mouth	0 1 (5.0%) 1 (5.0%) 0	0 0 3 (15.0%) 0	0 0 18 (90.0%) 0	2 (10.0%) 0 10 (50.0%) 1 (5.0%)
Eyes Area around eye / eyelids red Eyes discoloured Excessive lacrimation	0 0 1 (5.0%)	0 0 0	0 0 1 (5.0%)	4 (20.0%) 1 (5.0%) 3 (15.0%)

The mean numbers of corpora lutea and implantations in each of the treated groups were comparable with the concurrent control and the mean historical control values (Table B.6.6.3-3). Therefore, although not calculated in the historical control data, the % pre-implantation losses in this study would be comparable with the expected losses in NZW rabbits in the testing facility.

Table B.6.6.3-3: Implantation data (mean $\pm$ standard deviation)

		0 ppm (Control)	0.4 ppm	1.2 ppm	2.0 ppm	Historical control ¹
Animals on Study	N	20	20	20	20	979
Number Evaluated	N	18	19	16	8	910
Corpora Lutea *	Mean	13.3 $\pm$ 3.53	14.5 $\pm$ 4.62	12.8 $\pm$ 3.11	16.1 $\pm$ 4.64	13.1
	N	18	19	16	8	
	Total	239	275	204	129	
Implantation Sites *	Mean	7.6 $\pm$ 2.91	8.0 $\pm$ 2.69	7.4 $\pm$ 3.76	8.3 $\pm$ 3.54	7.0
	N	18	19	16	8	
	Total	136	152	118	66	
Post-implantation Loss*	Mean	0.2 $\pm$ 0.43	1.0 $\pm$ 1.86	0.6 $\pm$ 0.73	0.8 $\pm$ 0.71	0.9
	N	18	19	16	8	
	Total	4	19	9	6	
Pre-implantation Loss ^a	%	43.1	44.7	42.2	48.8	-
Post-implantation Loss ^b	%	2.9	12.5	7.6	9.1	-
Resorptions *	Mean	0.2 $\pm$ 0.43	1.0 $\pm$ 1.86	0.4 $\pm$ 0.63	0.8 $\pm$ 0.71	0.9
	N	18	19	16	8	
	Total	4	19	7	6	
Early	Mean	0.2 $\pm$ 0.43	0.9 $\pm$ 1.73	0.4 $\pm$ 0.63	0.4 $\pm$ 0.52	0.6
	N	18	19	16	8	
	Total	4	17	7	3	
Late	Mean	0.0 $\pm$ 0.00	0.1 $\pm$ 0.32	0.0 $\pm$ 0.00	0.4 $\pm$ 0.52	0.3
	N	18	19	16	8	
	Total	0	2	0	3	
Dead Fetuses	N	0	0	2	0	-
Animals with Viable Fetuses	N	18	19	16	8	-
Live Fetuses *	Mean	7.3 $\pm$ 2.99	7.0 $\pm$ 3.28	6.8 $\pm$ 3.53	7.5 $\pm$ 3.63	-
	N	18	19	16	8	
	Total	132	133	109	60	

^a $\frac{\text{Corpora Lutea} - \text{Implantations}}{\text{Corpora Lutea}} \times 100$

^b $\frac{\text{Implantations} - \text{Viable Fetuses}}{\text{Implantations}} \times 100$

* Statistical analysis applied, no significance observed

N – Number of animals

¹ Historical control data from 56 studies in NZW rabbits from 1985 to 1990 (Appendix D in report)

The slightly increased % post-implantation losses in treated groups compared with the control showed no dose response relationship. The post-implantation losses expressed as total, early and late resorptions were variable between groups, and although the treated group mean values were generally higher than the concurrent control group, they were comparable with the historical control mean values. The concurrent control mean values appeared to be lower than expected. Overall, there is considered to be no treatment related effect on post-implantation loss.

Although a slightly increased proportion of foetuses at 1.2 and 2 ppm was noted with variations this was not statistically significant. The incidence of developmental variations (supernumerary presacral vertebra, ribs and unossified tail bones) in all dose groups was increased over that of the concurrent control group but this increase was not dose-related or statistically significant.

The historical control data showed that the incidence of developmental variations is very variable. Overall, it is considered unlikely that there was a treatment related effect on the proportion of foetuses with developmental variations

Table B.6.6.3-4: Fetal Developmental variation data

	0 ppm (Control)	0.4 ppm	1.2 ppm	2.0 ppm
Number of litters examined	18	19	16	9
Number of fetuses examined externally	132	133	111	68
Number of fetuses examined viscera	132	133	111	68
Number of fetuses examined skeletally	132	133	111	68
Developmental variations observed	No. of fetuses (No. of litters)			
Carpal flexure			1 (1)	
Hemorrhagic iris		1 (1)		
Azygous lobe of lung absent	4 (3)	5 (3)	10 (5)	2 (2)
Left carotid arises from innominate	28 (13)	39 (10)	45 (12)	20 (6)
Spleen hypoplasia	1 (1)			
Gallbladder hypoplasia	5 (4)			1 (1)
Bilobed gallbladder			1 (1)	
Renal papillae not developed	2 (1)			
Distended ureter	1 (1)			
Hyoid bone unossified			3 (1)	4 (3)
Hyoid arch (es) bent	8 (6)	2 (2)	5 (4)	2 (2)
Accessory skull bone	3 (2)	1 (1)		
Misshapen skull bone: interparietal			1 (1)	
28 presacral vertebrae				1 (1)
27 presacral vertebrae	33 (10)	37 (13)	39 (11)	39 (9)
Extra vertebral ossification site				2 (1)
Greater than 12 pairs of full ribs	76 (16)	76 (18)	86 (16)	54 (9)
13th rudimentary rib(s)	27 (9)	22 (11)	11 (6)	6 (3)
7th cervical rib(s)	3 (2)	1 (1)	3 (2)	1 (1)
Sternebra # 5 or # 6 unossified	2 (1)	4 (4)	3 (2)	
Extra sternebra			2 (2)	
Extra sternal ossification site	3 (3)	1 (1)	2 (1)	
Misaligned sternebra(e): # 4 and/or # 5	2 (2)	1 (1)		
Tail unossified				5 (1)
Pubic bones unossified				2 (1)

Total foetuses (litters) with variations	114 (18)	112 (18)	109 (16)	65 (9)
% of pups (litters) with variations	86.4 (100)	84.2 (95)	98.2 (100)	95.6 (100)

Conclusion:

The no observable adverse effect level (**NOAEL**) of Chloropicrin when administered by whole body inhalation to inseminated rabbits was **0.4 ppm with regard to maternal toxicity**, based on deaths, clinical signs, necropsy findings, reduced body weight and food consumption and body weight loss at the higher exposure groups. Group differences in post-implantation losses or in the incidence of developmental variations are considered minimal and unlikely to be treatment related. Therefore, **the developmental NOAEL was established at 1.2 ppm** based on reduced foetal bodyweight at 2 ppm.

The study is considered acceptable.

B.6.6.4 Summary of reproduction and developmental toxicity

Chloropicrin has been evaluated in standardized regulatory protocols and tested under US EPA Good Laboratory Practices Regulations for the potential to produce developmental toxicity or adversely affect reproductive function. Testing has been via the most important route of exposure, inhalation, and conducted in sensitive species: rats and rabbits. The two-generation study on rat showed no effects on fertility and the effects on foetuses were present only at dose levels with maternal toxicity. Developmental toxicity studies in the rat and rabbit revealed minor developmental effects at maternally toxic doses only. On the basis of the available data, Chloropicrin does not induce specific effects on reproduction and development.

B 6.7 DELAYED NEUROTOXICITY (IIA 5.7)

No study available to address this point.

B 6.8 FURTHER TOXICOLOGY STUDIES (IIA 5.8)

B 6.8.1 Toxicity studies on metabolites (IIA 5.8.1) (EU – 5.8.1)

No study is available to address the point.

The metabolite dichloronitromethane (CHCl_2NO_2) was found in levels of approximately 20% of the parent compound. Further considerations as determined by the European Commission Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater for Substances Regulated under Council Directive 91/414/EEC have to be taking into account to assess the relevance of this metabolite in groundwater.

The toxicological significance of dichloronitromethane is assessed below.

Regarding acute toxicity, dichloronitromethane should be considered less toxic than Chloropicrin. This would be expected when comparing the molecular structure of the two compounds as dehalogenation in halonitromethanes has been shown to correlate with decreasing toxicity in this group (Sparks et. al., 1997 and 2000).

Sparks et. al. (1997) have demonstrated that the acute toxicity of dichloronitromethane to mice following intraperitoneal injection is 6 times less than that of its halogenated parent Chloropicrin with neurological signs similar for both compounds. Further investigations of the potential mechanism of toxicity have shown that dichloronitromethane acts as an 11 to 25 fold less effective inhibitor of pyruvate dehydrogenase (PDH) and succinate dehydrogenase (SDH) complexes compared with thiol reactive Chloropicrin (Sparks et. al. 2000). The

authors also determined that, unlike Chloropicrin, dichloronitromethane does not elevate oxyhaemoglobin levels. They concluded that the acute toxicity of Chloropicrin in mice was due to the parent compound or metabolites other than dichloronitromethane and chloronitromethane, and may be associated with inhibition of PDH and elevated oxyhaemoglobin. The acute oral LD₅₀ in rats of Chloropicrin is reported to be 250 mg/kg (IIA, 5.2.1) and given the findings discussed above the acute oral LD₅₀ of dichloronitromethane would be expected to be significantly higher than this.

Plewa *et. al.* (2004) showed that dichloronitromethane had a lower ability than Chloropicrin to cause DNA damage in hamster ovary cells. This study also concluded that the cellular cytotoxicity action of dichloronitromethane is similar to that of Chloropicrin.

Metabolism studies in mice have shown that dichloronitromethane is likely to be a major metabolite formed during the metabolism of Chloropicrin (see B.6.1). Therefore, it is considered that the toxic effects of dichloronitromethane have been taken into account in the battery of toxicology studies carried out with Chloropicrin.

Considering the transient nature of dichloronitromethane, and similarity to its parent compound, its toxicological nature and the fact that it occurs as a 20% dechlorination breakdown product of Chloropicrin, it would be highly unlikely that this metabolite could be of any more concern than its parent compound.

In line with the European Commission Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater for Substances Regulated under Council Directive 91/414/EEC, if a metabolite is found in animals and is less toxic than the parent, the acceptable limit may be defined starting from the ADI values derived for the parent compound (by using a conservative approach). According to what suggested by WHO Guideline for drinking water quality, the MAC (Maximum Allowable Concentration) of a compound can be derived by its ADI value. Considering a daily water consumption of 2L, the MAC can be evaluated as:

$$MAC = \frac{ADI \times bw}{C_w \times LF}$$

where:

ADI= Acceptable daily intake (parent compound); bw = body weight (60 kg for an adult); C_w = daily water consumption (2 litres); LF = location factor of 10%. It is the percentage of ADI which can be attributable to drinking water as single exposure source.

According to WHO guidelines, this value can be present in drinking water for a lifetime without appreciable health risk.

Following such an approach and based on the PEC_{gw} values, calculated for dichloronitromethane, a refined toxicological evaluation, with the availability of specific toxicity tests on the dichloronitromethane, should be carried out to adequately address the risk assessment of the metabolite. In the absence of such a study, the possibility to re-consider some of the exposure scenario is necessary.

B 6.8.2 Other/special studies

Cain, W.S., 2004, Human Sensory Irritation Testing for Chloropicrin.

Unpublished report-No PIC-1. Owner European Chloropicrin Group. 2004. IIA, 5.10/02 (EU- 5.8.2)

GLP: yes

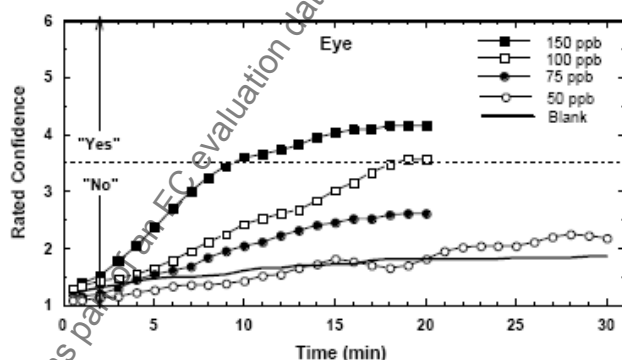
Methods:

Chloropicrin is used as a soil fumigant on its own or in conjunction with other substances. Chloropicrin can be sensed most intensely by the eyes where it can produce tearing and blepharospasm. This study sought to investigate this effect in relation to potential environmental and occupational exposures, and to establish thresholds for sensory responses.

A total of 127 young adult male and female human volunteers participated in three phases of testing. Young adults were selected for the study population. Phase 1 consisted of one of three tasks of odour detection, ocular detection and nasal localization, involving brief exposures of a few seconds to Chloropicrin at 356, 533, 800 or 1200 ppb to determine if Chloropicrin was detected via odour, feel in the eye and feel in the nose. Phase 2 consisted of exposures of 20 to 30 minutes in chambers to a blank, 50 ppb of Chloropicrin, lunch break, 75 ppb Chloropicrin, blank, 100 ppb Chloropicrin, blank and 150 ppb Chloropicrin to determine if concentrations which begin as undetectable become detectable over time across all modalities of exposure. Phase 3 consisted of exposures for an hour over four days with collection of exhaled nitric oxide in three cycles of six sessions of exposures to 100 ppb Chloropicrin, 150 ppb Chloropicrin and blank air to determine if repeated exposure to mildly irritating levels of Chloropicrin results in prolonged discomfort or inflammation in the mucosal tissue.

Results:

Taking into consideration data from the 3 phases of the study, phase 1 indicated that odour and eye irritation detection after a very short exposure was in average around 700 and 800 ppb, respectively (on the basis of 50% positive response). The dose response curve related to ocular irritation was particularly steep, so that small variation could lead to a wide difference in the response. Phase 2 demonstrated that the longer the period of exposure the lower was the concentration necessary to make people experiencing some effect, so that concentrations of chloropicrin that began as undetectable by feel became detectable over time (eye irritation being the most discernible end-point). Subjects could not discern between blank and 50ppb chloropicrin whereas they discerned between chloropicrin and blank exposure at concentrations as low as 75 ppb, but only over time during 20-min exposures. Levels of 75 to 100 and 150 ppb first became detectable in the eye after durations that varied inversely with concentration (see figure).



Phase 3 was designed to determine if repeated exposure to 100 and 150 ppb, mildly irritating (for the eyes and nose) levels of chloropicrin, resulted in prolonged discomfort and/or inflammation in the mucosal tissue. Measures of upper respiratory function (such as concentration of nitric oxide) showed some mild effect due to the exposures to 100 and 150 ppb, although measures of lower respiratory function did not.

Severity of symptoms experienced in the chamber during the one-hour exposures did not increase over a series of four successive days of exposure.

Conclusions:

It was found that the odour and ocular irritation of Chloropicrin became evident to most people within a minute or so after exposure to concentrations of 500 ppb or higher. If the concentration of Chloropicrin increased over time during a short exposure, odour was likely to become evident before ocular irritation and seemed to increase gradually in strength, with irritation increasing sharply.

On the basis of these considerations on results described in the study, it can be concluded that 75 ppb reasonably represents the NOAEL for the sensory irritation, being 50 ppb the NOEL.

Butala, J. H., 2007, Chloropicrin: use of human sensory irritation data in exposure standard setting. Report No.: ECG/04/05. Owner European Chloropicrin Group. 2007 (IIA, 5.10/03) (EU- 5.8.2)

The purpose of the human subject study conducted at the [REDACTED] (IIA, 5.10/02) was to address questions of human subjective and objective responses to environmentally-relevant levels of Chloropicrin vapour and to include in the methodology analytical verification of test material concentrations and standardised evaluation of subject responses.

A subsequent evaluation (IIA, 5.10/03) of the results obtained from this study has been carried out in order to use these data in exposure standard setting.

The results of the [REDACTED] study showed that exposure to Chloropicrin vapour concentrations at or below 75 ppb (0.075 ppm) is not likely to cause noticeable sensory irritation in the test population. Mild, reversible ocular irritation, a transient effect without long-term sequelae, is a uniform human response that is appropriate as a critical effect upon which human exposure limits can be set. Subject reporting of eye irritation proved to be the most sensitive endpoint for assessing effects of Chloropicrin exposure. That is, when exposed to environmentally-relevant levels of Chloropicrin vapour, human subjects reported eye symptoms (mild irritation) in the absence of other symptoms such as nose or throat irritation. This occurred when physical examination of the eyes, nose, throat and lung function revealed no adverse changes as a result of exposure. Eye irritation, then, is the effect of Chloropicrin exposure that is evident sooner than any other effect and at concentrations lower than those producing any other effect. A NOAEL for eye irritation could be estimated to be about 0.075 ppm based on all study data. In general, the NOAEL approach to dose-response assessment is limited because the NOAEL or LOAEL can only be one of the doses tested, does not take into account subject responses over the entire dose-response curve.

Therefore, a benchmark concentration for eye irritation and nasal nitric oxide (NO) levels was performed. The BMCL₁₀ was derived for a sensitive endpoint and represents the lower bound on a small percentage (10%) of a test population.

The BMCL₁₀ for nasal NO was 0.090 ppm, and the BMCL₁₀ for ocular symptoms was 0.073 ppm. The corresponding BMCs were 0.130 ppm and 0.110 ppm which together indicate that ocular symptoms are more sensitive than nasal effects, and represent the critical effect.

Since data have been obtained on human beings no uncertainty factor is needed for the interspecies extrapolation. However, it is of RMS opinion that for a risk assessment of the Operator/bystander exposure safe levels possible intra-individual differences have to be taken into account. Indeed, the number of subject used was limited, especially in the 3rd phase of the study (n=32; n ≈ 60 for the other 2 phases) not covering all the range of sensitivity. In addition, all the enrolled subject were healthy young adult volunteers, considered more susceptible than aged people to sensory irritation. However, it should be considered that sensory irritation was only one

of the effects noted in this study (being in some cases eye irritation the most discernible end-point). In addition, in RMS opinion bystander group consists not only of young adults and therefore people suffering respiratory discomfort/diseases (more frequent in aged people) and children may be more vulnerable and should be adequately protected.

In conclusion, exposure to Chloropicrin concentrations at or below 0.073 ppm is not likely to cause noticeable sensory irritation in the general population. Mild, reversible ocular irritation, a transient effect without long-term sequelae, is a uniform human response that is appropriate as a critical effect upon which human exposure limits can be set. No additional Safety Factor is warranted for the interspecies extrapolation. However, possible intra-individual differences have to be taken into account.

Butala, J. H., 2009a, Chloropicrin: Respiratory Sensitization. Report No.: ECG/05/09. Owner European Chloropicrin Group. 2009 (IIA, 5.10/04) (EU- 5.8.2)

An evaluation of the respiratory sensitisation potential of Chloropicrin is provided in this report and it is concluded that Chloropicrin is unlikely to be a respiratory sensitizer. Four lines of evidence were considered, as summarised below:

- 1.) Chloropicrin is not among the two dozen or so compounds known to be respiratory sensitizers and it is not structurally related to isocyanates, amines, acid anhydrides, metal salts, reactive dyes nor is it a protein. It does not contain functional groups as characteristic of respiratory sensitizers and it does not exhibit bifunctional reactivity which is associated with asthmagenicity. No references linking Chloropicrin with respiratory sensitisation were found in the literature. Allergic occupational asthma can be distinguished from "irritant-induced asthma" resulting from single, high intensity exposures to irritant fumes or gases such as Chloropicrin vapour.
- 2.) Repeated dose inhalation studies in rodents did not reveal any evidence of toxicological responses indicative of a respiratory sensitising effect or of changes to the immune system as a result of Chloropicrin exposures.
- 3.) The human subject study completed at the [REDACTED] did not specifically examine immune response to repeated Chloropicrin vapor exposures (IIA, 5.10/02). However, each of the three phases of the study involved repeated inhalation exposures to several concentrations of Chloropicrin vapour. Given that sensitization may occur following as little as one (or more) exposures to the sensitising agent, the human subject study design allowed for the possibility of sensitisation. Subject observations required by the study protocol were focused on breathing as well as sensory response and would have captured rhinitis or signs such as wheezing or difficulty in breathing, tightness in chest, etc. No such findings were reported. Foremost among the subject observations made in the study were recordings of exhaled nasal and pulmonary (lung) nitric oxide levels before and after repeated 1-hour exposures. All of the pulmonary nitric oxide levels were all unchanged as a result of Chloropicrin exposure and all levels were considered normal. This outcome does not match reports that increases in exhaled nitric oxide concentrations are associated with airway inflammation including airway inflammation associated with occupational respiratory sensitisation.
- 4.) The final piece of evidence supporting the claim that Chloropicrin does not act as a respiratory sensitizer comes from occupational medical monitoring programs used at Chloropicrin application companies in the US and Europe. Worker respiratory protection programs include requirements for periodic medical surveillance of employees with potential inhalation exposure to Chloropicrin. Results for surveillance programs of almost 300 employees at three applicator companies showed that respiratory function was normal in all

but 3 individuals who failed to qualify for respirator use because of pre-existing medical conditions since each of the three had no prior exposure to Chloropicrin. Thus all remaining employees with a history of working with Chloropicrin exhibited normal respiratory function.

Taken together these points support the position that Chloropicrin does not act as a respiratory sensitising agent and should not be classified and regulated as if it were.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL and DWL

Absorption, distribution, metabolism and excretion

In rats, Chloropicrin is rapidly ($C_{\max}$: 6-24 h) and extensively absorbed (>85%), based on data on radioactivity recovered in expired air, urine and carcass and widely distributed to different tissues. The excretion is mainly due to expired air as $^{14}\text{CO}_2$ (approximately 60% of the low dose and 40-50% of the high dose). Elimination via urine accounted for 20% (low dose) to 30% (high dose) and via faeces accounted for 10-15% (both doses). Small amounts of total radioactivity were found in tissues (< 1% in each one), with the highest values measured in the stomach and liver. No significant differences were reported between single and repeated dose treatment and between genders.

The presence of $^{14}\text{CO}_2$ in expired air and of polar metabolites in urine, indicated that the test substance is extensively metabolised and converted into several polar metabolites which have not been identified. However, results from literature data indicate that Chloropicrin is rapidly dechlorinated to CHCl_2NO_2 with conversion of GSH to GSSG. It is also proposed that the GS-adduct intermediate could cleave, leading to raphanusamic acid. A minor pathway has been identified involving adduct formation with thiol proteins such as Hb-adduct. Chloropicrin is also metabolised into CHCl_2NO_2 and CH_2ClNO_2 by liver microsomes and liver cytosol.

Acute mammalian toxicology

Undiluted Chloropicrin is harmful following ingestion and irritant to the skin and eyes. It is unlikely to be a skin sensitiser. Chloropicrin resulted highly toxic after inhalation, with a 4-hour nose-only inhalation LC_{50} in rats of 6.6 ppm (equivalent to 0.04 mg/l). The sensory irritation study, performed in mice inhaling Chloropicrin vapour for 30 minutes (head only exposure) established that Chloropicrin is a sensory irritant with a RD_{50} value of 2.34 ppm (0.016 mg/l). Other than sensory irritation (eye and nasal irritation), adverse health effects do not appear to occur in mice during and after exposure to Chloropicrin vapour at the RD_{50} concentration.

Short term toxicity

Oral toxicity studies have been carried out to investigate the short-term toxicity of Chloropicrin when administered to rats (90-days) and dogs (8 weeks and 1 year). On the basis of the results obtained from the 90-days oral study a NOAEL of 8 mg/kg/day was established for rats. In the 1-year dog study, the NOAEL value for oral toxicity was 0.1 mg/kg/day. From the 90-day inhalation toxicity studies performed on rats and mice it has been established that respiratory tissue is the major target of toxic effects due to exposure to Chloropicrin vapour. Due to adverse effects occurring in the upper respiratory tissue of animals inhaling chloropicrin vapour at the lowest concentrations tested, that is 0.3 ppm (equivalent to 0.54 mg/kg/day for rats and 0.96 mg/kg/day for mice) a reliable NOAEL could not be determined from the key study.

Genotoxicity

The results of mutagenicity tests in vitro indicate that pure (>99%) Chloropicrin is weakly mutagenic in bacteria in the presence of exogenous metabolic activation, and clastogenic in cultured mammalian cells, only in the absence of metabolic activation and at doses producing significant toxicity. Negative results were obtained in a mammalian cell gene mutation assay. In vivo, negative results were obtained in an adequately performed mouse bone marrow micronucleus test, with evidence of toxicity to target cells, and in the rat liver UDS. Based on these findings, it is concluded that the genotoxic potential observed in vitro is not expressed in detectable way in vivo, and therefore Chloropicrin can be evaluated as **not genotoxic** in vivo.

Long-term toxicity and carcinogenicity

Oral and inhalation long-term bioassays have been performed with Chloropicrin to evaluate the potential of this compound to cause chronic and/or carcinogenic effects.

From the long-term oral toxicity study performed via gavage with CD rats, a NOAEL of 0.1 mg/kg bw per day was established on the basis of the incidence of epithelial hyperplasia and hyperkeratosis in the nonglandular stomach.

Lifetime exposures to Chloropicrin vapour via whole body inhalation to CD-1 mice and CD rats indicate that respiratory tissue is the primary target organ for toxic effects. The NOEL was 0.1 ppm both for rats (0.18 mg/kg bw per day) and mice (0.32 mg/kg bw per day).

Three carcinogenicity studies have been performed with Chloropicrin. CD rats and CD-1 mice have been exposed by inhalation and CD rats have been administered Chloropicrin by gavage. Chloropicrin has shown no evidence of carcinogenic potential.

Reproduction and developmental toxicity

Chloropicrin has been evaluated in standardized regulatory protocols and tested under US EPA Good Laboratory Practices Regulations for the potential to produce developmental toxicity or adversely affect reproductive function. Testing has been via the most important route of exposure, inhalation, and conducted in two species: rats and rabbits. The two-generation study on rat showed no effects on fertility and the effects on foetuses were present only at dose levels with maternal toxicity. Developmental toxicity studies in the rat and rabbit revealed minor developmental effects at maternally toxic doses only. On the basis of the available data, Chloropicrin does not induce specific effects on reproduction and development.

Other/special studies

In a human subject study, it was concluded that Chloropicrin behaves as a mild irritant at the concentrations studied over time and that sensory (ocular) irritation, the most sensitive indicator of exposure. The results showed that exposure to Chloropicrin vapour at or below concentrations of 75 ppb (0.075 ppm) is not likely to cause noticeable sensory irritation. The BMCL₁₀ was derived for ocular irritation and represents the lower bound on a small percentage (10%) of the test population. The BMCL₁₀ for ocular symptoms was 0.073 ppm.

B.6.10.2 Proposal for an Acceptable Daily Intake

No suitable human data are available which would serve as a basis for setting an Acceptable Daily Intake (ADI) for Chloropicrin.

The calculation of the ADI is therefore based on data from studies in laboratory animals. Results from all available toxicology studies on the test substance are considered. The ADI is

derived using the most sensitive relevant effect in the most sensitive relevant animal model and an appropriate uncertainty factor. In the European Community, a no-observed adverse effect level (NOAEL) rather than a no-observed effect level (NOEL) is used in accordance with Directive 91/441/EEC.

An oral NOAEL value of 0.1 mg/kg bw was obtained from a two year oral study in the rat. This value was based on findings of epithelial hyperplasia and hyperkeratosis in the non-glandular stomach. The application of a safety factor of 100 is suggested to derive the ADI,

The proposed oral ADI is:

NOAEL/Safety factor = 0.10 mg/kg bw/day/100 = 1 µg /kg bw/day

The RMS agrees with The Notifier and other Member States that a TDI is not necessary for the reasons stated below.

The acceptable daily intake (ADI) is the amount of a chemical which can be ingested every day of an individual's entire lifetime in the practical certainty, on the basis of all known facts, that no harm will result. The ADI is expressed as mg/kg bw of the consumer. The risk from inhalation exposure to Chloropicrin by the general population, i.e. bystander occasionally exposed and for a short time, is dealt with using the specific AOEL calculated for bystanders (on the basis of the results obtained from the recent human study IIA, 5.10/02) as the appropriate reference dose. Therefore, it is believed that a TDI is not necessary for Chloropicrin and that an ADI is relevant for the assessment of long term consumer exposure by the dietary route.

B.6.10.3 Proposal for an ARfD (Acute reference dose)

In the original DAR the RMS considered the inhalation route as the major source of exposure for humans and then the 90-day inhalation study was taken as relevant.

However, if ARfD is considered limited to protect human health from exposure to the oral route according to the Draft Guidance Document "Guidance for the setting of an Acute Reference Dose (ARfD)", the NOAEL (0.1 mg/kg/d) from the 1 year dog study is to be considered for ARfD calculation.

The proposed oral ARfD is:

NOAEL/Safety factor = 0.10 mg/kg bw/day/100 = 0.001 mg/kg bw

B.6.10.4 Proposal for a Drinking Water Limit

According to the present EU rules a drinking water limit is 0.1 µg/l for active substances.

B.6.10.5 Proposal for an Acceptable Operator Exposure Level

According to the Draft Document "Guidance for the setting and application of acceptable operator exposure levels (AOELs)" (rev.10) AOELs should be set to ensure that the presence of an active substance in a PPP, used in a manner consistent with the label instructions and good plant production practice, has no harmful effects on the health of operators, workers, bystanders or residents.

Usually, the AOEL is derived from a complete package of mammalian toxicity and metabolism studies. The AOEL is based on the highest dose at which no adverse effect is observed (i.e., the NOAEL) in the most sensitive relevant animal species or, if appropriate data are available, in humans. As the most appropriate route of exposure for Chloropicrin in the inhalation route, a NOAEL from an inhalation study was deemed the most appropriate level.

Three population groups are considered for setting an AOEL:

1. Operators – tractor drivers, tiller operators, tarp sealers and assistants during application
2. Re-entry workers - tractor drivers at tarp cutting, tarp removers and workers transplanting in soil
3. Bystanders – members of the public or assistants who may be in the vicinity of the treated plot

Groups 1 and 2 are likely to work for up to 8-hours a day but would be exposed to Chloropicrin vapour for not more than 6 hours per day for a few months per year. Individuals would potentially be subject to daily exposure over several days during the application period. Therefore, the AOEL needs to consider potential systemic toxicity from repeated exposure as well as portal of entry effects to the respiratory tract. Group 3 (bystanders) could potentially be close to the edge of treated plots and exposed to Chloropicrin vapour for periods of up to 1-hour as a conservative default. In this case an AOEL based on appropriate acute inhalation portal of entry effects is considered appropriate.

Groups 1 and 2, are subject to sensory irritation, portal-of-entry and possibly systemic respiratory risk. Accordingly the AOEL for occupational exposures is based on animal toxicology study findings. Acute inhalation exposure is the greatest risk concern for Group 3, bystanders, and they can be adequately protected by maintaining their exposure at or below the threshold level for transient eye sensory irritation. The human subject study completed at the [REDACTED] most closely resembles the acute bystander inhalation scenario (1-8 hours of exposure) for human health risk assessment (IIA, 5.10/02). Eye irritation was the most sensitive endpoint determined for the sensitive subpopulation used in the human subject study, therefore, protecting against transient eye irritation would also protect against adverse irritation of the eyes, nose, and upper respiratory tract.

AOEL for operators and re-entry workers

The 90-day rodent inhalation studies are considered for establishing a systemic AOEL instead of the rat chronic study because the subchronic timeframe of the 90-day study is more appropriate for a fumigation applicator exposure scenario. In the key study (Point 5.3.6/01), a NOAEL could not be derived, a LOAEL of 0.3 ppm for rats (equivalent to 0.54 mg/kg bw/day) and 0.3 ppm for mice (equivalent to 0.96 mg/kg bw/day) was established. Therefore, the LOAEL to derive the AOEL is 0.3 ppm (2×10^{-3} mg/l) in the rat (equivalent to 0.54 mg/kg bw/day/day).

In deriving the AOEL a default position is used to account for uncertainties in extrapolation from toxicity data to the exposed human population. An assessment factor of 300 is used to account for interspecies variability (10X), for intraspecies variability (10X) and considering that the AOEL is based on a LOAEL rather than a NOAEL (3X).

Guidance on the establishment of a systemic AOEL (expressed in mg/kg bw/day) from animal inhalation studies is provided in the SANCO Guidance for the Setting and Application of Acceptable Operator Exposure Levels¹. By assuming 100% absorption of inhaled Chloropicrin and applying this guidance to the rat 90-day Chloropicrin inhalation study it is possible to express inhalation exposure as a systemic dose:

¹ EU (2006): Guidance for the Setting and Application of Acceptable Operator Exposure Levels (AOELs). SANCO 7531 – rev. 10

$$\begin{aligned}\text{LOAEL}_{\text{internal}} &= \text{LOAEC}_{\text{inhalation}} \times 45 \text{ l/kg bw/hr (rat respiration rate)} \times \text{daily} \\ &\quad \text{inhalation exposure} \\ &= 2 \times 10^{-3} \text{ mg/l} \times 45 \text{ l/kg bw/hr} \times 6 \text{ hr/day} \\ &= 0.54 \text{ mg/kg bw/day}\end{aligned}$$

Application of the proposed 300X Safety Factor results in a systemic AOEL of 0.002 mg/kg bw/day.

AOEL for bystander exposure

In the Human Subject Study with Chloropicrin it was established that sensory irritation of the eye is the most sensitive endpoint of Chloropicrin exposure and that it is a threshold event (IIA, 5.10/02). Since the human sensory irritation study showed that pulmonary inflammation does not appear to occur in humans even in the presence of sensory irritation, protection against sensory irritation in humans should be protective of respiratory system effects. Accordingly, a reasonable standard for short-term and intermediate-term exposure can be based on prevention of sensory irritation, the most sensitive endpoint of Chloropicrin exposure. A NOAEL of 0.075 ppm for eye irritation has been estimated based on all study data.

In deriving the AOEL for bystander a default position is used to account for uncertainties in extrapolation from toxicity data to the exposed human population. An assessment factor of 10 is used to account for interspecies variability. Application of the proposed 10X Safety Factor results in a systemic bystander AOEL of 0.0075 ppm.

B.6.11 Supplemental studies for combinations of plant protection products (IIIA 7.1)

Not relevant in the case of Chloropicrin

B.6.12 Dermal absorption

No study is available to address this point. However, considering the volatile and corrosive nature of Chloropicrin would be technically challenging to carry out a dermal absorption study. For these reasons the request for a dermal absorption study is thought not to be appropriate and a default value of 100% is proposed.

B.6.13 Toxicological data on non-active substances

Please refer to MSDSs for coformulants

B.6.14 Exposure data (ANNEX IIIA 7.2)

B.6.14.1 Operator exposure

Operator exposure is preferably assessed on the basis of representative field studies. When no representative field studies are supplied operator exposure is usually estimated using both the German model [Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Fortwirtschaft, Berlin-Dahlem, no. 277, 1992] and the UK model [Predictive Operator Exposure Model (POEM), UK MAFF, 1992].

However, as Chloropicrin 99 is applied pre-planting to the bare soil for the control of various fungal disease nematodes and germinating weed seeds, by soil injection or drip irrigation application methods, neither of these models can be used for calculation of operator exposure.

Chloropicrin 99 contains 990g/kg of the active substance Chloropicrin.

Chloropicrin is packaged in steel cylinders. A typical pack size is 28 kg (61L) cylinders. Chloropicrin 99 is applied with a single application per annum at a proposed maximum application rate of 272 l/ha (448 kg as/ha).

Product handling is restricted only to professional users. The application system is a closed system that does not require transfer of fumigant, which to a large extent mitigates operator exposure.

Two methods of application are proposed: soil injection and drip irrigation.

Injection application method

Chloropicrin 99 can be applied by direct injection into the soil using suitable fumigant application machines pulled by tractor, with delivering blades no more than 30 cm and cutting 15 cm into the soil. Soil must be covered with plastic films virtually impermeable to vapours (VIF), at the time of application. The VIF should be glued together, with the outer sides buried into the soil 15-20 cm deep. After the application the plastic cover must be maintained in place for at least 4-7 days. When removing the VIF, proceed first to lift the buried sides then, after 12-24 more hours, the whole plastic should be removed.

Drip application method

A steel cylinder containing Chloropicrin 99 is transported to the field to be treated. The cylinder is then connected to the drip (trickle) irrigation system (in place on the ground and under a layer of Tarp) by means of a pipeline. The cylinder is then pressurized to facilitate transfer of the product through the pump. The water is turned on, and then the pump is turned on. The Chloropicrin 99 is metered into the water by means of a flow controller. The dosage of the product is determined by the flow rate of the fumigant, the flow rate of the water used in the application, the duration of the application (usually 4-8 hours), and the area treated.

Operator exposure monitoring studies

Five operator exposure field studies have been carried out which measure the levels of exposure to the operator during application of Chloropicrin.

“Monitoring of Chloropicrin Emissions from Field and Greenhouse Drip Irrigation Applications, and Implied Worker Inhalation Exposure From Application of Chloropicrin by Shank Injection, Drip irrigation Systems and a Tree Replant sites”

“Monitoring of Potential Worker Exposure, Field Flux, and Off-Site Air Concentration during Chloropicrin Field Application”

“Research on Chloropicrin Residues in Air when the Plant Protection Product Tripicrin™ is Applied in Greenhouse Through the Drip Irrigation System”

“Analysis of Chloropicrin Residues in the Atmosphere during an Open Field Application to evaluate the Risk of Exposure to Operators and Bystanders”

“Chloropicrin: concentration in air and operators exposure assessment during and after soil application”

The previous four studies have been evaluated in the DAR, the fifth is summarized below:

Trevisan, M., 2009, Chloropicrin: concentration in air and operators exposure assessment during and after soil application. CERZOO - Centro Ricerche per la Zootecnia e l'Ambiente, Piacenza, Italia. Unpublished report-No

CZ/08/013/UCSC/CHLAIR/RF. Owner European Chloropicrin Group. 2009. (IIIA 7.3.3/01)

GLP: Yes

Methods:

The study was conducted at an experimental field site located near Alessandria in northern Italy. Chloropicrin (99%) was applied to a test field system (2 ha) according to normal application practices (shank application technique), at a rate equivalent to 432 kg/ha, and then covered with a virtually impermeable film (VIF) for 7 days. On Day 7, the VIF was opened by cutting and on day 8, the VIF was completely removed.

Twelve air samplers were installed beyond the field margins, three on each compass point aspect of the plot in the middle of the respective edge, located 1, 20 and 50 m away from the field margins. Another sampling position was located away from the treated area (1250 m in a South East direction) as a control. Air samplers were positioned 150 cm above the ground. Sampling was conducted continuously for 14 days after application.

In addition to field sampling, air samples were also taken from the operators/workers during Chloropicrin application, VIF cutting and VIF removal.

Field spiked air sampling tubes were fortified with nominally 3 µg of Chloropicrin for subsequent analysis to confirm the stability of any residues in study samples. Sampling tubes taken from the field were sealed and immediately placed in portable freezers until transport to the test facility where they were placed in frozen storage (<-20°C). All the samples collected during the study were analysed for Chloropicrin using a validated procedure employing extraction into hexane and GC-ECD detection. Air samplers were installed at three distances (1, 20 and 50 m) from the field margins in the four cardinal directions and the concentration of Chloropicrin in the sampled air was measured continuously for 14 days after the application. Air samples were also taken from the operators who were involved in the Chloropicrin application. Recovery tests from XAD-4 resin at different concentrations were carried out prior to analysis, spiking a measured amount of active ingredient on the sorbent resin. Different concentrations of Chloropicrin standard solutions were adsorbed on XAD-4 and were then submitted to air aspiration flow, for around ten hours, in order to simulate the field process.

Results:

During the recovery trials the temperature ranged from 22°C to 28°C, while the air humidity ranged from 40 to 75%. Overall mean recovery was 91.3% with an RSD of 6.4%. During the validation analysis the breakthrough for each spiked concentration was assessed. The rear sections of the XAD-4 tubes contained <10% of the Chloropicrin measured in the main section. Analysis of the field spike samples resulted in a mean recovery of 86% with an RSD of 12.7%. This confirmed that any residues of Chloropicrin taken at the field site could be accurately determined in the subsequent analysis.

Chloropicrin was determined in the air samples taken from the field monitoring positions and reported as daily weighted mean (DWM) concentrations in air. DWMs were calculated from the results of three consecutive 8-hour samples on Days 1 and 2 and of two consecutive 12-hour samples on each of Days 3 to 14, as mg/m³. A summary of the findings is shown in Table B.6.14.1-1 to Table B.6.14.1-3.

Table B.6.14.1-1: Chloropicrin air emission concentrations 1 m from plot margins following field shank application

Day	Chloropicrin concentration, daily weighted mean (DWM, mg/m ³) ¹			
	North	East	South	West
1	0.0491	0.0109	0.0161	0.0395
2	0.0174	0.0228	0.0108	0.0057
3	0.0078	0.0027	0.0065	0.0021
4	0.1702 ²	0.0329	0.0164	0.0188
5	0.0038	0.0009	0.0003	0.0006
6	0.0179	0.0218	0.0085	0.0226
7	0.0112	0.0009	0.0110	0.0511
8	0.0034	0.0363	0.0197	0.0334
9	0.0069	0.0014	0.0018	0.0007
10	0.0101	0.0092	ND	0.0004
11	0.0071	0.0041	0.0043	0.0086
12	ND	ND	ND	ND
13	ND	ND	ND	ND
14	ND	ND	ND	ND

1 – ND denotes no Chloropicrin residue detected

2 – Maximum DWM derived from two results of 0.3077 mg/m³ for 797 minutes and 0.008 mg/m³ for 647 minutes

The highest air DWM concentration reported for the North side of the plot on Day-4 was attributed in part to the higher recorded air temperatures recorded around this timepoint.

Table B.6.14.1-2: Chloropicrin air emission concentrations 20 m from plot margins following field shank application

Day	Chloropicrin concentration, daily weighted mean (DWM, mg/m ³) ¹			
	North	East	South	West
1	0.0900	0.0066	0.0285	0.0390
2	0.0319	0.0149	0.0040	0.0022
3	0.0281	0.0035	0.0041	0.0047
4	0.1636 ²	0.0413	0.0036	0.0003
5	0.0035	0.0010	0.0003	0.0007
6	0.0196	0.0052	0.0064	0.0063
7	0.0108	0.0039	0.0074	0.0197
8	0.1207	0.0374	0.0248	0.0148
9	0.0065	0.0006	0.0014	0.0003
10	0.0086	0.0017	ND	0.0023
11	0.0048	0.0020	0.0032	0.0053
12	ND	ND	ND	ND
13	ND	ND	ND	ND
14	ND	ND	ND	ND

1 – ND denotes no Chloropicrin residue detected

2 – Maximum DWM derived from two results of 0.2960 mg/m³ for 795 minutes and 0.0020 mg/m³ for 651 minutes

Table B.6.14.1-3: Chloropicrin air emission concentrations 50 m from plot margins following field shank application

Day	Chloropicrin concentration, daily weighted mean (DWM, mg/m ³) ¹			
	North	East	South	West
1	0.0371 ²	0.0026	0.0161	0.0302
2	0.0227	0.0093	ND	0.0027
3	0.0039	0.0020	0.0006	0.0052
4	0.0016	ND	0.0103	0.0003
5	0.0016	0.0004	0.0002	0.0003
6	0.0107	0.0024	0.0039	0.0052
7	0.0065	0.0023	0.0032	0.0094
8	0.0057	0.0157	0.0138	0.0055
9	0.0057	0.0005	0.0014	0.0031
10	0.0008	0.0010	ND	0.0014
11	0.0033	0.0014	0.0017	0.0033
12	ND	ND	ND	ND
13	ND	ND	ND	ND
14	ND	ND	ND	ND

1 – ND denotes no Chloropicrin residue detected

2 – Maximum DWM derived from three results of 0.0163 mg/m³ for 440 minutes, 0.0466 mg/m³ for 525 minutes and 0.0456 mg/m³ for 497 minutes

In the air samples taken from the field margins continuously after application for 14 days, levels of Chloropicrin were highest in the 1.5 m height sampling stations situated 1 m away from the treated field margins. The daily weighted mean (DWM) values were a maximum of 0.0491 mg/m³ on the day of application (day 1) reaching a DWM maximum of 0.1702 mg/m³ on day 4. There was a good correlation between the higher values observed to the orientation of the field with respect to the wind direction.

On days 12 to 14 no Chloropicrin was detected in any of the samples taken from 1 m, 20 m or 50 m away from the field margins.

Results of air samples taken from the operators during Chloropicrin application, VIF cutting and VIF removal are summarised in Table B.6.14.1-4.

Table B.6.14.1-4: Operator exposure to Chloropicrin following field shank application

Day (operation)	Operators	Description of the worker	Time of exposure (min)	Chloropicrin concentration (mg/m ³)		
				right	left	mean
Day 1 (application)	WRK1 Driver	Tractor driver	274	0.151	0.120	0.1355
	WRK2 Worker	Tiller operator	275	0.340	0.435	0.3875
	WRK3 Worker	Tiller operator sometimes sealed the tarp stripes	275	0.467	0.481	0.474
	WRK4 Assistant	At the side of the plot, sometimes sealed the start/end of the tarp stripes	274	0.006	0.025	0.0155
Day 7 (VIF cutting)	WRK1 Driver	Tractor driver	77	0.322	0.284	0.303
	WRK2 Assistant	At the side of the plot, sometimes refined the tarp cutting	76	0.016	0.019	0.0175
	WRK3 Assistant	At the side of the plot	77	0.020	0.018	0.019
Day 8 (VIF removal)	WRK1 Worker	Tarp remover	147	0.0039	0.0029	0.0034
	WRK2 Worker	Tarp remover	147	0.0099	0.0076	0.00875
	WRK3 Assistant	At the side of the plot,	147	0.0029	0.0026	0.00275

Conclusions:

Under the conditions of this study, Chloropicrin applied in a field at a rate of 432 kg/ha resulted in mean operator exposures to air of between 0.016 (assistant working mainly to side of plot) and 0.474 mg/m³ (tiller operator) during the application process. Chloropicrin was also measured in the air samples of the operators who were involved in opening or cutting of the VIF covering (Day 7) with between 0.018 (assistant working mainly to side of plot) and 0.303 mg/m³ (tractor driver) during this process. Finally, reduced Chloropicrin levels were measured in the air samples of the operators who were involved in removing the VIF covering (Day 8) with between 0.0028 (assistant working mainly to side of plot) and 0.0088 mg/m³ (VIF remover) during this process.

In the air samples taken from the field margins continuously after application for 14 days, levels of Chloropicrin were highest in the 1.5 m height sampling stations situated 1 m away from the treated field margins. The daily weighted mean (DWM) values were a maximum of 0.0491 mg/m³ on the day of application (day 1) reaching a DWM maximum of 0.1702 mg/m³ on day 4. There was a good correlation between the higher values observed to the orientation of the field with respect to the wind direction.

On days 12 to 14 no Chloropicrin was detected in any of the samples taken from 1 m, 20 m or 50 m away from the field margins.

Assessment of Operator Exposure

The field generated data for exposure of operators to Chloropicrin can be used to assess the potential risks associated with this method of application.

Determination of the Tolerable Exposure

Derivation of the AOEL is presented in B.6.10.5. The following LOAEL was obtained in a toxicological study relevant to operator safety with Chloropicrin.

Study type	LOAEL
90-day rat inhalation	0.54mg/kg bw/day

In deriving the AOEL an assessment factor of 300 is used to account for interspecies variability (10X), for inter-individual variability (10X) and considering that the AOEL is based on a LOAEL rather than a NOAEL (3X). Thus, a systemic AOEL of 0.002 mg/kg bw/day is calculated.

The only route of exposure to chloropicrin is inhalation and 100% systemic absorption is assumed.

Comparison of Measured and Tolerable Exposure

From the study results, the various activities associated with first the application and then the cutting and removal of the VIF present air concentrations to which the operators / workers were exposed. In this section the data are considered for the operators present during application only and for this operation the worst case exposure was for Worker 3 (tiller operator).

The average air concentration to which Worker 3 was exposed was 0.474 mg/m³ Chloropicrin. Worker 3 was engaged in these activities for a total of 275 minutes (4.58 hours) but it is necessary to estimate the exposure data to a realistic worst case working day for continued exposure to Chloropicrin (6 hours).

The breathing rate for a worker is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which Worker 3 would have been exposed for a 6-hour day is given by:

Air concentration (mg/m³) x Breathing rate (m³/hour) x Working day (hours)

For this study the total exposure for Worker 3 equals:

0.474 (mg/m³) x 1.25 (m³/hour) x 6 (hours)

= 3.555 mg Chloropicrin

On the basis of the estimated exposures, the portion of the proposed AOEL_{systemic} (0.054 mg/kg bw/day) accounted for is shown in the following table. Systemic exposures were estimated assuming a worst-case 100% inhalation absorption. This estimation is assuming that no respiratory protective equipment (RPE) is worn.

Total Systemic Exposure – 60 kg person (mg/kg bw/day)	% of AOEL (0.002 mg/kg bw/day)
3.555 / 60 = 0.05925	2962%

A second tier operator exposure assessment is conducted considering the use of RPE.

It is proposed to use the “assigned protection factors” (APF) as deduced by BSI and ANSI to calculate inhalation exposure loading.

Since these values are somewhat at variance and since in agricultural settings efficient control and proper training and education with respect to inhalation protection devices, is generally absent, it is good to err on the safe side and use the lowest of both values, if available².

For a half mask fitted with a gas filter the APF value is 10 and for a full mask fitted with a gas filter the APF value is 20. For a power assisted full face gas filtering device (TM 3) the APF value is 40.

The exposure assessment can therefore be refined further as shown in the following table.

Total Systemic Exposure 60 kg person (mg/kg bw/day)	RPE type	RPE reduction factor (APF)	% of AOEL (0.002 mg/kg bw/day)
3.555 / 60 = 0.05925	Half mask with gas filter	10 (x 0.1)	296%
3.555 / 60 = 0.05925	Full mask with gas filter	20 (x 0.05)	148%
3.555 / 60 = 0.05925	Power assisted full face gas filtering device (TM 3)	40 (x 0.025)	74%

Assessment

The exposure estimates shown above demonstrate that operator exposures to Chloropicrin during field application according to the GAP are above the AOEL_{systemic} only if a power assisted full face gas filtering device (TM 3) is worn.

See B.6.14.1 for the assessment of exposure to Chloropicrin for workers involved in the associated application activities of VIF cutting/puncturing and VIF removal.

B.6.14.2 Bystander exposure

Due to the nature of Chloropicrin, it is advised that bystanders are excluded from the area being treated. Only trained handlers should be permitted to the area during fumigation. Chloropicrin is injected directly into the ground, which is then immediately covered with tarp (VIF) for a period of 7-8 days, thus ensuring that exposure to workers or bystanders, either directly or through vapour transport is minimised. Despite this, it is of value to understand what potential risks may exist for a bystander in the vicinity of a field either being treated or

² Rianda Gerritsen-Ebben, Derk H. Brouwer, Jopp J. Van Hemmen; Effective Personal Protective Equipment (PPE). Vision paper on the use of PPE for registration purposes of handling of agrochemical, microbiological and biocidal pesticides. June 2006

having recently been treated with Chloropicrin. Existing exposure scenarios or models are not appropriate for estimating such potential risk but a field study to measure environmental emissions of Chloropicrin has been conducted (B.6.14.1. Trevisan, M., 2009) and an assessment based on this study data is presented below.

Assessment of Bystander Exposure

The field generated data for environmental emissions of Chloropicrin can be used to assess the potential risks to bystanders located at different distance from the treated area (1 m, 20 m and 50 m).

Determination of the Tolerable Exposure

In the Human Subject Study with Chloropicrin it was established that sensory irritation of the eye is the most sensitive endpoint of Chloropicrin exposure and that it is a threshold event (IIA, 5.10/02). Since the human sensory irritation study showed that pulmonary inflammation does not appear to occur in humans even in the presence of sensory irritation, protection against sensory irritation in humans should be protective of respiratory system effects. Accordingly, a reasonable standard for short-term and intermediate-term exposure can be based on prevention of sensory irritation, the most sensitive endpoint of Chloropicrin exposure. A NOAEL of 0.075 ppm for eye irritation has been estimated based on all study data.

In deriving the AOEL for bystander a default position is used to account for uncertainties in extrapolation from toxicity data to the exposed human population. An assessment factor of 10 is used to account for inter-individual variability. Application of the proposed 10X Safety Factor results in a systemic bystander AOEL of 0.0075 ppm.

Comparison of Measured and Tolerable Exposure

From the study results, the worst case air emission concentration of Chloropicrin occurred at 1, 20 and 50 m have been considered for assessment. The maximum air concentration to which a bystander in proximity to the treated area might have been exposed was 0.046 ppm Chloropicrin. A conservative assumption for the duration of exposure is that a bystander may have been present for a period of 1 hour.

From the study result, the maximum level of 0.046 ppm for a bystander located 1 m from the treated area is 613% of the proposed bystander AOEL.

From the study result, the maximum level of 0.044 ppm for a bystander located 20 m from the treated area is 587% of the proposed bystander AOEL.

From the study result, the maximum level of 0.0069 ppm for a bystander located 50 m from the treated area is 92% of the proposed bystander AOEL.

Alternative approach using systemic AOEL

Derivation of the AOEL is presented in B.6.10.5. The following LOAEL was obtained in a toxicological study relevant to operator safety with Chloropicrin.

Study type	LOAEL
90-day rat inhalation	0.54mg/kg bw/day

In deriving the AOEL an assessment factor of 300 is used to account for interspecies variability (10X), for inter-individual variability (10X) and considering that the AOEL is based on a LOAEL rather than a NOAEL (3X). Thus, a systemic AOEL of 0.002 mg/kg bw/day is calculated.

The only route of exposure to chloropicrin is inhalation and 100% systemic absorption is assumed.

The maximum air concentration to which a bystander in proximity (1 m) to the treated area might have been exposed was 0.3077 mg/m³ Chloropicrin. A conservative assumption for the duration of exposure is that a bystander may have been present for a period of 1 hour.

The breathing rate for a bystander is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which a bystander located 1 m from the treated area may have been exposed for a 1-hour period is given by:

$$\text{Air concentration (mg/m}^3\text{)} \times \text{Breathing rate (m}^3\text{/hour)} \times \text{Exposure duration (hours)}$$

From the study data the total exposure for a bystander equals:

$$0.3077 \text{ (mg/m}^3\text{)} \times 1.25 \text{ (m}^3\text{/hour)} \times 1 \text{ (hour)}$$

$$= 0.3846 \text{ mg Chloropicrin}$$

The maximum air concentration to which a bystander located 20 m from the treated area might have been exposed was 0.2960 mg/m³ Chloropicrin. A conservative assumption for the duration of exposure is that a bystander may have been present for a period of 1 hour.

The breathing rate for a bystander is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which a bystander located 20 m from the treated area may have been exposed for a 1-hour period is given by:

$$\text{Air concentration (mg/m}^3\text{)} \times \text{Breathing rate (m}^3\text{/hour)} \times \text{Exposure duration (hours)}$$

From the study data the total exposure for a bystander equals:

$$0.2960 \text{ (mg/m}^3\text{)} \times 1.25 \text{ (m}^3\text{/hour)} \times 1 \text{ (hour)}$$

$$= 0.37 \text{ mg Chloropicrin}$$

The maximum air concentration to which a bystander located 50 m from the treated area might have been exposed was 0.0466 mg/m³ Chloropicrin. A conservative assumption for the duration of exposure is that a bystander may have been present for a period of 1 hour.

The breathing rate for a bystander is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which a bystander located 50 m from the treated area may have been exposed for a 1-hour period is given by:

$$\text{Air concentration (mg/m}^3\text{)} \times \text{Breathing rate (m}^3\text{/hour)} \times \text{Exposure duration (hours)}$$

From the study data the total exposure for a bystander equals:

$$0.0466 \text{ (mg/m}^3\text{)} \times 1.25 \text{ (m}^3\text{/hour)} \times 1 \text{ (hour)}$$

= 0.0583 mg Chloropicrin

On the basis of the estimated exposures, the portion of the proposed AOEL_{systemic} (0.002 mg/kg bw/day) accounted for is shown in the following table. Systemic exposures were estimated assuming a worst-case 100% inhalation absorption. This estimation is assuming that no protective equipment (PE) is worn.

Bystander distance from treated area (m)	Total Systemic Exposure – 60 kg person (mg/kg bw/day)	% of AOEL (0.002 mg/kg bw/day)
1	0.3846 / 60 = 0.0064	320%
20	0.37 / 60	308%
50	0.0583 / 60	49%

Assessment

The exposure estimates shown above demonstrate that bystander exposures to Chloropicrin are below the proposed systemic AOEL for bystanders only if bystanders are located 50 m from the treated area.

B.6.14.3 Worker exposure

Two scenarios are assessed:

1. Estimation of worker exposure by dermal contact with treated soil at time of transplanting
2. Assessment of inhalation exposure of workers involved in activities post application, i.e. VIF cutting and VIF removal.

B.6.14.3.1 Estimation of worker exposure by dermal contact with treated soil at time of transplanting

At least 14 days must lapse between tarp removal and the time when the crops are sown or transplanted. Since Chloropicrin is volatile, it is proposed that only minimal residues will be left on the treated field surface when the workers enter the treated areas. As Chloropicrin is not intended for use by foliar application, it is not envisaged that exposure to DFRs will be a point of concern.

However, estimation of the potential for exposure to Chloropicrin residues that may be present in the topsoil at the time of transplanting when workers are involved in manual activities can be considered using predicted soil residues and available surrogate transfer values.

The PEC soil value for the topsoil 14 days after injection treatment at the supported GAP is 16.7 mg/kg (see B.8). This value can be used as a worst case assessment but it should again be noted that due to volatilisation the actual concentration available to workers manually touching the upper soil layer is likely to be much lower.

Data are available from the UK CRD (Guidance for Post-Application (Re-Entry Worker) Exposure Assessment) for scenarios where workers may be exposed to soil borne residues. Field studies investigating dermal exposure to soil by direct gravimetric measurements

(Kissel et al)³ suggest that an appropriate hand soil loading for a worker would be 0.44 mg/cm² (geometric mean peak value for farmers involved in hand weeding).

Exposure may therefore be estimated as:

Dermal exposure = Conc._{soil} x Dermal adherence_{soil} x Skin area contaminated x Transfer_{soil/skin}

and

Absorbed dose = Dermal exposure x Dermal absorption (%)

It is accepted that not all of the active substance in a layer of soil exposed to skin may be bioavailable. However, in the absence of specific information on the transfer of the active substance from soil to the skin conservative assumptions should be used.

Assuming a surface area of the hands of 820 cm², complete coverage of the hands (Transfer_{soil/skin} = 100%), and a soil retention value of 0.44 mg/cm², the daily maximum hand loading would be 361 mg of soil. This amount of soil containing the PEC of Chloropicrin, at a concentration of 16.7 mg/kg of soil, would contain 0.00603 mg Chloropicrin.

Assuming a dermal absorption value of 100% (in the absence of data) and a 60 kg body weight, the systemic exposure for a worker handling Chloropicrin treated soil would be 1 x 10⁻⁴ mg/kg bw/day. This estimated exposure is for workers handling treated soil with bare hands, i.e. no PPE.

Comparison of Measured and Tolerable Exposure

On the basis of the estimated dermal exposures, the portion of the proposed AOEL_{systemic} (0.002 mg/kg bw/day) accounted for is shown in the following table. Systemic exposures were estimated assuming a worst-case 100% dermal absorption. This estimation is assuming that no personal protective equipment (PPE) is worn.

Total Systemic Exposure – 60 kg person (mg/kg bw/day)	% of AOEL (0.002 mg/kg bw/day)
1 x 10 ⁻⁴	5%

Assessment

The exposure estimates shown above demonstrate that when working with treated soil worker exposures to Chloropicrin are negligible when compared to the AOEL_{systemic}. These results indicate that Chloropicrin can be used in a manner consistent with label recommendations without potential risks to workers re-entering a treated plot for manual transplanting of crops, without the use of PPE (gloves).

B.6.14.3.2 Assessment of inhalation exposure of workers involved in activities post application, i.e. VIF cutting and VIF removal.

The field generated data for exposures of operators and workers to Chloropicrin can be used to assess the potential risks to workers involved in activities post application, i.e. VIF cutting and VIF removal (B.6.14.1).

³ Kissel, J., Richter, K. & Fenske, R., (1996). Field measurements of dermal soil loading attributable to various activities: implications for risk assessment. Risk Anal. Feb 16(1), 115-125

Comparison of Measured and Tolerable Exposure

From the study results, the various activities associated with first the application and then the cutting and removal of the VIF present air concentrations to which the operators / workers were exposed. In this section the data are considered for the operators present during cutting and removal of the VIF and for these operations the worst case exposures were for Worker 1 (tractor driver) during VIF cutting and Worker 2 (VIF remover) during VIF removal. Exposures to workers present at the site who were assisting in activities to the side of the plot were lower. Where appropriate exposures estimates are refined with the use of RPE.

VIF Cutting

The average air concentration to which Worker 1 (tractor driver) was exposed was 0.303 mg/m³ Chloropicrin. Worker 1 was engaged in these activities for a total of only 77 minutes (1.28 hours) but it is necessary to estimate the exposure data to a realistic worst case working day for continued exposure to Chloropicrin for this activity (6 hours).

The average air concentration to which Worker 3 (assistant) was exposed was 0.019 mg/m³ Chloropicrin. Worker 3 was engaged in these activities for a total of only 77 minutes (1.28 hours) but it is necessary to estimate the exposure data to a realistic worst case working day for continued exposure to Chloropicrin for this activity (6 hours).

The breathing rate for a worker is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which these workers may have been exposed for a 6-hour period is given by:

$$\text{Air concentration (mg/m}^3\text{)} \quad \times \quad \text{Breathing rate (m}^3\text{/hour)} \quad \times \quad \text{Exposure duration (hours)}$$

From the study data the total exposure for Worker 3 and Worker 1 equals:

Worker 3 (tractor driver)

$$0.303 \text{ (mg/m}^3\text{)} \quad \times \quad 1.25 \text{ (m}^3\text{/hour)} \quad \times \quad 6 \text{ (hours)}$$

$$= 2.2725 \text{ mg Chloropicrin}$$

Worker 1 (assistant)

$$0.019 \text{ (mg/m}^3\text{)} \quad \times \quad 1.25 \text{ (m}^3\text{/hour)} \quad \times \quad 6 \text{ (hours)}$$

$$= 0.1425 \text{ mg Chloropicrin}$$

On the basis of the estimated exposures, the portion of the proposed AOEL_{systemic} (0.002 mg/kg bw/day) accounted for is shown in the following table. Systemic exposures were estimated assuming a worst-case 100% inhalation absorption. This estimation is assuming that no respiratory protective equipment (RPE) is worn.

Worker	Total Systemic Exposure – 60 kg person (mg/kg bw/day)	% of AOEL (0.002 mg/kg bw/day)
Worker 3 (tractor driver)	2.2725 / 60 = 0.0379	1895%
Worker 1 (assistant)	0.1425 / 60 = 0.0024	120%

The product label for Chloropicrin advises wearing of suitable respiratory protection for activities associated with VIF removal. The exposure assessment for the tractor driver can be refined further using the RPE APF values noted in B.6.14.1, as shown in the following table.

Total Systemic Exposure 60 kg person (mg/kg bw/day)	RPE type	RPE reduction factor (APF)	% of AOEL (0.002 mg/kg bw/day)
$2.2725 / 60 = 0.0379$	Half mask with gas filter	10 (x 0.1)	189%
$2.2725 / 60 = 0.0379$	Full mask with gas filter	20 (x 0.05)	95%
$2.2725 / 60 = 0.0379$	Power assisted full face gas filtering device (TM 3)	40 (x 0.025)	47%

Assessment

The exposure estimates shown above demonstrate that worker exposures to Chloropicrin during field activities associated with VIF cutting are significantly less than the AOEL_{systemic} only if power assisted full face gas filtering device (TM 3) is worn. These results indicate that Chloropicrin can be used in a manner consistent with label recommendations without potential risks to workers.

VIF Removal

The average air concentration to which Worker 2 (VIF remover) was exposed was 0.00875 mg/m³ Chloropicrin. Worker 2 was engaged in these activities for a total of 147 minutes (2.45 hours) but it is necessary to estimate the exposure data to a realistic worst case working day for continued exposure to Chloropicrin for this activity (6 hours).

The average air concentration to which Worker 3 (assistant) was exposed was 0.00275 mg/m³ Chloropicrin. Worker 3 was engaged in these activities for a total of 147 minutes (2.45 hours) but it is necessary to estimate the exposure data to a realistic worst case working day for continued exposure to Chloropicrin for this activity (6 hours).

The breathing rate for a worker is taken to be 1.25 m³/hour.

Therefore the amount of Chloropicrin to which these workers may have been exposed for a 6-hour period is given by:

$$\text{Air concentration (mg/m}^3\text{)} \times \text{Breathing rate (m}^3\text{/hour)} \times \text{Exposure duration (hours)}$$

From the study data the total exposure for Worker 2 and Worker 3 equals:

Worker 2 (VIF remover)

$$0.00875 \text{ (mg/m}^3\text{)} \times 1.25 \text{ (m}^3\text{/hour)} \times 6 \text{ (hours)}$$

$$= 0.0656 \text{ mg Chloropicrin}$$

Worker 3 (assistant)

$$0.00275 \text{ (mg/m}^3\text{)} \times 1.25 \text{ (m}^3\text{/hour)} \times 6 \text{ (hours)}$$

$$= 0.0206 \text{ mg Chloropicrin}$$

On the basis of the estimated exposures, the portion of the proposed AOEL_{systemic} (0.002 mg/kg bw/day) accounted for is shown in the following table. Systemic exposures were estimated assuming a worst-case 100% inhalation absorption. This estimation is assuming that no respiratory protective equipment (RPE) is worn.

Worker	Total Systemic Exposure – 60 kg person (mg/kg bw/day)	% of AOEL (0.002 mg/kg bw/day)
Worker 2 (VIF remover)	$0.0656 / 60 = 0.00109$	54.5%
Worker 3 (assistant)	$0.0206 / 60 = 0.00034$	17%

Assessment

The exposure estimates shown above demonstrate that worker exposures to Chloropicrin during field activities associated with VIF removal are significantly less than the AOEL_{systemic}. These results indicate that Chloropicrin can be used in a manner consistent with label recommendations without potential risks to workers.

B.6.14.4 Conclusions

Comparisons of measured exposure data and AOEL indicate that risks to operators and workers are acceptable under the conditions of use of Chloropicrin. Risk to bystander is acceptable only if bystanders are located 50 m from the treated area.

B.6.15 REFERENCES RELIED ON

Active ingredient

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection claimed Y/N	Owner
IIA, 5.1/02	Kidd, G.G., Davidson, J.	2005	[¹⁴ C]-Chloropicrin: Absorption, Distribution, Metabolism and Excretion in the Rat Report No. 24991 GLP, Unpublished	Y	European Chloropicrin Group
IIA, 5.1/03	Sparks, S.E., Quistad, G.B., Casida, J.E.	1997	Chloropicrin: Reactions with Biological Thiols and Metabolism in Mice University of California, Berkeley <i>Chem. Res. Toxicol.</i> , 10, 1001-1007 No GLP, Published	N	-
IIA, 5.1/04	Sparks, S.E., Quistad, G.B., Li, W., Casida, J.E.	1997	Chloropicrin Dechlorination in Relation to Toxic Action University of California, Berkeley <i>J. Biochem. Molecular Toxicology</i> , 14, 1: 26-32 No GLP, Published	N	-
IIA, 5.2.6/01	Basketter D. A., Gerberick, G. F. and Kimber I.	1998	Strategies for identifying false positive responses in predictive skin sensitization tests <i>Food and Chemical Toxicology</i> 36, 327-333 No GLP, Published	N	-
IIA, 5.5.2/02	Giknis, M.L.A and Clifford, C.	2004	Compilations of Spontaneous Neoplastic Lesions and Survival in the Rats from Control Groups No GLP, Unpublished	Y	-
IIA, 5.5.3/02	Kovatch, R. M.	1996	Pathology peer review of Chloropicrin: vapour inhalation oncogenicity study in CD-1 mice Study no. 92N1105 No GLP, Unpublished	Y	European Chloropicrin Group
IIA, 5.5.3/03	Giknis, M.L.A and Clifford, C.	2005	Spontaneous Neoplastic Lesions in the Mouse in Control Groups from 18 Month to 2 year studies No GLP, Unpublished	Y	-
IIA, 5.6.10/02	Lang, P. L	1993	Historical control data for developmental and reproductive toxicity studies using the rat No GLP, Unpublished	Y	-
IIA, 5.6.10/03	Schardein, J.L.	1994	Letter (refers to IIA, 5.6.10/01)	Y	European Chloropicrin

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection claimed Y/N	Owner
			[REDACTED] No GLP, Unpublished		Group
IIA, 5.8/01	Sparks, S.E., Quistad, G.B. and Casida, J.E.	1997	Chloropicrin: Reactions with Biological Thiols and Metabolism in Mice Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720-3112, USA <i>Chem. Res. Toxicol.</i> 10, 1001-1007 No GLP, Published ⇒ IIA, 5.1.1/02	Y	-
IIA, 5.8/02	Sparks, S.E., Quistad, G.B., Li, W. and Casida, J.E.	2000	Chloropicrin Dechlorination in Relation to Toxic Action Department of Environmental Science, Policy and Management, University of California, Berkeley, CA 94720-3112, USA <i>J. Biochem. Molecular Toxicology</i> , 14 (1), 26-32 No GLP, Published ⇒ IIA, 5.1.1/03	Y	-
IIA, 5.8/03	Plewa, M.J., Wagner, E.D. and Jazwierska, P.	2004	Halonitromethane Drinking Water Disinfection Byproducts: Chemical Characterization and Mammalian Cell Cytotoxicity and Genotoxicity Department of Crop Science, college of Agricultural, Consumer and Environmental Sciences, University of Illinois at Urbana-Champaign, 1101 West Peabody Drive, Urbana, Illinois 61801, USA National Exposure Research Laboratory, U.S. Environmental Protection Agency, Athens, Georgia 30605, USA CanSyn Chem. Corp. 200 College Street, Toronto, Ontario, M5S 3E5, Canada <i>Environ. Sci. Technol.</i> 38, 62-68 No GLP, Published	Y	-
IIA, 5.10/01	Hoffman, G.E.	1999	Chloropicrin: A Sensory Irritation Study in the Mouse via Head-Only Exposure [REDACTED] Report No. 99-5388 GLP, Unpublished	Y	European Chloropicrin Group
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Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection claimed Y/N	Owner
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IIA, 5.14/01	Butala, J. H.	2009b	Chloropicrin: Evaluation of the human sensory irritation threshold in relation to animal inhalation toxicity data and determination of safety factors Report No. ECG/05/10 No GLP, Unpublished	Y	European Chloropicrin Group

Plant Protection Product: Chloropicrin 99

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection claimed Y/N	Owner
IIIA 7.3.3/01	Trevisan, M.	2009	Chloropicrin: concentration in air and operators exposure assessment during and after soil application. CERZOO - Centro Ricerche per la Zootecnia e l'Ambiente, Piacenza, Italia. Report-No CZ/08/013/UCSC/CHLAIR/RF. GLP, Unpublished	Y	European Chloropicrin Group