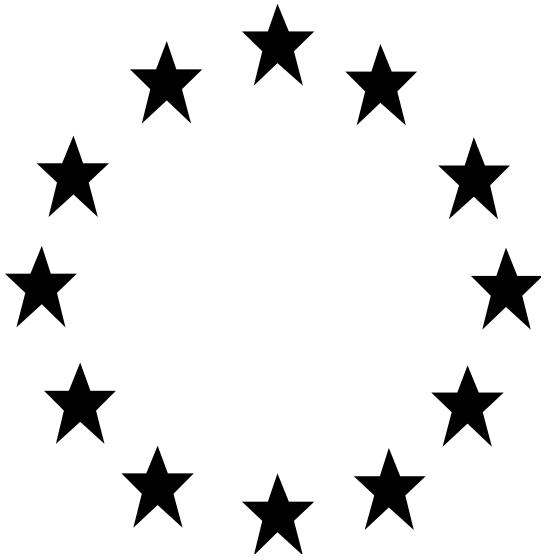

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Spain for the existing active substance

RAPESEED OIL

of the fourth stage of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, part 2, B.6

September 2008

ANNEX B

RAPESEED OIL

B - 6: TOXICOLOGY AND METABOLISM

Introduction

Refined Rapeseed oil is a dietary vegetable oil derived from seeds of *Brassica napus.* The quality of low-erucic acid refined rapeseed oil (no more thant 2% erucic acid) is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore, no toxicological studies performed with the active substance and according to EU guidelines and GLP compliant have been submitted. However, acute toxicity studies performed with the preparation NEU 1160 I (90 % Rapeseed oil) and according to GLP and EU guidelines were submitted and evaluated. In addition published information related with toxicokinetics, acute toxicity, genotoxicity, long-term, and reproductive toxicity data performed with rapeseed oil has been evaluated and included in the D.A.R.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (IIA 5.1)

Summary

Rapeseed oil is a dietary vegetable oil derived from seeds of *Brassica napus.* No studies are presented, since absorption, distribution, metabolism, and excretion of esterified fatty acids (lipids) in mammals are basic knowledge and as such are presented in all relevant handbooks of biochemistry (e.g. Berg et al. 2002). In summary, rapeseed oil is, like all vegetable oils, metabolized by hydrolysis of the glycerol ester to release glycerol and fatty acids. These are incorporated as normal body constituents or degraded via ß-oxidation. Dietary lipids are processed by known metabolic pathways within the body and contribute to normal physiological functions. They are utilized as a carbon and energy source.

Due to these reasons no toxicokinetic studies with Rapeseed oil in rats, with single oral doses or repeated oral doses were conducted. These cannot be expected to lead to other than the already existing scientific conclusions.

B.6.2 Acute toxicity (IIA 5.2)

Summary

The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore no studies on acute toxicity (acute oral, dermal or inhalation toxicity, skin or eye irritation and skin sensitisation) were undertaken with rapeseed oil. However, acute toxicity studies were conducted with the formulation NEU 1161 I with the content of 90 % Rapeseed oil and 2 % Pyrethrum Extract (Refer to Annex IIIA, point 7.1.1 to 7.1.6, see assessment in chapter B.6.11). These studies are summarised in Table 6.2-1.

The lack of toxicity reported in these studies is supporting the view that Rapeseed oil has a low acute oral, dermal or inhalation toxicity and has no skin and eye irritating or dermal sensitizing potential.

In addition, the opinion of the the EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to rapeseed oil high in unsaponifiable matter as a novel food ingredient (The EFSA Journal (2005) 304, 1-11) indicated that the novel food is considered equivalent to its source (refined rapeseed oil) as regards fatty acid composition and contaminants. With regard to acute toxicity information on the new food, acute oral toxicity was tested in accordance with OECD Guideline 401 in mice and rats (in each case 5 males and 5 females). LD50 values were higher than 10 mL/kg body weight (mice) and 5000 mg/kg body weight (rats). (EViC-CEBA, 1999 a and b)

According to EU Commission Directive 2001/59/EC, classification for acute toxicity of Rapeseed oil is not required.

Table 6.2-1: Summary of Acute Toxicity, Primary Irritation and Dermal Sensitisation Studies

Route. Species / Sex	Dosage	Vehicle	Results	Reference	A*
Acute oral toxicity					
Wistar rats of both sexes	2000 mg/kg	Undiluted	Oral LD_{50} > 2000 mg/kg bw	Assessment of acute oral toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996a) (IIIA, 7.1.1/01, Report No. 170764)	Yes
Acute dermal toxicity					
Wistar rats of both sexes	2000 mg/kg	Undiluted	Dermal LD_{50} > 2000 mg/kg	Assessment of acute dermal toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996b) (IIA 7.1.2 /01, Report No. 170775)	Yes
Acute inhalation toxicity					
SD Crl: CD rats of both sexes	2.36 mg/L	None	LC_{50} for four hours was >3.26 mg/L	Acute inhalation toxicity - NEU 1161 I. Lenz, G. (1996), (IIA 7.1.3 /01, Report No. 96 5041 804)	Yes
Acute dermal irritation					
New Zealand White Rabbits /males	0.5 mL	Undiluted	Non Irritant	Primary skin irritation/corrosion study with NEU 1161 I in the rabbit (4-hour semi-occlusive application). Rijken, W.R.P.(1997), (IIA 7.1.1 /01: Report No. 170775)	Yes
Acute eye irritation					
New Zealand White Rabbits / males	0.1 mL	Undiluted	Non Irritant	Acute eye irritation/corrosion study with Neu 1161 I in the rabbit. Rijken, W.R.P. (1996c), (IIA 7.1.5 /01, Report No. 170797)	Yes
Skin sensitisation					
Dunkin Hartley Albino Guinea Pig / female	20% for intradermal injection and 100% for topical induction and challenger	Water	No sensitizer	Test for sensitization (Guinea pig maximisation Test) with NEU 1160 I. Otterdijk van, F.M (2002), (IIIA 7.1.6/01 Report No: 356052)	Yes

A*: Acceptability

B.6.3 Short-term toxicity (IIA 5.3)

Summary

Notifier has not presented short-term toxicity studies performed with rapeseed oil.

The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001).

Therefore, short term oral toxicity studies are not necessary.

Neverteless and as additional information, as it can see in the scientific report presented in chapter B.6.6 to evaluate reproductive success, the main advers effect observed in female hamsters feeding during 110 days with 25% rapeseed oil rich in erucic acid (41.4%) in diet, was decreased bile flow. Moreover, depleted linoleic acid and increased erucic acid moderately in the liver and kidney and noticeably in the heart was seen in pregnant hamsters.

It shoud be noted that the refined rapeseed oil accepted as food according to Codex Alimentarius (FAO-WHO, 2001) have a content in erucic acid ≤ 2%, while in the rapeseed oil tested the content is 41.4%.

B.6.4 Genotoxicity (IIA 5.4)

Summary

Notifier has not presented genotoxicity studies performed with rapeseed oil. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Rapeseed oil consists of esters of glycerol with saturated and unsaturated long chain fatty acids. These are natural body constituents and there is no indication for a genotoxic potential.

In addition, the opinion of the EFSA Scientific Panel of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to rapeseed oil high in unsaponifiable matter as a novel food ingredient (The EFSA Journal (2005) 304, 1-11) indicated that the novel food is considered equivalent to its source (refined rapeseed oil) as regards fatty acid composition and contaminants. With regard to genotoxicity information on the new food, The Ames test was carried out in accordance with OECD Guideline 471 with five strains of *Salmonella typhimurium* up to 5000 µg/plate showed no mutagenic activity with and without metabolic activation (Marzin, 1999).

B.6.5 Long term toxicity and carcinogenesis (IIA 5.5)

Summary

Only two publications had been submitted in long-term section, however these reports were not according to OECD guidelines and GLPs. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore, long term toxicity and carcinogenesis studies are not necessary.

Long-term study was conducted with male rats, fed with diets with 20% Rapeseed oil that contained low or high levels of erucic acid or soybean oil to investigate ultrastructural characteristics of the myocardium. Long-term feeding of high erucic acid rapeseed oil (30.9%) resulted in alteration of mitochondrial morphology, disorganization of myofibrils, and degeneration or necrosis of the cardiac muscle fiber. Low erucic acid rapeseed oil (0.9%) induced less severe cardiopathologic changes but the nature of the alterations was similar to that high levels of erucic acid.

Long term feeding experiments with ICR mice (6% Rapeseed oil in the diet) for 18 months resulted in an increased survival rate as compared to a control group with a diet containing equal amounts of palm oil.

Table 6.5-1: Summary Long-term toxicity

Study/Reference/Purity of test substance	Specie	Dose Level		NOAEL		LOAEL		Target organ/main effect	Comments	A (*)
		ppm	mg/kg/d	ppm	mg/kg/d	ppm	mg/kg/d			
Myocardial ultrastructure of rats fed high and low erucic acid rapeseed oils	SD rats (male)	20% SBO 20% LER 20% HER		Not applicable				High erucic acid rapeseed oil (30.9%)resulted in alteration of mitochondrial morphology, disorganization of myofibrils, and degeneration or necrosis of the cardiac muscle fiber. Low erucic acid rapeseed oi l(0.9%) induced less severe cardiopathologic changes.	It's a publication that only investigate ultrastructural characteristics of the myocardium	A.I
Effect of lard, palm and rapeseed oil life conservation in aged mice	CD1 Mice (male, female)	Palm oil (6%) Lard diet (6%) Rapeseed oil (6%)		Not applicable				The results of fatty acid analyses seen to reveal that intensity of n-3 PUFA deficiency is ranking in the following order; the palm oil diet fed-male>the female mice>the lard diet fed-male and female mice>the rapeseed oil diet fed male and female mice. Group fed with a diet deficient in n-3 PUFA had a decrease in survival rate.	Only test the effects of dietary fat and oils with different amounts of n-3 PUFAs on the survival and fatty acid composition of brain and liver lipids in mice.	A.I

A*: Acceptance

B.6.5.1 Long-term oral and carcinogenicity in the rat

B.6.5.1.1 Myocardial ultrastructure of rats fed high and low erucid acid rapeseed oils. Yamashiro, S., Clandinin,M.T. 1980. (KA, 5.5/02, Experimental and molecular pathology 33, 55-64)

Date of experimental work: not reported. Date of report: 1980.

Objective: investigate ultrastructural characteristics of the myocardium of rats fed different types of vegetale oils for longer periods.

Guidelines: not applicable it's a published study.

Deviations: not applicable.

GLP: No

This study is considered acceptable as additional information.

Materials and methods.

Sixty male rats of the Sprague Dawley strain were allocated to three dietary oil treatments and fed a methionine-supplemented purified diet *ad libitum* for 16 or 28 weeks (10 animals for each treatment). The diets contained 20% (w/w) of either soybean oil (SBO), low erucic rapeseed oil (LER) or high erucic acid rapeseed oil (HER). The fatty acid compositions of the oils utilized are in table below.

Table 6.5.1.1-1 Fatty acid composition of the experimenta oils.

Fatty acid (%,w/w)	SBO	LER	HER
$C_{14:0}$		TRc	TR
$C_{16:0}$	11.1	4.6	5.4
$C_{18:0}$	2.7	0.7	0.6
$C_{18:1}$	23	54	24.2
$C_{18:2}$	51.1	25.5	17.9
$C_{18:3}$	11.6	13	7.8
$C_{20:0}$		TR	
$C_{20:1}$	0.2	1.2	12.6
$C_{22:1}$ (erucic acid)		0.9	30.9
$C_{24:1}$		TR	TR
Others	0.3	0.1	0.5

Animals were stunned by a sharp cranial blow and their hearts immediately removed. Small pieces ($1mm^3$) of ventricular myocardium were fixed in 2.5% glutaraldehyde in phosphate buffer and post fixed in 2.0% osmium tetraoxide. Samples were embedded and sectioned with an ultramicrotome, contrasted with uranyl and lead citrate. The sections were examined in a electron microscope. Larger pieces of the tissue were fixed in 10% neutral buffered formalin and were processed for light microscopic examination.

Findings

Gross examination of the hearts during the tissue collection revealed no apparent lesions.

Histologically, myocardium of the affected animals showed focal necrosis of the cardiac muscle fiber often accompanying an infiltration by macrophages. Hearts with necrotic lesions often showed loosening of arteriolar walls. Incidence of myocarcial necrosis are in table 6.5.1.1-2. myocardium of LER- and HER fed rats with no apparent necrotic foci often showed aggregation of mesenchymal cells in the interstitium.

Table 6.5.1.1-2: Incidence of myocardial necrosis in rats fed experimental diets for 16 or 28 weeks.

Diets	16 weeks	28 weeks
SBO	0	0
LER	5	6
HER	9	10

Ultrastructurally, cardiac muscle fiber of SBO-fed animals showed regular myofibrillar arrays.

Mitochondria were present in rows between the myofibrils. Occasionally a few lysosomes containin lipid droplets were observed. The myocytes were similar for both 16 and 28 weeks of SBO feeding.

Myocardium form rats fed LER for 16 weeks exhibited a mixture of apparently normal cardiac muscle fibers and some middly degenerative fibers. Lipid droplets were occasionally seen near mitochondria.

Mitocondria of LER treatment were of various sizes. Degenerating cardiac muscle fibers were obvious in myocardium of rats which showed focal necrosis in histological preparations. Interstitium exhibited a mild to moderate edema. Endothelial cells of capillaries showed numerous pinocytotic vesicles. Rats fed LER for 28 weeks showed an increase in collagen fibrils in the lesions; accompanying fibroblasts displayed dilated cisternae of granular endoplasmic reticulum.

All animals fed HER showed prominent degenerative changes in the myocardium. Myocites of rats fed HER for 16 weeks contained many lipid droples. Some of degenerating fibers showed separation of intercalated disc. Myofibrils of degenerating myocytes were disorganized and such myocytes were surrounded by citoplasmic processes of macrophages. Mitochondria were often seen in cluster. These unusual mitochondria were occasionally observed in myocytes of rats fed HER for 28 weeks. Necrotic lesion of rats fed HER for 28 weeks showed edema and proliferation of collagen fibrils accompanied by fibroblast, mast cells, and macrophages. Cytoplasm of myocytes exhibed many vesicles containing an amorphous substance. Similar vesicles were also seen in the interstitium.

Conclusions

Long-term feeding of high erucic acid rapeseed oil resulted in alteration of mitochondrial morphology, disorganization of myofibrils, and degeneration or necrosis of the cardiac muscle fiber. Low erucic acid rapeseed oil induced less severe cardiopathologic changes but the nature of the alterations was similar to that HER.

B.6.5.2 Long-term oral toxicity and carcinogenicity in the mice

B.6.5.2.1 Effect of lard, palm and rapeseed oil life conservation in aged mice. Suzuki, H., Yamazaki, M., Arai, S., Nagao, A., Terao, J.1991. (K 5.5/01, *Mechanism of ageing and development, 60 (1991) 267-274*)

Date of experimental work: not reported. Date of report: 1991.

Objective: to test the effects of dietary fat and oils with different amounts of n-3 PUFAs on the survival and fatty acid composition of brain and liver lipids in mice.

Guidelines: not applicable, it's a published report.

Deviations: not applicable

GLP: No

This study is considered acceptable as additional information.

Materials and methods

Animals used were male and female mice Crj:CD-1 (5 weeks old). The animals were divided into three groups of ten animals/sex/ group. The animals were fed palm oil (n-3 PUFA deficient) diet, lard diet, or rapeseed oil (n-3 PUFA sufficient) diet for 15 months. Each diet contained 6% lipid sources, and remaining components were as follows: corn starch, 41.5%; casein, 25%; α-starch, 10%; cellulose powder, 8%; granulated sugar, 5%; salt mixture, 3.5%; vitamin mixtured, 1%.

The dietary lipids consisted of the characteristic fatty acids derived from the fat and oils used as ingredients. Lard and rapeseed oil containes 0.2% and 9.8% α-linolenic acid (18:3, n-3), respectively. However, n-3 PUFAs were not found in the palm oil. In order to prevent changes in fatty acid composition and the formation of peroxides during storage, each experimental diet was inserted into a pounch with an oxygen absorber and stored at 5ºC.

Diets and water were provided *ad libitum*.

Body weights and number of surviving mice were measured once a month. At the end of feeding trial, all mice were fasted for 24h and sacrificed. The whole brain and liver were removed immediately and homogenized. The homogenates were used for fatty acid analyses.

Fatty acid analyses: the brain and liver homogenates were directly saponified in KOH-ethanol with heat for 1h. The resuling mixtures of fatty acids were removed and the isolated fatty acids were esterified with N,N-dimethyl acetal. The fatty acids methyl esters were analyzed using gas chromatography.

Findings

No marked differences in changes of body weights among dietary groups were observed. Female mice tended to weigh more than male mice at the end of feeding trial.

Surviving number of male mice fed of palm oil diet rapidly diminished after 13 months, and the proportion of surviving mice became 40% at the end of the feeding trial. A similar survival curve was shown in female mice fed the same diet but the curve in females was less dramatic than that in males.

Over 80% of male and female mice fed on lard and rapeseed oil (n-3 PUFA sufficient) diets had survived to the end.

n-3 PUFA was not found in the liver of male and female mice fed on the palm oil diet. A small amount of docosahexaenoic acid alone was detected in the lard diet fed-mice liver. Certain amounts of α-linolenic and docasahexaenoic acids were present in the liver of rapeseed oil diet fed-mice. The fatty composition of female liver lipids showed smaller tendency toward these percentages of linoleic (18:2, n-6), arachidonic and docasahexaenoic acids than those of males, but it was caused by a more abundant amount of oleic acid (18:1, n9) in female liver lipids.

Little change in the fatty acid compositions of brain lipids occurred due to the the diets as compared with those of liver lipids. Thus, there were no appreciable differences in the percentages of brain palmitic (16:0) to arachidonic acids between the dietary groups. However, the percentages of docosahexaenoic acid in brain lipids of the palm oil diet fed-mice was apparently less than that of the other diet fed-animals. Moreover, the percentage of n-3 PUFA in male mice fed on palm oil diet was smaller than that in the female animals. The docosapentaenoic acid (22:5, n-6) in the rapeseed oil group tended to decrease as compared to the other diet groups.

Conclusions

There were no difference in body weight between dietary groups.

A marked decrease in the percentage of survival between male and female mice fed on palm oil diet was observed compared with rapessed oil group. The results of fatty acid analyses seen to reveal that intensity of n-3 PUFA deficiency is ranking in the following order; the palm oil diet fed-male>the female mice>the lard diet fed-male and female mice>the rapeseed oil dieet fed male and female mice.

B.6.6 Reproductive toxicity (IIA 5.6)

Summary

Notifier has not presented conventional studies to assess reproductive and developmental effects after rapeseed oil administration. Instead, a scientific report was presented in order to evaluate reproductive success and outcomes after rapeseed oil administration.

The experimental survey administered a diet containing 25% rapeseed oil or corn oil (controls).

Rapeseed oil in the diet was rich in erucic acid (41.4%). Both males and females were provided with the diets for 90 days in pre-mating phase and during gestation. Half of the animals were continued until day 110 and the remaining were paired. At day 20 of gestation (rat) or day 14 (hamster), pregnant females were sacrificed and examined for reproductive outcomes. In addition, all animals (both pregnants or non-pregnants) were examined for the weight and histology of some organs, bile flow, acid contents, lithogenic index and hepatic organic anion excretory capacity examined with sulfobromophthalein in order to compare this aspects with control animals.

Effects on the mothers attributed to rapeseed oil diet consisted of decreased bodyweight in non-pregnant rats (8.7%) and hamsters (7.8%). However, female fertility index, the number and the weight of fetuses were not affected. In addition, fetuses were macroscopically considered as normal. When adult rats were examined for macroscopic/microscopic lesions and the weight of liver, kidney, heart and adrenal glands, the report did not show abnormalities.

Rapeseed oil administration decreased bile flow in pregnant hamsters. No differences could be observed in the concentration of bile acids, biliary lipids, lithogenic index and the hepatic organic anion excretory capacity examined with sulfobromophthalein.

Results of the fatty acid proportion were only presented for the hamster heart. Administering witth rapeseed oil rich in erucic acid depleted linoleic acid and increased erucic acid moderately in the liver and kidney and noticeably in the heart.

B.6.6.1. Reproductive and developmental studies

B.6.6.1.1 Is Dietary Erucic Acid Hepatotoxic in Pregnancy?. An Experimental Study in Rats and Hamsters. Reyes, H; Ribalta, J; Hernánadez, I; Arrese, M; Pak, N; Wells, M; Kirsch, RE. 1995. (KII/5.6/01)

Report: Hepatology 21, 1373-1379. (1995).

Objective: Test whether prolonged feeding of diets containing rapeseed oil rich in erucic acid affect bile flow and the excretion of biliary lipids particularly at the end of pregnancy.

Guidelines: not applicable it's a published report.

Deviations: not applicable

Comments:

GLP: No

This study is considered acceptable

Materials and methods:

Animals: Weanling Wistar rats and Weanling Golden Syrian hamsters. They were acclimatized for 1 week, after which, they were paired and maintained in wire-botton cages in a temperature and light-controlled room (22-25ºC and 12 h light/dark cycle).

Diet: The experimental diet contained 11.5% casein, 53.5% cornstarch, 1% mineral mixture, 5% non-nutritive cellulose, 5% vitamin-mineral admixture, and **25% test oil**.

Two dietarly oils were used, rapeseed oil for the test groups, and corn oil for controls. The composition was determined by capillary glass chromatography with flame-ionization detection.

Table 6.6.1.1-1: Fatty acid composition of dietary oils

Fatty acid		**Dietarly oil (% by weigh of total fatty acids)**	
C	Name	Rapeseed oil	Corn oil
16:0	Palmitic	3.9	9.6
16:1	Palmitoleic	0.2	0.1
18:0	Stearic	1.4	1.9
18:1	Oleic	20.2	28.3
18:2	Linoleic	14.2	58.1
18:3	Linolenic	16.2	1.3
20:1	Eicosaenoic	-	-
20:4	Araquidonic	-	-

Fatty acid		Dietarly oil (% by weigh of total fatty acids)	
22:0	Docosanoic	0.6	-
22:1	ERUCIC	41.4	0.5
22:2	Docosadienoic	0.3	-
24:0	Tetracosanoic	0.8	-
20:1	nervonic	-	-
S/U*		0.06	0.13

* ratio of total percentage of saturated fatty acids to total percentage of unsaturated fatty acids.

The diet was administered for 90 days. After this period, one half of the females were mated with males and the diet was continued until the end of gestation.

Procedure: At day 20 of gestation in the rat and at day 14 of gestation in hamsters, and in similar periods for non-pregnant animals, the animals were bile cannulated.

-Bile flow, biliary lipids concentration, and lithogenic index of bile: Bile was collected at 10-minute intervals for 80 minutes, in rats, and at 15-minute intervals, for 75 minutes, in hamsters and several parameters were analyzed:

-bile acid, phospholipid and cholesterol concentration

-lithogenic index of bile

-Hepatic organic anion excretory capacity: The capacity of nonpregnant female rats fed rapeseed or corn oil diets to excrete an exogenous organic anion into bile was assessed. For this purpose, rats were vein and bile cannulated. The animals were infused intravenously with sulfobromophthalein (BSP). Blodd samples were taken every 5 minutes and bile was collected every 15-minute intervals for 90 minutes and:

-BSP concentration in plasma and in bile was measured

-Biliary BSP excretion and maximal transport into bile were calculated.

-Fatty acid analysis of tissue homogenates: The liver, heart, or kidneys of pregnant and nonpregnant hamsters were obtained and analysed for:

-the content of fatty acids (GC) in the organ homogenates.

-Fatty acid analysis of isolated liver cells: The liver of non-pregnant rats were perfused and the liver cells were isolated and stored at –20ºC. Further, they were analysed for:

-fatty acid content.

Results

Body weight females: A significant bodyweight depression occurred in non-pregnant females fed with rapeseed oil in both species, but not observable for pregnant animals. Non-pregnant rats consuming rapeseed oil decreased the bw gain by 8.7% regarding those fed with corn oil, and in the case of non-pregnant hamster, the bodyweight gain depression achieved 7.8%.

Organs weight: The report confirmed no statistical significant differences for liver, kidney or heart weight between groups of dosing, indicating that rapeseed oil did not affect such parameters. Relative liver weight was observed to be statistically higher in pregnant animals regarding non-pregnants, both in rat or hamster females and independently of the oil treatment.

Foetal parameters: There were no differences in the number of foetus and fetal weight at cesarean in both rats or hamster administered with rapeseed oil regarding those provided with corn oil. In addition, no morphological abnormalities in the fetuses were reported.

Histological abnormalities and microscopic fatty deposition in the viscera: The report stated that the microscopic examination of liver, heart, kidneys and adrenals from rats and hamsters did not evidence morphological abnormalities. The microscopic deposit of fat was comparable between the groups of dosing. Only pregnant females of hamster showed a significant increase in the proportion of liver cells containing fatty vacuoles (three fiedls randomly examined per sample), when compared to non-pregnant females, however, this difference was observed in either rapeseed oil or corn oil administered females, indicating a non-relationship with rapeseed oil treatement.

Table 6.6.1.1-2: Maternal and foetal parameters evaluated after 110 days rapeseed oil administration

		Rat		**Hamster**	
		Pregnant	Non-pregnant	Pregnant	Non-pregnant
Bodyweight (g)	Rapeseed oil	250	179	173	139
	Corn oil	281	215	170	152
liver weight (g)	Rapeseed oil	9.8	5.8	7.9	5.6
	Corn oil	9.3	6.1	7.4	6.1
Relative liver weight	All	3.61	3.03	4.51	4.06
Heart weight (g)	Rapeseed oil	0.7	0.6	0.7	0.6
	Corn oil	0.6	0.6	0.6	0.6
Kidney weight (g)	Rapeseed oil	1.6	1.3	1.2	1.1
	Corn oil	1.5	1.5	1.3	1.2
Number of foetuses	Rapeseed oil	9.7		2.4	
	Corn oil	8.7		2.6	
Foetal weight (g)	Rapeseed oil	14.4		0.8	
	Corn oil	13.4		0.9	
% liver cells containing fatty vacuoles	Rapeseed oil			4.5	0.4
	Corn oil			8.5	0.4

-Bile flow, biliary lipids concentration, and lithogenic index of bile:

Rat: The bile flow was more or less stable for the period of collection (80 min) in both groups of dosing or considering pregnancy. The statistical study did not reveal any difference between the dosing groups, and pregnancy did not influence upon bile flow results. Nevertheless, administering rapeseed oil appeared to increase the mean biliary flow in both pregnant and non-pregnant rat females regarding the groups administered with corn oil. Note also that non-pregnant rats had higher bile flow than pregnant animals fed with both rapeseed oil or corn oil.

Hamster: The bile flow was decreasing slightly for the period of collection (70 minutes) in all females studied, independently of pregnancy or the oil administered. The effect of feeding with rapeseed oil produced different results regarding bile flow. Considering the status, pregnant hamster females decreased considerably the bile flow regarding non-pregnant animals (statistically from 0 to 15 minutes, $p<0.05$), which effect was not observed in females fed with corn oil. However, the bile flow was similar in non-pregnant females fed with rapeseed oil or corn oil.

The concentration of biliary lipids and the lithogenic index of the bile were not generally modified by the rapeseed oil treatment (table 6.6.1.1-5). Common to both oil treatment, the pregnant rats decreased the average bile acid and cholesterol concentration and lithogenic index with regard to the nonpregnant females. In pregnant hamster and common to all oil treatments, the bile acid contents decreased, increasing levels of cholesterol and the lithogenic index, this latter no more than 1.

Table 6.6.1.1-3: Biliary lipids concentration and lithogenic index in rat and hamster

			Biliary lipids (mmol/L)			
			Bile acids	**Phospholipids**	**Cholesterol**	**Lithogenic Index**
Rat	Non-pregnant	Rapeseed oil	29.6	2.26	0.65	0.27
		Corn oil	27.9	2.63	0.73	0.33
	Pregnant	Rapeseed oil	26.0	2.52	0.49	0.22
		Corn oil	22.8	2.07	0.39	0.20
Hamster	Non-pregnant	Rapeseed oil	31.2	6.31	0.88	0.25
		Corn oil	38.8	8.60	0.90	0.20
	Pregnant	Rapeseed oil	25.9	7.37	1.10	0.33
		Corn oil	25.1	7.01	1.04	0.32

-Hepatic organic anion excretory capacity: During intravenous infusion of BSP, bile flow diminished progressively in non-pregnant rats fed with the oil diets. BSP excretion into bile and the maximal transport capacity of BSP into bile were similar in non-pregnant rat females fed with rapeseed or corn oil diets.

-Fatty acid analysis of tissue homogenates/isolated liver cells:

Erucic acid higher proportions were found in the heart of hamster, while minor proportions were found in the liver or kidney. Regarding erucic acid concentration in the organs studied, there was no difference between pregnant or non-pregnant animals, indicating that this condition did not influence upon the erucic acid accumulation in the organs studied. However, there was a noticeable difference in the accumulation of erucic acid when the animals were dosed with rapeseed oil when compared to animals dosed with corn oil. For example, erucic acid concentration in the heart of females fed with rapeseed oil accounted for 14-14.4% of the total fatty acid, while in corn oil females, erucic acid accounted only 0.1-0.3%. In a minor proportions, liver or kidney also accumulated more erucic acid in those hamster fed with rapeseed oil (see table 6.6.1.1-3). By contrast, hamster females dosed with corn oil deposited more linoleic acid in the organs studied than the females dosed with rapeseed oil.

Table 6.6.1.1-4: Fatty acid composition of total lipids in liver, heart, and kidney in homogenates from female hamster

Non-pregnant hamsters							
		Dietary oil (% by weight of total fatty acids)					
Fatty acid		**Rapeseed oil**			**Corn oil**		
C	**Name**	**Liver**	**Heart**	**Kidney**	**Liver**	**Heart**	**Kidney**
16:0	Palmitic	14.2	9.0	16.2	18.9	14.1	15.5
16:1	Palmitoleic	0.9	0.6	0.8	-	0.2	0.3
18:0	Stearic	15.4	10.7	15.2	19.2	14.6	13.4
18:1	Oleic	22.1	25.7	20.2	11.9	15.7	19.1
18:2	Linoleic	14.0	20.5	12.3	21.1	37.8	29.3
18:3	Linolenic	1.4	1.1	0.7	0.4	0.5	0.4
20:1	Eicosaenoic	4.1	5.4	2.5	0.8	0.2	0.9
20:4	Araquidonic	11.2	6.8	15.4	15.6	8.3	15.9
22:0	Docosanoic						
22:1	ERUCIC	2.5	14.4	3.1	0.3	0.3	-
22:2	Docosadienoic						
24:0	Tetracosanoic	-	-	-	5.6	2.3	2.3
20:1	nervonic	11.2	4.2	5.3	5.3	4.8	2.2
pregnant hamsters							
		Dietary oil (% by weight of total fatty acids)					
Fatty acid		**Rapeseed oil**			**Corn oil**		
C	**Name**	**Liver**	**Heart**	**Kidney**	**Liver**	**Heart**	**Kidney**
16:0	Palmitic	21.7	9.7	16.2	24.0	16.0	18.8
16:1	Palmitoleic	0.6	0.4	0.8	0.2	0.2	0.2
18:0	Stearic	15.2	11.0	15.2	16.4	16.4	15.4
18:1	Oleic	16.3	23.6	20.2	9.7	18.0	15.8
18:2	Linoleic	10.1	20.8	12.3	15.7	30.9	21.2
18:3	Linolenic	0.5	0.7	0.7	1.3	0.5	0.2
20:1	Eicosaenoic	1.4	4.8	2.5	-	0.6	0.7

20:4	Araquidonic	17.5	7.8	15.4	22.5	9.9	15.4
22:0	Docosanoic						
22:1	ERUCIC	2.7	14.0	3.1	0.3	0.1	0.2
22:2	Docosadienoic						
24:0	Tetracosanoic	0.2	-	-	2.7	1.3	2.1
20:1	nervonic	11.1	4.2	5.3	4.2	3.9	2.2

Isolated liver cells analysis confirmed the findings of the homogenate liver analysis (table 6.6.1.1-4)

Table 6.6.1.1-5: Fatty acid composition determined in liver cells

Fatty acid		**Dietarly oil (% by weigh of total fatty acids)**	
C	**Name**	**Rapeseed oil**	**Corn oil**
16:0	Palmitic	12.1	14.6
16:1	Palmitoleic	2.0	-
18:0	Stearic	17.1	19.6
18:1	Oleic	27.7	11.2
18:2	Linoleic	10.7	23.6
18:3	Linolenic	0.8	0.7
20:1	Eicosaenoic	2.8	-
20:4	Araquidonic	15.8	21.7
22:0	Docosanoic	-	-
22:1	ERUCIC	3.9	-
22:2	Docosadienoic	-	-
24:0	Tetracosanoic	-	2.9
20:1	nervonic	6.7	5.5
S/U*		0.42	0.60

* ratio of total percentage of saturated fatty acids to total percentage of unsaturated fatty acids.

Conclusions:

- The administration of rapeseed oil, containing 25% w/w rapessed oil (41.4% erucic acid) produced a significant bodyweight depression of 8.7% in non-pregnant rats, and a non-statistical decrease of 11% in pregnant females. The effect was also observable in non-pregnant hamsters, reducing statistically an 7.8% the bodyweight regarding those receiving corn oil. Pregnant hamsters was not affected by the treatment.

- There were no modifications in the liver, kidney or heart weight and macroscopic/microscopic alterations after the diet treatment with rapeseed oil.

- Bile flow in pregnant hamsters decreased after rapeseed oil administration. The effect of rapeseed oil administration on bile flow was not observed in the rat, although pregnancy status was observed to reduce the bile flow, independently of the oil treatment.

- The administration of a diet containing 25% rapeseed oil, rich in erucic acid (41.4%) did not affect on the concentration of biliary lipids, lithogenic index or the hepatic organic anion excretory capacity using sulfobromophthalein.

- In the hamsters, the liver and kidney increased slightly the % of erucic acid in their composition, while the heart of these animals increased noticeably the % of erucic acid.

- After the administration of rapeseed oil in a 25% in diet containing erucic acid (41.4%) did not affect in the rat or hamster reproductive success, the number and weight of the fetuses, and produced no observable adverse macroscopical effects in foetuses.

B.6.7 Neurotoxicity (IIA 5.7)

Notifier has not presented neurotoxicity studies performed with rapeseed oil. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore, neurotoxicity studies are not necessary.

B.6.8 Further toxicological studies (IIA 5.8)

B.6.8.1 Metabolites

Notifier has not presented toxicity studies performed with metabolites. Rapeseed oil is, like all vegetable oils, metabolized by hydrolysis of the glycerol ester to release glycerol and fatty acids. These are incorporated as normal body constituents or degraded via ß-oxidation.

B.6.8.2 Other risk assessments

B.6.8.2.1 Canola Oil; Exemption from the Requirement of a Tolerance, Section III, Toxicological Profile, April 3, 1998 (40 CFR Part 180, [OPP-300623; FRL-5773-9]2070-AB78.). Environmental Protection Agency.

"Data waivers were requested for acute oral, dermal, inhalation, and eye toxicity, dermal sensitization, genotoxicity, reproductive and developmental toxicity, subchronic (90-day) oral and inhalation toxicity, and teratogenicity for NEU1160 Vegetable Oil Insecticide. The waivers were accepted based on the long history of use of canola as an edible fat and oil in food without any indication of deleterious effects; its low toxicity; its natural occurrence as an oil extracted from plants; its low erucic acid (less than 2%) content; its conformity with 21 CFR 184.1555(c); and its classification by FDA as ``generally recognized as safe" (GRAS) for use as an edible fat or oil in human food. Available toxicity data on vegetable oils from the open literature and the Reregistration Eligibility Decision document for Flower and Vegetable Oils (EPA 738-R-93-031) support this finding."

B.6.8.2.2 IIFG Decision Documents on Reassessment of Exemptions from the Requirement of a Tolerance for Fatty Acids, Section 16, Determination of Safety, July 31, 2002. Environmental Protection Agency

"Based on its review and evaluation of the available information, EPA concludes that there is a reasonable certainty that no harm will result to the general population, and to infants and children from aggregate exposure to residues of the C8 to C18 fatty acids. Therefore, the following exemptions from the requirement of a tolerance are reassessed: In 40 CFR 180.1001 (c), fatty acids, conforming to 21CFR 172.860, palmitic acid, stearic acid, and oleic acid. In 40CFR 180.1001 (e), stearic acid, and oleic acid conforming to 21CFR 172.862."

B.6.9 Medical Data (IIA 5.9)

Summary

Since there is no toxicity associated with Rapeseed oil there are no medical data regarding occupational and accidental, long term or acute toxic effects.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD (IIA 5.10)

B.6.10.1 Summary of mammalian toxicology

Toxicokinetics

Rapeseed oil is, like all vegetable oils, metabolized by hydrolysis of the glycerol ester to release glycerol and fatty acids. These are incorporated as normal body constituents or degraded via ß-oxidation.

Acute toxicity

The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore no studies on acute toxicity (acute oral, dermal or inhalation toxicity, skin or eye irritation and skin sensitisation) were undertaken with rapeseed oil. However, acute toxicity studies conducted with the formulation NEU 1161 I with the content of 90 % Rapeseed oil and 2 % Pyrethrum Extract (Refer to Annex IIIA, point 7.1.1 to 7.1.6, see assessment in chapter B.6.11).

The lack of toxicity reported in these studies is supporting the view that Rapeseed oil has a low acute oral, dermal or inhalation toxicity and has no skin and eye irritating or dermal sensitizing potential.

According to EU Commission Directive 2001/59/EC, classification for acute toxicity of Rapeseed oil is not required.

Short-term toxicity

Notifier has not presented short-term toxicity studies performed with rapeseed oil. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore, short term oral toxicity studies are not necessary.

Genotoxicity

Notifier has not presented genotoxicity studies performed with rapeseed oil. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Rapeseed oil consists of esters of glycerol with saturated and unsaturated long chain fatty acids. These are natural body constituents and there is no indication for a genotoxic potential.

Long-term toxicity and carcinogenicity

Only two publications had been submitted in long-term section, however these reports were not according to OECD guidelines and GLPs. The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). Therefore, Long term toxicity and carcinogenesis studies are not necessary.

Long-term study was conducted with male rats, fed with diets with 20% Rapeseed oil that contained low or high levels of erucic acid or soybean oil to investigate ultrastructural characteristics of the myocardium. Long-term feeding of high erucic acid rapeseed oil (30.9%) resulted in alteration of mitochondrial morphology, disorganization of myofibrils, and degeneration or necrosis of the cardiac muscle fiber. Low erucic acid rapeseed oil (0.9%) induced less severe cardiopathologic changes but the nature of the alterations was similar to that high levels of erucic acid.

Long term feeding experiments with ICR mice (6% Rapeseed oil in the diet) for 18 months resulted in an increased survival rate as compared to a control group with a diet containing equal amounts of palm oil.

Reproductive and developmental toxicity

Notifier has not presented conventional studies to assess reproductive and developmental effects after rapeseed oil administration. Instead, a scientific report was presented in order to evaluate reproductive success and outcomes after rapeseed oil administration.

The experimental survey administered a diet containing 25% rapeseed oil or corn oil (controls). Rapeseed oil in the diet was rich (41.4%) in erucic acid. Both males and females were provided with the diets for 90 days in pre-mating phase and during gestation. Half of the animals were continued until day 110 and the remaining were paired. At day 20 of gestation (rat) or day 14 (hamster), pregnant females were sacrificed and examined for reproductive outcomes. In addition, all animals (both pregnants or non-pregnants) were examined for the weight and histology of some organs, bile flow, acid contents, lithogenic index and hepatic organic anion excretory capacity examined with sulfobromophthalein in order to compare this aspects with control animals.

Effects on the mothers attributed to rapeseed oil diet consisted of decreased bodyweight in non-pregnant rats (8.7%) and hamsters (7.8%). However, female fertility index, the number and the weight of fetuses were not affected. In addition, fetuses were macroscopically considered as normal. When adult rats were examined for macroscopic/microscopic lesions and the weight of liver, kidney, heart and adrenal glands, the report did not show abnormalities.

Rapeseed oil administration decreased bile flow in pregnant hamsters. No differences could be observed in the concentration of bile acids, biliary lipids, lithogenic index and the hepatic organic anion excretory capacity examined with sulfobromophthalein.

Results of the fatty acid proportion were only presented for the hamster heart. Administering with rapeseed oil rich in erucic acid depleted linoleic acid and increased erucic acid moderately in the liver and kidney and noticeably in the heart.

Neurotoxicity

The quality of rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). The mode of action of Rapeseed oil as a plant protection product does not target the nervous system, therefore neurotoxic effects of Rapeseed oil are not expected.

Other studies

Metabolites: Notifier has not presented toxicity studies performed with metabolites. Rapeseed oil is, like all vegetable oils, metabolized by hydrolysis of the glycerol ester to release glycerol and fatty acids. These are incorporated as normal body constituents or degraded via ß-oxidation.

Other risk assessments: EPA (1998) accepted data waivers requested for acute oral, dermal, inhalation, and eye toxicity, dermal sensitization, genotoxicity, reproductive and developmental toxicity, subchronic (90-day) oral and inhalation toxicity, and teratogenicity for NEU 1160 Vegetable Oil Insecticide based on the long history of use of canola as an edible fat and oil in food without any indication of deleterious effects; its low toxicity; its natural occurrence as an oil extracted from plants; its low erucic acid (less than 2%). In addition, EPA (2002) concluded that there is a reasonable certainty that no harm will result to the general population, and to infants and children from aggregate exposure to residues of the C8 to C18 fatty acids.

Medical data

Since there is no toxicity associated with Rapeseed oil there are no medical data regarding occupational and accidental, long term or acute toxic effects.

B.6.10.2 Proposed ADI, AOEL and ArfD

The quality of low-erucic acid refined rapeseed oil (no more thant 2% erucic acid) is accepted as food according to Codex Alimentarius (FAO-WHO, 2001). The applicant stated that the setting of references values is not applicable. In addition EPA (1998) accepted data waivers requested for acute oral, dermal, inhalation, and eye toxicity, dermal sensitization, genotoxicity, reproductive and developmental toxicity, subchronic (90-day) oral and inhalation toxicity, and teratogenicity for NEU 1160 Vegetable Oil Insecticide based on the long history of use of canola as an edible fat and oil in food without any indication of deleterious effects; its low toxicity; its natural occurrence as an oil extracted from plants; its low erucic acid (less than 2%).

According to Product Health and Safety Data (Document JIII 1.4.1/01) there is a **Occupational exposure limit (OEL) for oil mist of 5mg/m^3** (TWA, 8h – workday) recommended based upon the ACGIH TLV (Analysis according to US NIOSH Method 5026, NIOSH Manual for Analytical

Methods, 3rd Ed.).

RMS agree with the Applicant that rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001) and the setting of references values seems to be not applicable.

Nevertheless, since there is an OEL and the method kind of application for NEU1160 I is spraying a exposure risk assessment could be done.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (IIIA7.1)

Summary

Neudorff GmbH KG has presented and sponsored six studies for evaluating the acute toxicity, primary irritation and sensitisation of NEU 1160 I. All studies were reported over the period 1996 to 2002. All of them were guideline and GLP compliant and all were accepted.

NEU 1160 I is non-hazardous by the oral (oral LD_{50} in rats> 2000 mg/kg), dermal (dermal LD_{50} in rats> 2000 mg/kg) or inhalation (LC_{50} in rats> 3.26 mg/L) route. It is not irritant to the eyes and to the rabbit skin and does not induce delayed contact hypersensitivity in guinea pigs.

Based on these results, and in accordance with Annex I of Council Directive 67/548/EEC (Dangerous Substances Directive) and 99/45/EC (Dangerous Preparations Directive), classification for acute toxicity of NEU 1160 I is not required.

These studies are summarised in Table 6.11-1.

Table 6.11-1: Summary of Acute Toxicity, Primary Irritation and Dermal Sensitisation Studies

Route. Species / Sex	Dosage	Vehicle	Results	Reference	A*
Acute oral toxicity					
Wistar rats of both sexes	2000 mg/kg	Undiluted	Oral LD_{50} > 2000 mg/kg bw	Assessment of acute oral toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996a) (IIIA, 7.1.1/01, Report No. 170764)	Yes
Acute dermal toxicity					
Wistar rats of both sexes	2000 mg/kg	Undiluted	Dermal LD_{50} > 2000 mg/kg	Assessment of acute dermal toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996b) (IIA 7.1.2 /01, Report No. 170775)	Yes
Acute inhalation toxicity					
SD Crl: CD rats of both sexes	2.36 mg/L	None	LC_{50} for four hours was >3.26 mg/L	Acute inhalation toxicity - NEU 1161 I. Lenz, G. (1996), (IIA 7.1.3 /01, Report No. 96 5041 804)	Yes
Acute dermal irritation					
New Zealand White Rabbits /males	0.5 mL	Undiluted	Non Irritant	Primary skin irritation/corrosion study with NEU 1161 I in the rabbit (4-hour semi-occlusive application). Rijken, W.R.P.(1997), (IIA 7.1.1 /01: Report No. 170775)	Yes
Acute eye irritation					
New Zealand White Rabbits / males	0.1 mL	Undiluted	Non Irritant	Acute eye irritation/corrosion study with Neu 1161 I in the rabbit. Rijken, W.R.P. (1996c), (IIA 7.1.5 /01, Report No. 170797)	Yes
Skin sensitisation					
Dunkin Hartley Albino Guinea Pig / female	20% for intradermal injection and 100% for topical induction and challenger	Water	No sensitizer	Test for sensitization (Guinea pig maximisation Test) with NEU 1160 I. Otterdijk van, F.M (2002), (IIIA 7.1.6/01 Report No: 356052)	Yes

A*: Acceptability

B.6.11.1 Assessment of acute oral toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996a) (IIIA, 7.1.1/01, Report No. 170764)

Date of experimental work: 6 to 20 March 1996. Date of report: 23 May 1996

Objectives: To assess the acute oral toxicity of NEU 1161 I after single gavage administration to rats.

Guideline: The study was checked for compliance OECD 401 Guideline (1987).

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch 2/96 and a purity of 90% in Rapeseed oil and 2 % of Pyrethrum Extract.

Test animals: Wistar Crl: (WI) BR rats of both sexes (approx. 6 weeks and 174-212 g males, 146-155 g females). No vehicle was used.

NEU 1161 I, was administered by oral gavage to five rats by sex at a dose of 2000 mg/kg bw.

All the animals were observed for mortality and clinical signs daily during 15 days. Individual body weights were recorded on days 1, 8 and 15. At the end of the test, all animals were sacrificed and subjected to necropsy. All macroscopic abnormalities were recorded.

Not statistical analysis was performed.

Findings

No mortality occurred, no clinical signs of toxicity were observed.

The mean body weight gain during the observation period was within the range expected for untreated rats of the same age and strain.

No abnormalities were found in the animals upon macroscopic post mortem examination.

Conclusion

The oral LD_{50} value of NEU 1161 I in rats of both sexes was greater than 2000 mg/kg bw. In accordance with the EU criteria, it does not require classification for acute oral toxicity.

B.6.11.2 Assessment of acute dermal toxicity with NEU 1161 I in the rat. Rijken, W.R.P.(1996b) (IIA 7.1.2 /01, Report No. 170775)

Date of experimental work: 7 to 21 March 1996. Date of report: 23 May 1996

Objectives: To assess the acute dermal toxicity of NEU 1161 I after single application to rats.

Guideline: OECD 402 Guideline (1987).

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch 2/96 and a purity of 90% in Rapeseed oil and 2 % of Pyrethrum Extract.

Test animals: Wistar Crl: (WI) BR rats of both sexes (approx. 8 weeks and 309 – 333 g males, 197-225 g females). No vehicle was used.

NEU1161 I was applied occlusively onto the shaved skin of five rats by sex at a dose of 2000 mg/kg bw for 24 hours after that, residual test substance was removed with tap water.

All the animals were observed for mortality and clinical signs daily during 15 days. Individual body weights were recorded on days 1, 8 and 15. At the end of the test, all animals were sacrificed and subjected to necropsy. All macroscopic abnormalities were recorded.

Not statistical analysis was performed.

Findings

No mortality occurred.

Red staining on the head or in the neck was noted in one female from day 2 onwards.

The mean body weight gain during the observation period was within the range expected for rats used in this type of study.

No abnormal findings were noted at necropsy.

Conclusion

The dermal LD_{50} value of NEU 1161 I in rats was greater than 2000 mg/kg bw. In accordance with the EU criteria, it does not require classification for acute dermal toxicity.

B.6.11.3 Acute inhalation toxicity - NEU 1161 I. Lenz, G. (1996), (IIA 7.1.3 /01, Report No. 96 5041 804)

Date of experimental work: 6 to 25 March 1996. Date of report: 16 April 1996

Objectives: To determine the acute inhalation toxicity of NEU 1161 I to rats of both sexes exposed once nose-only to a solid aerosol for four hours.

Guideline: OECD 403 Guideline (1981).

Deviations: The batch of the test substance was not reported.

Comments: Due to the oily nature of the test substance problems occurred during the sampling for determination of the concentration. Therefore, concentration measurements are based on only one measurement.

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch not reported and a purity of 90% in Rapeseed oil and 2 % of Pyrethrum Extract.

Test animals: Sprague-Dawley (CD Crl:CD rats of both sexes (179-194g males, 185-196g females. Vehicle: PEG 400 (0.8 g/200 ml).

Five rats by sex were exposed nose-only for four hours to an aerosol at a nominal concentration of the test substance of 50 mg/L (measured concentration: 2.36 mg/L, the highest technical achievable concentration).

Temperature, relative humidity and the airflow in the inhalation chamber were recorded. The aerosol concentration and the particle size distribution were analysed during the exposure. Mass median

aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined. Two samples of test atmosphere were taken during exposure to determine analytically by GC the actual concentration of NEU 1161 I.

All the animals were observed for 14 days for mortality and clinical signs of toxicity. Body weights were recorded prior to exposure and on days 8 and 15. All animals were necropsied and subjected to gross macroscopic examination.

Findings

Due to the oily nature of the test substance problems occurred during the sampling for determination of the concentration. Therefore, concentration measurements are based on one measurement.

Exposure conditions:

Nominal concentration (mg/L air)	50
Measured concentration (mg/L air)	2.36
Air flow exposure (L/min)	15
Chamber temperature (° C)	19-23
Relative humidity (%)	30-70
MMAD (µm) GSD (µm)	1.1-1.7 1.4-1.5
Particles < 4µm (%)	> 95

No mortality or acute toxicological symptoms were observed over a 14-day observation period. The post-mortem findings after euthanasia did not show any macroscopic organ changes

Conclusions

Under the test conditions, the acute LC_{50} of NEU 1161 I to rats for four hours was >2.36 mg/L. In accordance with the EU Commission Directive 2001/59/EC, it does not require classification for inhalation toxicity.

B.6.11.4 Primary skin irritation/corrosion study with NEU 1161 I in the rabbit (4-hour semi-occlusive application). Rijken, W.R.P.(1997), (IIA 7.1.1 /01: Report No. 170775)

Date of experimental work: 21 to 28 of May 1997. Date of report: 22 June 1997

Objectives: To determine the potential skin irritation of NEU 1161 I when applied to the shaved intact skin of rabbits.

Guideline: OECD 404 Guideline (April 2002)

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch 2/96 and a purity of 90% in Rapeseed oil and 2 % of Pyrethrum Extract.

Test animals: Male new Zealand White albino rabbits (at least 6 weeks, 1928 – 2010 g).

No vehicle was used.

Three male rabbits were exposed to 0.5 mL of NEU 1161 I, applied onto the clipped dorsal skin for four hours using a semi-occlusive dressing. After that, residual test item was removed with tissue moisturized with water and subsequently a dry tissue.

Mortality and signs of toxicity were observed at least once daily. The skin reactions were evaluated at 1, 24, 48, 72 hours and 7 days after removal of the dressings using Draize system.

Findings

No symptoms of systemic toxicity were found and no mortality occurred.

Very slight erythema and slight oedema were observed in the treated skin-areas of the three treated rabbits. Oedema could not be scored from days 2 onwards.

The skin irritation had resolved within 72 h in two animals and within 7 days after exposure in the third animal. Greasy remnants of the test substance was present on the skin on day 1. The results were summarized in table below:

Table 6.11.4-1: Individual and mean skin irritation scores

	Erythema			Oedema		
Animal no	**1021**	**1023**	**1025**	**1021**	**1023**	**1025**
After 1 hr	1	1	1	2	2	2
After 24 hr	1	1	1	1	1	1
After 48 hr	1	1	1	0	0	0
After 72 hr	1	0	0	0	0	0
After 7 d	0	0	0	0	0	0
Mean score 24 – 72 h	0.78			0.34		

Conclusion

Under the conditions of this study, NEU 1161 I in not irritating to the rabbit skin. In accordance with EU Commission Directive 2001/59/EC, it does not require classification.

B.6.11.5 Acute eye irritation/corrosion study with Neu 1161 I in the rabbit. Rijken, W.R.P. (1996c), (IIA 7.1.5 /01, Report No. 170797)

Date of experimental work: 18 to 21 March 1996. Date of report: 23 May 1996

Objectives: To determine the potential eye irritation of NEU 1161 I after application to the eyes of rabbit.

Guideline: OECD 405 Guideline (April 2002).

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch 2/96 and a purity of 90% in Rapeseed oil and 2 % of Pyrethrum Extract.

Test animals: Male new Zealand White albino rabbits (approximately 7 weeks, 1207 – 1396 g).

No vehicle was used.

A volume of 0.1 mL of the test substance was instilled in the conjunctival sac of one eye of each of 3 young adult male albino rabbits. The other eye remained untreated and served as the reference control.

After the 24 hour observation, a solution of 2% fluorescein in water was instilled into both eyes of each animal to quantitatively determine corneal epithelial damage. Observations were done on mortality/viability (twice per day), clinical signs of toxicity (at least once daily) and on eye irritation (1, 24, 48 and 72 hours after instillation of the test substance). Eye irritation was scored using the Draize

scheme for eyes.

Findings

No symptoms of systemic toxicity were found and no mortality occurred.

Instillation of the test substance resulted in slight irritation of the conjunctival tissue, which had resolved within 24 hours. The results were summarized in table below:

Table 6.11.5-1: Mean values of eye irritation scores (24, 48 and 72 h after instillation)

Animal no.	Mean 24-72 hours				
	Corneal opacity	Iris	Conjunctivae		
			Redness	Chemosis	Discharge
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	0

Conclusion

Under the conditions of this study, NEU 1160 I produced slight irritation that rapidly resolved. No corrosive effect to the eye was induced. In accordance with EU Commission Directive 2001/59/EC, NEU 1160 I does not require classification as irritating to eyes.

B.6.11.6 Test for sensitization (Guinea pig maximisation Test) with NEU 1160 I. Otterdijk van, F.M (2002), (IIIA 7.1.6/01 Report No: 356052)

Date of experimental work: 23 July to 13 September 2002. Date of report: 1 November 2002

Objectives: To evaluate the sensitisation potential of NEU 1160 I in guinea pig using a the Maximisation test.

Guideline: OECD Guideline 406 (1992).

GLP: Yes

This study is considered accepted

Material and methods

Test substance: NEU 1161 I, a yellow liquid, batch 205020 and a purity of 96% in Rapeseed oil.

Test animals: females Dunkin Hartley Albino Guinea Pig (approximately 4 weeks, 366 ± 26 g).

Vehicle: water.

Test substance concentrations selected for the main study were based on the results of a preliminary study in which 4 animals and concentrations ranging from 10% to 100% were used for intradermal injection (each of 2 animals received 2 different concentrations in duplicate (0.1 mL/site) in the clipped scapular region, dermal reactions were assessed 24 and 48 hours after treatment) and by epidermal application (two different concentrations (0.5 mL each) per animal to the clipped flank using semi-occlusive dressings (dermal reactions were assessed 24 and 48 hours later).

Main study

A group of 15 female albino guinea pigs were used (10 test - 5 control).

Concentrations of NEU 1160 I used: 20% for intradermal injection, 100% for topical induction and for challenge. The test was preformed according the guideline procedure and the dosing scheme is summarised in table 6.11.6-1

Table 6.11.6-1: Dosing scheme of NEU 1160 I in the Magnusson and Kligman test

	Test	**Control**	**Application site**
Induction intradermal injection (0.1 ml) (Day 1)			
1	FCA /distilled water 1:1	FCA /distilled water 1:1	Top of shoulder region
2	Test sample at 20% in water	Water	Middle of shoulder region
3	Test sample at 40%/FCA 1:1	Water / FCA 1:1	Bottom of shoulder region
Induction topical application (0.5 mL) (Day 8)*	Test sample at 100%	Water	Scapular area between injection sites
Challenge topical application (0.1 mL) (Day 22)**			
	Test sample at 100%	Test sample at 100%	Shorn flank

*Applied in a patch held in contact by occlusive dressing for 48 hours. Afterwards, the skin was cleaned with water.
** Applied in a patch held in contact by occlusive dressing for 24 hours. Afterwards, the skin was cleaned with water.

On Day 3 dermal reactions were assessed for irritation.

On day 7 the scapular area between the injection sites was clipped and rubbed with 10% sodium dodecylsulfate in Vaseline (this concentration causes a mild inflammatory reaction).

The treated sites were assessed for challenge reactions according to the Magnusson and Kligman grading scale, 24 and 48 hours after removal of the dressing.

Findings

The results of the preliminary study were summarized in tables 6.11.6-2 and 6.11.6-3

Table 6.11.6-2: Skin reactions after intradermal injection

Animal Nº	Conc. (%)	48 hours after injection		48 hours after injection	
		Erythema (grade)	Necrosis (grade)	Erythema (grade)	Necrosis (mm)
695	100 50	nr nr	3 2	nr nr	4 3
700	20 10	2 2	nr nr	2 2	nr nr

Nr: no reaction

Table 6.11.6-3: Skin reactions after epidermal exposure

Animal Nº	Conc. (%)	48 hours after injection		48 hours after injection	
		Erythema (grade)	Necrosis (grade)	Erythema (grade)	Necrosis (mm)
685	100 50	nr nr	nr nr	nr nr	nr nr
690	100 50	nr nr	nr nr	nr nr	nr nr
695	20 10	nr nr	nr nr	nr nr	nr nr
700	20 10	nr nr	nr nr	nr nr	nr nr

Nr: no reaction

Main study:

No mortality occurred and no symptoms of systemic toxicity were observed. Body weights and body weight gain remained in the same range as controls.

No signs of irritation were observed with the undiluted test substance. Therefore, 10% sodium dodecylsulfate was employed 24 hours before the epidermal induction to provoke a mild inflammatory reaction.

No skin reactions were observed in treated or control animals upon challenge with undiluted NEU 1160 I at 24 and 48-hours observation.

An earlier test with alpha-hexylcinnamic aldehyde as positive reference resulted in allergic reactions and has shown the sensitivity of the guinea pig strain used.

Conclusion

Based on the results of the Magnusson and Kligman Maximisation test, NEU 1160 I did not induce delayed contact hypersensitivity in guinea pigs and in accordance with EC criteria, NEU 1160 I is not classified as a sensitising agent.

B.6.12 Dermal absorption (IIIA 7.6)

Summary

No data available.

B.6.13 Toxiclogical data on non active substances (IIIA 7.9)

Summary

Detailed specifications of the preparation NEU 1160 I (composition and material safety data sheets for coformulants) are provided in Annex C of monograph.

The toxicological properties of the non-active substances contained in NEU 1160 I are provided in the material safety data sheet of each substance.

It can be concluded that the non-active substances with toxicological properties are present in amounts that do not represent a health hazard and thus, NEU 1160 I does not require to be classified except for the properties of the active substance.

B.6.14 Data on exposure: Operator, Bystander and Worker exposure

NEU 1160 I (883 g/L Rapeseed oil) is an Acaricide/insecticide against spider mites, mealy bugs and scales. According to Aplicant, rapeseed oil is assumed to be of very low toxicity and its content in NEU 1160 I does not warrant operator, bystander and worker exposure estimations.

According to Product Health and Safety Data (Document JIII 1.4.1/01) there is a **Occupational exposure limit (OEL) for oil mist of 5mg/m^3** (TWA, 8h – workday) recommended based upon the ACGIH TLV (Analysis according to US NIOSH Method 5026, NIOSH Manual for Analytical Methods, 3rd Ed.).

RMS agrees with the Applicant that rapeseed oil is accepted as food according to Codex Alimentarius (FAO-WHO, 2001) and the setting of references values seems to be not applicable.

Nevertheless, since there is an OEL and the method kind of application for NEU1160 I is spraying a exposure risk assessment could be done.

B.6.15 Refence relied on

Annex point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data Protec-tion Claimed yes/no	Owner
IIA 5.1/01	Berg, J.M., Tymoczko, J.L., Stryer,L.	2002	BIOCHEMISTRY not applicable W.H. Freeman and Co, New York, 5th Edition Report-no. - GLP: no published: yes	no	--
IIA 5.2/01	Anonymous	2005	STATEMENT LAMOTTE not applicable not applicable Report-no. not stated GLP: no published: yes	no	-
IIA 5.2/02	Anonymous	2001	CODEX ALIMANTARIUS FATS, OILS AND RELATED PRODUCTS not applicable not applicable Report-no. - GLP: no published: yes	no	-
IIA 5.2.1/01	Rijcken, W.R.P.	1996a	ASSESSMENT OF ACUTE ORAL TOXICITY WITH NEU 1161 I IN THE RAT [redacted] W. Neudorff GmbH KG Report-no. [redacted] Project 170764 GLP: yes published: no Submitted in: KIIIA 7.1.1/01	yes	NEU
IIA 5.2.2/01	Rijken, W.R.P.	1996b	ASSESSMENT OF ACUTE DERMAL TOXICITY WITH NEU 1161 I IN THE RAT [redacted] W. Neudorff GmbH KG Report-no. [redacted] Project 170775 GLP: yes published: no Submitted in: KIIIA 7.1.2/01	yes	NEU
IIA 5.2.3/01	Lenz, G.	1996	ACUTE INHALATION TOXICITY - NEU 1161 I [redacted] W. Neudorff GmbH KG Report-no. 96 50 41 804 GLP: yes published: no Submitted in: KIIIA7.1.3/01	yes	NEU

Annex point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed yes/no	Owner
IIA 5.2.4/01	Rijken W.R.P.	1997	PRIMARY SKIN IRRITATION/CORROSION STUDY WITH NEU 1161 I IN THE RABBIT (4-HOUR SEMI-OCCLUSIVE APPLICATION) [redacted] W. Neudorff GmbH KG Report-no[redacted]Project 205739 GLP: yes published: no Submitted in: KIIIA7.1.4/01	yes	NEU
IIA 5.2.5/01	Rijken, W.R.P.	1996c	ACUTE EYE IRRITATION/CORROSION STUDY WITH NEU 1161 I IN THE RABBIT [redacted] W. Neudorff GmbH KG Report-no[redacted]Project 170797 GLP: yes published: no Submitted in: KIIIA7.1.5/01	yes	NEU
IIA 5.2.6/01	Otterdijk van, F.M	2002	ASSESSMENT OF CONTACT HYPERSENSITIVITY TO NEU 1160 IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST) [redacted] W. Neudorff GmbH KG Report-no[redacted]project 356052 GLP: yes published: no Submitted in: KIIIA7.1.6/01	yes	NEU
IIA 5.5./01	Suzuki, H., Yamazaki, M., Arai, S., Nagao, A., Terao, J.	1991	EFFECT OF LARD, PALM AND RAPESEED OILS LIFE CONSERVATION IN AGED MICE - Mechanisms of Ageing and Development 60, pp 267-274 Report-no. GLP: no published: yes	no	-
IIA 5.5./02	Yamashiro, S., Clandinin, M.T.	1980	MYOCARDIAL ULTRASTRUCTURE OF RATS FED HIGH AND LOW ERUCIC ACID RAPESEED OILS - Experimantal and Molecular Pathology 33, pp 55-64 Report-no. GLP: no published: yes	no	-

Annex point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data Protec-tion Claimed yes/no	Owner
IIA 5.6/01	Reyes, H., Ribalta, J., Hernandez, I., Arrese, M., Pak, N., Wells, M., Kirsch, R.E.	1995	IS DIETARY ERUCIC ACID HEPATOTOXIC IN PREGNANCY? AN EXPERIMANTAL STUDY IN RATS AND HAMSTERS Hepatology 21, 1373-1379 Report-no. GLP: no published: yes	no	
IIA 5.11/01	Pfau, W.	2005	SUMMARY OF THE TOXICOLOGICAL AND METABOLISM STUDIES ON THE ON THE ACTIVE SUBSTANCE not applicable W. Neudorff GmbH KG Report-no. not stated GLP: no published: no	yes	NEU
IIIA 7.1.1/01	Rijken, W.R.P.	1996a	ASSESSMENT OF ACUTE ORAL TOXICITY WITH NEU 1161 I IN THE RAT [redacted] W. Neudorff GmbH KG Report-no [redacted] Project 170764 GLP: yes published: no	yes	NEU
IIIA 7.1.2/01	Rijken, W.R.P.	1996b	ASSESSMENT OF ACUTE DERMAL TOXICITY WITH NEU 1161 I IN THE RAT [redacted] W. Neudorff GmbH KG Report-no [redacted] Project 170775 GLP: yes published: no	yes	NEU
IIIA 7.1.3/01	Lenz, G.	1996	ACUTE INHALATION TOXICITY - NEU 1161 I [redacted] 6185 Karlsruhe W. Neudorff GmbH KG Report-no. 96 50 41 804 GLP: yes published: no	yes	NEU
IIIA 7.1.4/01	Rijken, W.R.P.	1997	PRIMARY SKIN IRRITATION/CORROSION STUDY WITH NEU 1161 I IN THE RABBIT (4-HOUR SEMI-OCCLUSIVE APPLICATION) [redacted] W. Neudorff GmbH KG Report-no [redacted] Project 205739 GLP: yes published: no	yes	NEU

Annex point/ reference number	Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Data Protec-tion Claimed yes/no	Owner
IIIA 7.1.5/01	Rijken, W.R.P.	1996c	ACUTE EYE IRRITATION/CORROSION STUDY WITH NEU 1161 I IN THE RABBIT [redacted] The Netherlands W. Neudorff GmbH KG Report-no [redacted]Project 170797 GLP: yes published: no	yes	NEU
IIIA 7.1.6/01	Otterdijk van, F.M.	2002	ASSESSMENT OF CONTACT HYPERSENSITIVITY TO NEU 1160 I IN THE ALBINO GUINEA PIG (MAXIMISATION-TEST) [redacted] The Netherlands W. Neudorff GmbH KG Report-no [redacted]roject 356052 GLP: yes published: no	yes	NEU

Other documents reviewed by the RMS

EFSA Scientific Panel. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to rapeseed oil high in unsaponifiable matter as a novel food ingredient (The EFSA Journal (2005) 304, 1-11).

Canola Oil; Exemption from the Requirement of a Tolerance, Section III, Toxicological Profile, April 3, 1998 (40 CFR Part 180, [OPP-300623; FRL-5773-9]2070-AB78.). Environmental Protection Agency.

IIFG Decision Documents on Reassessment of Exemptions from the Requirement of a Tolerance for Fatty Acids, Section 16, Determination of Safety, July 31, 2002. Environmental Protection Agency.

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