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Technical Information

Summary of Toxicity Studies with Fenoxaprop-ethyl

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DESCRIPTION OF THE TEST CHEMICAL

Fenoxaprop-ethyl is the active ingredient of corresponding commercially available herbicide formulations. It has been synthesized by Hoechst AG (FRG) since 1982 and is used for postemergence selective control of annual and perennial grasses. It acts predominantly by inhibition of lipid biosynthesis in the susceptible grass weed.

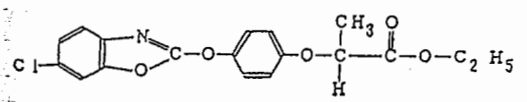
The toxicological profile of the technical substance has been established in a large number of in-life studies. These studies provide an adequate data basis for an evaluation of health risks for users of crop protection agents containing fenoxaprop-ethyl and for an assessment of the health risk for consumers of treated food products.

The chemical structure and physico-chemical properties of fenoxaprop-ethyl are given below.

Common name: fenoxaprop-ethyl

Chemical name: ethyl 2-(4-(6-chloro-2-benzoxazolyloxy) - phenoxy) - propanoate (IUPAC); = racemate; (D/L)-enantiomer

Structural formula:



Molecular formula: $C_{18}H_{16}ClNO_5$

Molecular weight: 361.8

Appearance: colourless powder

Melting point: 85–87°C

Solubility (20°C): hexane 5 g/*l*, cyclohexane 15 g/*l*, ethanol and 1-octanol 20 g/*l*, sesame oil 25 g/*l*, toluene 340 g/*l*, acetone 510 g/*l*, water 0.8 mg/*l*.

ACUTE TOXICITY STUDIES

Fenoxaprop-ethyl exhibited slight toxic properties following acute treatment. Testing for acute toxicity in various species by various routes of administration resulted in the following LD_{50}-Values: Table 1.

Lethally intoxicated mice and rats died between one and seven days after dosing. The rats reacted more sensitively than the mice. Symptoms of intoxication included passivity, drowsiness, disequilibrium and abdominal position. Necropsy revealed bright spots and lobular markings on the liver. Beagle dogs tolerated an oral dose of 1500 mg/kg bodyweight without any signs of intoxication, higher doses caused emesis. There was no dermal toxicity in rats or rabbits at the doses as high as those tested. Inhalational exposure of rats for 4 hours yielded an LC_{50} higher than 511 mg/m³ which was the highest possible technically feasible concentration.

LOCAL IRRITATION STUDIES

Fenoxaprop-ethyl was only slightly irritant to the skin and eye mucosa in rabbits[13] and exhibited no allergic property in a test conducted with guinea pigs according to the method of Buehler.[14]

(Hoechst AG, 1979[13] and 1982[14])

REPEATED-DOSE INHALATION AND DERMAL TOXICITY

The 'No Observable Effect Level' (NOEL)

Table 1

Species	Sex	Route	Vehicle	LD_{50}[a] (mg/kg)	Ref. no.
Mouse	M	oral	sesame oil	4670 (4180–5130)	1)
Mouse	F	oral	sesame oil	5490 (5010–6140)	2)
Rat	M	oral	sesame oil	2357 (2240–2479)	3)
Rat	F	oral	sesame oil	2500 (2230–2780)	4)
Rat	F	oral	sesame oil	3646 (2837–4685)	5)
Dog	M	oral	sesame oil	>1500 (emesis)	6)
Dog	F	oral	sesame oil	>1500 (emesis)	6)
Rat	F	dermal	sesame oil	>2000	7)
Rat	F	dermal	sesame oil	>2000	8)
Rabbit	M	dermal	—	>1000	9)
Rabbit	F	dermal	—	>1000	9)
Rat	M	i.p.	sesame oil	739 (253–1150)	10)
Rat	F	i.p.	sesame oil	864 (691–1079)	11)
Rat	M, F	inhal. (4 hr)	ethanol/polyglycol	>511[b]	12)

[a] ( ) 95% confidence limit.
[b] mg/m³ (LC_{50}).

determined in a six week inhalation toxicity study was 14.3 mg/m³ air. Higher concentrations (≧73 mg/m³) caused a significant increase in liver and kidney weights. Microscopic examination revealed a dose-dependent (248 and 727 mg/m³) centrilobular hepatocellular hypertrophy at the end of the treatment period.[15] 30-days dermal toxicity study in rats yielded a NOEL of 20 mg/kg bodyweight. At a dose of 100 mg/kg bodyweight an increase of liver weights was evident. 500 mg/kg bodyweight caused reduced bodyweights and increased liver and kidney weights.[16] In these studies,[15,16] a reversible decrease in total lipids and cholesterol was observed. Five repeated dermal applications of 1000 mg/kg fenoxaprop-ethyl to the shaved nape skin of 6 rabbits resulted in one death after 11 days, reduction of bodyweights, passiveness, ataxia, diarrhoea and local dermal irritations.[17]

(Hoechst AG, 1979[17] and 1984[15,16])

SUBCHRONIC AND CHRONIC FEEDING STUDIES

Toxicity testing of fenoxaprop-ethyl was carried out in a large number of feeding studies in accordance with the OECD, EPA and Japanese MAFF guidelines, which are tabulated as follows: Table 2.

Results

In Rats from 80 ppm onwards, a first sign on lipid metabolism could be seen. In the subchronic studies from 315 ppm onwards the cholesterol and total lipids were significantly decreased. The liver weights[18,19] and alkaline phosphatase (AP) were increased.[18] At 1250 ppm histopathology of the liver indicated single cell necrosis and hepatocellular hypertrophy with a fine-granulated eosinophilic staining of the cytoplasm. In all of the rat studies performed there were no pathological lesions in the kidneys, although the kidney weights were increased at higher concentrations. In the chronic study, the magnitude of the lipid lowering effect seen at 180 ppm diminished up to the end of the study (106 weeks) indicating an adaptive response to the pharmacodynamic activity of the test compound. Biochemical examinations showed that dietary concentrations of 5, 30 or 180 ppm over a 12-month period did not lead to any induction of foreign substance metabolism or to peroxisomal proliferation.[21] Life-time feeding of 180 ppm fenoxaprop-ethyl in the diet caused no carcinogenic effects in rats. The NOEL of 30 ppm in the diet is equivalent to a daily intake of 1.58 and 2.0 mg/kg bodyweight for the male and female rats.

In mice, at concentrations from 20 to 315 ppm cholesterol and total lipids were in-

Table 2

Species	Duration	Concentration (mg/kg food)	NOEL	LEL	TEL	Ref. no.
Rat	32 d	0–80–315–1250–5000	80	315	1250	18)
	90 d	0–20–80–320	80	320	320	19)
	24 M	0–5–30–180	30	>30	180	20–24)
Mouse	32 d	0–80–315–1250–5000	<80	80	315	25)
	30 d	0–5–10–20–80	>10	20	80	26)
	24 M	0–2.5–10–40	>10	40	40	27–29)
Dog	30 d	0–80–400–2000	≦400	400	400	30)
	90 d	0–16–80–400	16	80	400	31)
	12 M	0–3–15–75	75			32)
	24 M	0–3–15–75	15	75	75	33)

NOEL=No observable effect level.
LEL=Lowest effect level (Changes in lipid status).
TEL=Toxic effect level.

creased,[25,26] which returned to normal at 1250 ppm.[25] From 315 ppm onwards, absolute and relative liver weights were increased. Hepatocellular hypertrophy and liver cell necrosis were seen histopathologically indicating progressive liver toxicity. The liver enzymes (AP, SGPT) were increased. In addition, tubular kidney necrosis occurred from 315 ppm onwards.[25] At the one year interim sacrifice of the carcinogenicity study[27] a statistically significant increase in the absolute and relative kidney weights was observed in the 40 ppm group females. This might be relevant, because the same pattern was observed in the rat chronic and oncogenicity studies. However, these findings did not show any histological correlation. Since the catalase activity was not increased up to and including a concentration of 40 ppm, peroxisomal proliferation could be excluded.[26] Based on all data generated in mice, it is clear that fenoxaprop-ethyl is not carcinogenic. The NOEL for the mouse at >10 ppm in the diet is equivalent to a daily intake of >1.38 and 1.61 mg/kg bodyweight for the male and female mice.[29]

In dogs, fenoxaprop-ethyl was tolerated up to and including a concentration level of 400 ppm.[30] No special pharmacodynamic effect on lipid metabolism could be detected.[30-33] The neurologic and ophthalmoscopic examinations, hearing and dental checks, haematology and clinical chemistry examinations, urinalysis, liver (BSP) and renal (PSP) function tests revealed no substance related changes. In contrast to the findings in rats and mice, the liver and kidneys showed no morphological changes and no change in the organ weights, indicating that the interaction with normal physiology by fenoxaprop-ethyl differs considerably in rodent and non-rodent animals. Since reduced bodyweights were observed in the chronic study in males and females at dietary concentrations of 75 ppm, the NOEL was established at 15 ppm, equivalent to a daily intake of 1.1 and 0.9 mg/kg bodyweight for the male and female dog.[33]

Toxicological profile

Based on the results, it can be stated that the pharmacodynamic activity of fenoxaprop-ethyl affects the lipid metabolism in rats and mice although the way of interaction shows some species-specific differences. The effects on lipid metabolism were already seen at non-hepatotoxic doses. The liver and kidneys are the target organs in mice and rats, but not in dogs. It should be emphasized that the comparable hepatomegalic action and the subtle effects on kidneys seen in rats and mice at higher doses can be considered mainly as "adaptive response" of the target organs to changes in physiological homeostasis induced by the test compound that might be the result of peroxisomal proliferation. However, neither induction of drug metabolizing enzymes nor proli-

feration of peroxisomes could be seen in any of the chronic feeding studies. In addition, the lipid-lowering effect and the severity of the lesions diminished up to the end of the studies.

EVALUATION OF THE EMBRYOTOXICITY AND REPRODUCTION-STUDIES

The embryotoxic and/or teratogenic potential of fenoxaprop-ethyl was investigated in a variety of studies in mice, Wistar- and Sprague-Dawley rats, rabbits and non-human primates and by two different routes of administration, namely oral and dermal in rats rabbits in order to yield as much information as possible. Based on all data generated the following 'No Observable Effect Levels' were established: Table 3.

In mice, repeated oral treatment with fenoxaprop-ethyl during the period of major organogenesis did not affect embryonic or foetal development even though maternal toxic dosages were given.[34]

In rats and rabbits oral treatment with the test substance during the phase of organogenesis gave indications of an embryotoxic potential only at dose levels markedly toxic and partially lethal to the dams. It can be assumed that abnormalities, anomalies and variations in foetuses treated with fenoxaprop-ethyl were of spontaneous nature or related to the overall toxicity in the dams and embryos.[35,36,38,39] This position is supported by the fact that in embryotoxicity studies of fenoxaprop-ethyl in rats and rabbits using dermal application, the test substance did not reveal any disturbance of embryonic or foetal development even at dose levels exhibiting clear signs of intoxication to the mother animals.[37,40] The latter aspect is extremely important, since dermal contamination represents a major route of exposure during application and use, and is thus relevant for evaluating the possible risk to human health. The results of these studies were consistent and clearly indicated that fenoxaprop-ethyl does not exhibit any teratogenic potential in the absence of maternal toxicity.

Due to the similarities in reproductive physiology during pregnancy, in the sequence of events during morphogenesis and in the type of placentation between primates and human beings, the outcome of the oral embryotoxicity study in Cynomolgus monkeys (*Macaca fascicularis*) is the most relevant for predicting an extremely low risk in regard to possible embryotoxic potential of fenoxaprop-ethyl. Furthermore, the examinations of the foetuses—even at dose levels partly lethal to the mother animals—gave no indication of any teratogenic potential of the test substance.[41]

The data of the multigeneration studies in rats permitted the conclusion that fenoxaprop-

Table 3

Species	Route	Dosage[a]	NOEL Dams	Fetuses	Ref. no.
Mouse	oral	0–2–10–50	10	>50	34)
Rat	oral	0–10–32–100	10	>10	36)
		0–10–32–100	32	32	35)
Rat	dermal	0–100–300–1000	>1000	>1000	37)
Rabbit	oral	0–12.5–50–200	<50	50	38)
		0–2–10–50	>10	>10	39)
Rabbit	dermal	0–100–300–1000	100	>1000	40)
Monkey	oral	10–50	10	>50	41)
Rat/Multigeneration	oral	0–5–30–180[b]	30 (>180)+	30 (>180)+	42–44)
		0–5–30–180[b]			

[a] (mg/kg bodyweight/day).

[b] mg/kg diet (ppm).

Duration of treatment: mouse (day 6–15 p.c.), rat (day 7–16 p.c.), rabbit (day 7–19 p.c.) and monkeys (day 20–50 p.c.).

( )+ NOEL with respect to reproduction and fertility.

ethyl does not interfere with reproduction and fertility of parents. In the first study,[42] the reduction in viability of the offspring observed at 180 ppm could not be attributed to a definite assessment of the risk to the offsprings due to the fact that RCV/SDA-like virus infection could have influenced this parameter. In order to clarify this problem, a comparable study was carried out using Wistar rats.[43,44] Following dietary exposure to fenoxaprop-ethyl, reproductive performance, development and maturation of progenies were not impaired at any time during the study. No changes in the reproductive organs could be detected macroscopically and/or microscopically.[43,44] Due to slight changes in the lipid status at 180 ppm in the parents and their progeny, the "No Toxic Effect Level" is considered to be 30 ppm.

EVALUATION OF THE MUTAGENICITY STUDIES

The following short-term *in vitro*-tests taking different end-points into consideration were carried out to evaluate the possible mutagenic potential:

Point mutation (*in vitro*)

Procaryotes: An Ames test was carried out in *Salmonella typhimurium* (strains: TA100, TA1535, TA1537, TA1538 and TA98) and in *Escherichia coli* (strain: WP2uvrA) with and without metabolic activation (S9-mix) in a dose range of 4000–5000 μg/plate.

Result: non-mutagenic[45]
(Hoechst AG, 1982)

Eucaryotes: in *Schizosaccharomyces pombe* (strain: SP ade 6–60/rad 10–198, h-) with and without metabolic activation (S9-mix) in a dose range of 125–1000 μg/plate.

Result: non-mutagenic[46]
(Istituto di Ricerche Biomediche, 1982)

Chromosomal aberration

Mammalian cells: A chromosomal aberration assay in cultured human lymphocytes was carried out with and without metabolic activation (S9-mix) in a dose range of 1–1000 μg/ml.

Result: non-mutagenic[47]
(Istituto di Ricerche Biomediche, 1982)

DNA-Repair

Eucaryotes: Gene conversion–DNA-Repair test in *Saccharomyces cerevisiae* (D4-strain) with and without metabolic activation (S9-mix) in a dose range of 125–1000 μg/ml.

Result: non-mutagenic[48]
(Istituto di Ricerche Biomediche, 1982)

Mammalian cells: UDS-Test in HeLa-cells with metabolic activation (S9-mix) in a dose range of 5–500 μg/ml and without metabolic activation (S9-mix) in a dose range of 5–50 μg/ml.

Result: non-mutagenic[49]
(Istituto di Ricerche Biomediche, 1982)

Micronucleus test (*in vivo*)

Twice administration of 0, 18, 180 and 1800 mg/kg bodyweight of the test substance to NMRI mice and 100 mg/kg bodyweight Endoxan (Cyclophosphamide) serving as positive control. Evaluation of the number of polychromatic erythrocytes with micronuclei.

Result: non-mutagenic[50]
(Hoechst AG, 1984)

No mutagenic activity could be detected in any of the studies. Therefore fenoxaprop-ethyl does not exhibit any mutagenic potential.

CONCLUSION

The presented experimental data clearly defined the toxicological profile and hazard potential of fenoxaprop-ethyl. Based on the fact that the active ingredient exhibits no mutagenic, teratogenic or carcinogenic potential and does not interfere with reproduction performance, the detected 'No Observable Effect Levels' (NOEL) following subchronic and chronic treatment indicate no unexpected risk to human health under the recommended condition of use. Fenoxaprop-ethyl was registered in Japan for various crops and vegetables (*e.g.* sugar beets, soy beans, carrots, garden peas, sweet potatoes, cabbages, strawberries, onions) in 1988 and for chrysanthemums in 1989. Registrations have also been granted in many other countries since 1987.

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要　　約

フェノキサプロップエチルの毒性試験の概要

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フェノキサプロップエチルはドイツヘキスト社によって合成されたイネ科雑草に有効な茎葉処理型選択性除草剤である．本原体の各種動物に対する毒性試験の結果によると，ラットの急性経口毒性において LD_{50} は 2357～3646 mg/kg，マウスにおいては，4670～5490 mg/kg であった．ラットの急性経皮毒性では LD_{50} は 2000 mg/kg 以上であった．また眼および皮膚への一次刺激性は軽度であり，皮膚感作性は陰性であった．さらに亜急性毒性，慢性毒性，発癌性，繁殖毒性，催奇形性およ

び変異原性においても，特記すべき本品投与の影響は認められなかった．したがって，フェノキサプロップエチルは，定められた使用方法および注意事項を遵守することにより，安全性を確保できる薬剤であると言える．

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