

CYCLOXYDIM

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EXPLANATION

Cycloxydim is a systemic cyclohexanedione herbicide used for the control of grass weeds in many agricultural and horticultural broad-leaved crops. Cycloxydim was reviewed for the first time by the present Meeting.

EVALUATION FOR ACCEPTABLE DAILY INTAKE**BIOLOGICAL DATA****Biochemical aspects****Absorption, distribution, and excretion****Rats**

Radiolabelled (4,6-¹⁴C) cycloxydim was administered as both acid and sodium salt in toxicokinetic studies. Five Sprague-Dawley rats per sex were given single oral doses via stomach tube of 10 or 300 mg/kg bw in PEG 400 for the acid and similar groups were given 10 mg/kg bw of the sodium salt as an aqueous solution. In order to investigate enterohepatic circulation these experiments were repeated using groups of 3 males and 3 females in bile duct cannulated rats. The effects of repeated dosing were investigated in groups of 5 rats/sex given single radiolabelled doses of 10 mg/kg bw/days acid after 14 previous daily doses of unlabelled material at 10 mg/kg bw/day and in groups of 3 rats of each sex given 7 daily doses of 10 or 300 mg/kg bw/day radiolabelled acid. Furthermore, 5 rats per sex were given a single intravenous dose of 10 mg/kg bw radiolabelled sodium salt (Hawkins *et al.*, 1986).

Both the acid and sodium salt were well absorbed and almost completely excreted within 5 days after dosing. Elimination proceeded predominantly via the urine with 74 to 86% of the applied radioactivity being excreted within 5 days. Faeces contained approximately 12 to 25% of the applied radiolabel. No volatile radiolabel was detected in expired air and less than 1% of the administered dose was retained in the body. In rats with cannulated bile ducts, 55 to 66% of the dose was excreted into the bile within 48 h regardless of the dose level or type of formulation. In the same time period, renal excretion amounted to 23 to 37%, and faecal excretion to < 3%. Therefore, enterohepatic recirculation occurred in rats

and almost all radioactivity excreted in faeces of intact rats would have been excreted via the bile. It may also be concluded that both excretion and bioavailability of the two types of formulation are comparable. The sodium salt seemed to be more rapidly absorbed after oral administration, but an influence of the different type of carrier cannot be excluded.

After oral administration of 300 mg/kg bw to rats, normalised areas under plasma radiolabel concentration against time curves (AUCs) were approximately twice as high as after administration of 10 mg/kg bw. This indicates a non-linear relationship between dose level and AUC. Following multiple oral dosing for 7 days, at 10 mg/kg bw/day, normalized AUC figures were double compared to those figures obtained after single oral administration of 10 mg/kg bw. Following both single and multiple oral administration of radiolabelled cycloxydim the highest residue concentrations were detected in liver and kidneys. The level of residues in the various tissues was not influenced by single oral administration of either acid or sodium salt. Compared to the plasma levels, multiple dosing did not result in essential accumulation of radiolabel.

Radiolabelled cycloxydim was applied to shaved skin areas of Sprague-Dawley rats in nominal doses of 0.014, 0.14 or 1.4 mg/cm² (corresponding nominally to 0.9, 9 or 90 mg/kg bw) at a constant dose volume of approximately 1.3 ml/kg to an area of approximately 12 cm² on the back. The duration of treatment at the various dose levels was 0.5, 1, 2, 4 and 10 h with additional groups of animals treated for 10 h and sacrificed at 72 h (collection of excreta for 62 hours after removal of dermal dose). The results indicated that within 10 h up to 36%, 36% and 24% of radiolabel is absorbed through the skin at dose levels of 0.014, 0.14 and 1.4 mg/cm² respectively. After oral doses of 10 mg/kg bw a mean of 89.5% was found to be absorbed within the 10 h after treatment. With the exception of untreated skin, mean quantities of radiolabel in tissues and plasma of all dermal dose groups were at all sacrifice times at or below 1% of the applied dose. Unexpected high mean quantities of radiolabel were found consistently in untreated skin. The design of experiments and additional tests provided no evidence that cross-contamination from treated skin to untreated skin occurred. From data presented in this study it was concluded that dermal doses of 0.9, 9.0 and 90 mg/kg bw (0.014, 0.14, 1.4 mg/cm²) could be considered equivalent to oral doses of approximately 0.3, 3.0 and 30 mg/kg bw, respectively, for the purpose of toxicological evaluation assuming that the distribution and metabolism of a dermal dose was the same as an oral dose (Hoffmann *et al.*, 1989).

Biotransformation

Rats

Metabolism of cycloxydim in rats was largely unaffected by dose levels or dosage form (free acid or sodium salt). In all cases the major metabolite in urine was the sulfoxide (TSO). The next most important metabolite was T1SO resulting

from N-de-ethoxylation of TSO. Other less important metabolites in urine were the sulfones of T1SO and T2SO (Beckmann rearranged product of T1SO). The identities of these major metabolites were confirmed by mass spectrometry. Minor components in some urine samples corresponded chromatographically to the sulfone of TSO and unchanged cycloxydim. The presence of minor metabolites hydroxylated at the 5-position of the cyclohexone ring of cycloxydim was indicated after oxidation and methylation of a urine sample. The patterns of radioactive components in bile and tissue residues were generally similar to the pattern in urine. A postulated biotransformation pathway for cycloxydim in the rat is shown in Figure 1.

Toxicological studies

Acute toxicity studies

The results of acute toxicity studies on cycloxydim are summarised in Table 1. Deaths generally occurred 1-2 days after dosing and the non-specific signs of toxicity were characterized by reversibility within the observation period. The gross-pathological examination of the animals did not show any consistent substance-induced changes.

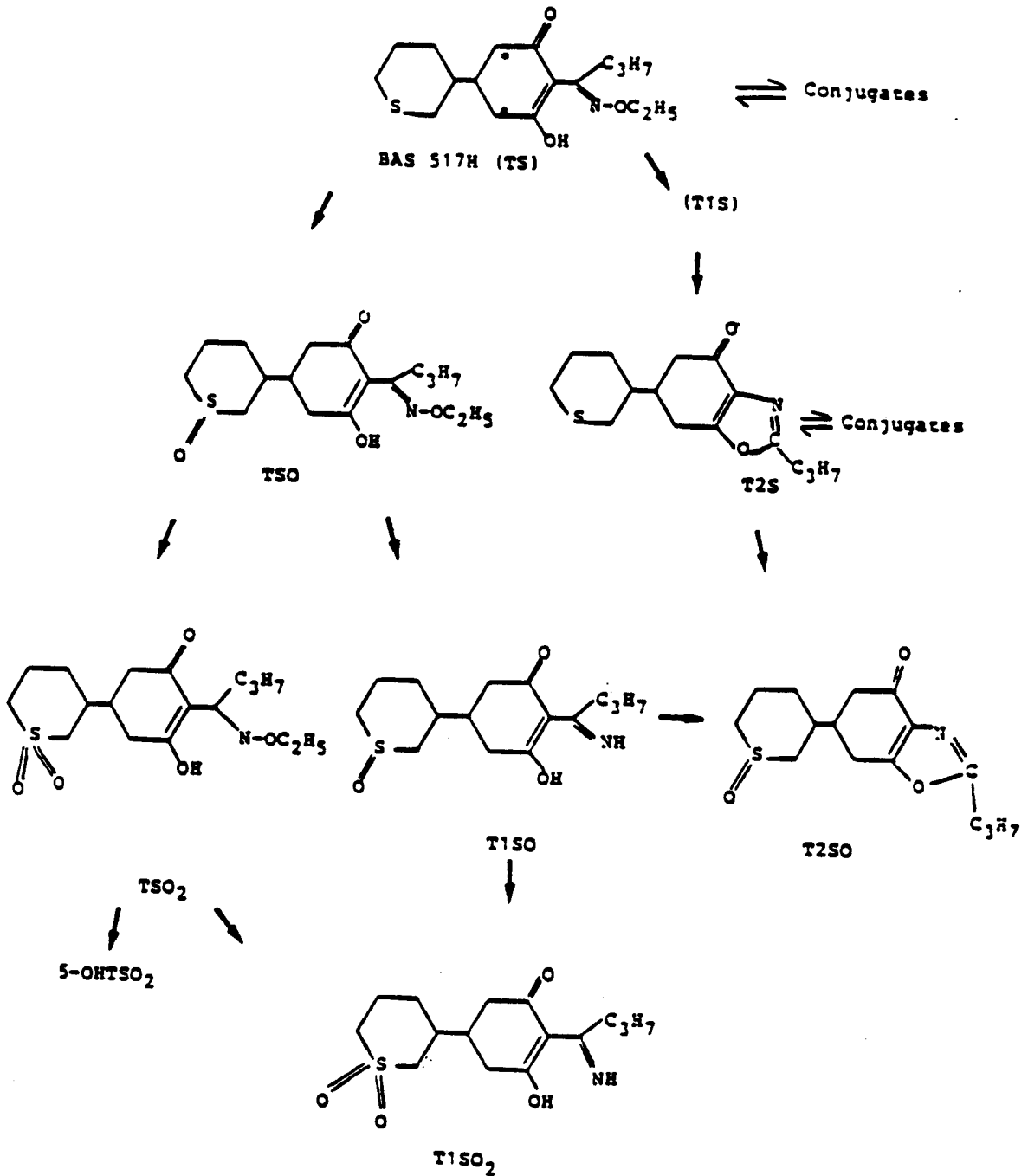
Table 1: Acute toxicity of cycloxydim (acid)

Species	Sex	Route	LD ₅₀ mg/kg bw	LD ₅₀ mg/l	Reference
Rat	M+F	Oral	~5000		Hildebrand & Kirsch (1987)
Rat	M	Oral	4420		Kirsch & Kieczka (1984a)
	F		3830		
Mouse	M+F	Oral	>5000		Kirsch & Kieczka (1985a)
Rat	M+F	Dermal	>2000		Kirsch & Kieczka (1984b)
Rat	M+F	Inh		>5.28	Klimisch, et al. (1985)
Rat	M	ip	2370		Kirsch & Kieczka (1985b)
	F		1943		

Short-term toxicity studies

Subacute toxicity studies with rats were carried out for 4 weeks and 3 months. Mice were used as a second rodent species for 4 weeks. Cycloxydim (as the acid or sodium salt) could not be administered to rodents via the feed because of insufficient stability nor was it possible to administer cycloxydim as the acid in the drinking water on account of its low solubility. After only one day the active ingredient concentration in the mixture of feed and test substance was only about 78% of the initial value, and after 8 days only about 50% was detected. Therefore, following demonstration of adequate stability by chemical analysis, the test substance was administered as the sodium salt via the drinking water in all short- and long-term investigations in rodents. In dogs the test substance could

Figure 1. Biotransformation pathway for cycloxydim in the rat



be administered as sodium salt in the feed since the mixture was prepared freshly each day shortly before feeding, and sufficient stability could be verified analytically for the short period until feed was consumed completely (food was generally consumed within one hour, at which time the analyzed concentration was 91% of intended). Altogether, 3 feeding studies were carried out with beagle dogs for 4 weeks, 3 months and 1 year. In all studies, the data in ppm or mg/kg bw refer to cycloxydim as acid rather than sodium salt.

Mice

In a 4-week range-finding study, groups of 10 B₆C₃F₁ mice per sex were administered doses of 0, 300, 1000, 3000 or 9000 ppm cycloxydim as the sodium salt (purity 94.8%) via the drinking water. Drinking water consumption was reduced, in a dose dependent fashion, up to a maximum of 50%, whereas feed consumption and body-weight gain were decreased only at 9000 ppm (approximately 14%). The clinical chemistry examinations revealed reduced cholesterol values, compared to controls, at a dose of 3000 ppm and above and increased urea values in both sexes of the highest dose group. At the end of the study, an increase in the relative liver weight was observed in the males of all dose groups. The histopathological examination showed hydropic vacuolar degeneration of hepatocytes only at the highest dose level (Kuhborth *et al.*, 1986a).

Since a NOAEL could not be determined in the previous study, a further range-finding study was carried out in mice of the same strain using a similar experimental design and doses of 0, 30, 100, 300 or 900 ppm. The absolute and relative liver weights of the males were increased at doses of 100 ppm and above. Reduced drinking water consumption (4-9%) was observed in the females at doses of 300 ppm and above, but feed consumption or body-weight gain were not affected even at 900 ppm. Clinical chemistry values and other organ weights were not changed by the test substance at any dose level. No histopathological examinations were carried out in this study since no histopathological findings were detected in the first range-finding study in the mouse within a similar dose range. The NOAEL was difficult to establish since the liver weight increase occurred without any other indications of toxicity, such as changes in clinical chemistry parameters or gross-pathological or histopathological findings. Assuming that the organ weight change was indicative of toxicity the NOAEL was 30 ppm in males and 100 ppm in females, equal to 7 mg/kg bw/day in males and 28 mg/kg bw/day in females, respectively (Kuhborth *et al.*, 1986b).

Rats

In a dose range-finding study, groups of 5 Wistar rats per sex were administered cycloxydim as the sodium salt (purity 92.8%) for 28 days via the drinking water at levels of 0, 300, 1000, 3000 or 9000 ppm. At the lowest two dose levels the only difference from controls was a reduction in drinking water intake. On account of the presence of test substance in the drinking water, the isolated

finding of reduced drinking water consumption is regarded as a problem of palatability rather than as a sign of toxicity of the test substance. Reduced feed consumption mainly in the females (approximately 15%), reduced drinking water consumption in general (approximately 20-28%) and a slight reduction in body-weight gain (5-12%) in the females were observed at 3000 ppm. Slightly increased urea levels in the blood were detected in the clinical chemistry examinations at 3000 ppm, along with increased relative liver weights in the females and increased relative kidney weights in the males, but there was no correlation with the histopathological evaluation. The highest dose of 9000 ppm showed pronounced toxicity, which was evident in the form of poor general state, reduced feed consumption (20-50%), reduced drinking water consumption (42-69%) and reduced body-weight gain of more than 20% compared to the control. The clinical chemistry examination revealed slightly increased urea and sodium values in both sexes, increased cholesterol and chloride values in the serum of the males and lowering of the triglyceride levels and alkaline phosphatase activity in both sexes compared to controls. Investigative, analytical studies confirmed that an apparent bilirubinaemia was due to test material or metabolite in serum interfering with the routine analytical methodology. Among the relative organ weights, the increased liver and kidney weights were particularly noticeable. No histopathological correlation was found at this dose level either. The changes in the clinical chemistry parameters described were probably due to the reduced feed and drinking water consumption and the resulting depression of body-weight gain. One female rat receiving 9000 ppm died after 11 days of treatment. Pathological investigations revealed heart and lung lesions which were not considered to be treatment-related. The NOAEL was 1000 ppm, equal to 100 mg/kg bw/day, with the liver and kidneys being regarded as possible target organs (Kuhborth *et al.*, 1986c).

Groups of 10 Wistar rats/sex were administered 0, 30, 100, 300, 900 or 2700 ppm of cycloxydim as the sodium salt (purity 94.8%) in the drinking water for 13 weeks. A further 10 animals per sex were added to the control group and the groups receiving 900 or 2700 ppm and were maintained for a 6-week withdrawal period after completion of treatment. The animals tolerated 30, 100 and 300 ppm without any substance-induced effect. Signs of slight toxicity were observed at 900 ppm. In the clinical chemistry examinations, the creatinine values were increased in the females and plasma ALAT activities were increased in both sexes. Plasma ALP activity was reduced in the males. Body-weight and feed consumption were unaffected, while the drinking water consumption of the females was reduced by a maximum of 17%. At the highest dose level of 2700 ppm a reduction in feed consumption (11%) and drinking water consumption (a maximum of 35%) were recorded. The body-weight of the males was reduced by 8% compared with the control group. The creatinine, urea and cholesterol values of the females and the plasma ALAT activity of both sexes were increased, while the activity of alkaline phosphatase in the plasma was reduced. There were no treatment-related changes in organ weight, gross-pathology or histopathological findings at any dose level. All changes described showed a tendency to reversibility during the 6-week

withdrawal period. The NOAEL was 300 ppm, equal to 25 mg/kg bw/day (Kuhborth *et al.*, 1986d).

Dogs

In a range-finding study, 2 beagle dogs per sex and dose were given 0, 300, 1200, 3600 or 10 800 ppm as the sodium salt of cycloxydim (purity 94.8%) in their feed for 4 weeks (equal to doses of 0, 10, 40, 120 or 360 mg/kg bw/day). At the highest dose level, the feed consumption of the females was occasionally reduced, while body-weight remained unaffected. The plasma ALP activity and the plasma cholinesterase activity were increased in the males. On several occasions of the red blood count were marginally reduced. The relative liver weight was increased compared with the untreated control and histopathological examination showed enlargement of hepatocytes. The findings were less pronounced at a dose level of 120 mg/kg bw/day. The only treatment-related findings at this dose were increased plasma ALP in the males and a tendency to an increased relative liver weight. The NOAEL in this study was 40 mg/kg bw/day (Hellwig *et al.*, 1985).

Groups of 4 beagle dogs/sex/dose were used for a subchronic feeding study in dogs over 13 weeks using doses of 0, 60, 300, 1500 or 7500 ppm as the sodium salt (purity 94.8%). No findings related to the test substance administered were obtained at the 3 lowest dose levels but toxicity was pronounced at 7500 ppm. However, there were no clinical changes and body-weight gain remained unaffected by treatment. The clinical chemistry examination revealed increased serum activities of ALP and a reduced albumin concentration. The females had increased globulin values in the serum, while the sodium level in the blood was reduced in the males. Among the haemato-logical changes, the reduced erythrocyte counts and presence of Heinz bodies were of note. However, the number of reticulocytes and platelets was increased suggesting a compensatory reaction in the bone marrow. In the males, the MCH and MCV were also increased. Of the organ weights, the absolute and relative liver weights were above those of the untreated control in both sexes. The bile was reddish and histopathological examination revealed an enlargement of hepatocytes. The NOAEL was 1500 ppm, equal to 50 mg/kg bw/day (Hellwig *et al.*, 1986).

In a 12-month feeding study, groups of 6 beagle dogs/sex were given doses of 0, 400, 1600 or 6400 ppm cycloxydim (acid) as sodium salt (purity 93.9%). The lowest dose of 400 ppm did not lead to any substance-induced changes. At 1600 ppm, the number of Heinz bodies was increased in both sexes, indicating a disturbance in the haemoglobin metabolism. Male animals showed an increased activity of ALP in plasma and a lowering of the albumin level. The absolute and relative liver weights of these animals were significantly increased, but no corresponding histopathological changes were observed. This finding was also detected in both sexes of the highest dose group and some further clinical chemistry parameters were also changed. There was an increase in plasma ALP and a lowering of the albumin and protein concentrations (in the males only). The

haematological examinations showed signs of slight anaemia with a compensatory bone marrow reaction. These findings were manifest in the females in the form of haemosiderosis of Kupffer's cells in the liver as a histopathological correlation. The NOAEL was 400 ppm in the diet, equal to 20 mg/kg bw/day (Hellwig *et al.*, 1988a).

Long-term toxicity/carcinogenicity studies

Mice

In a 24-month study in B₆C₃F₁ mice, cycloxydim was administered as the sodium salt (purity 93.9%) in the drinking water at doses of 0, 10, 20, 60 or 240 ppm to groups of 50 mice/sex (controls 100 mice/sex). There were no clinical findings which were induced by the test substance. Body-weight gain, food and water intake and the incidence of mortality remained unaffected by treatment. The histopathological examination at the end of the study did not reveal any changes related to the test substance. The tumours that occurred corresponded to those of the spontaneous range of the animal strain used so that no carcinogenic potential of the test substance was apparent, up to a dose level of 240 ppm, equal to 32 mg/kg bw/day (Kuhborth *et al.*, 1988c).

Rats

In a study to determine chronic toxicity, groups of 20 Wistar rats per sex/dose were administered 0, 100, 400, 1600 or 2700 ppm of cycloxydim as sodium salt (purity 93.9%) via the drinking water for 18 months. At the highest dose of 2700 ppm, reduced food and drinking water consumption were observed in both sexes together with a decrease in body-weight gain of up to 21% compared with the control during the major part of the study period. Doses of 400 and 1600 ppm also led to reduced body-weight gain compared with the control, but drinking water consumption and feed consumption (males only) were reduced only at 1600 ppm. In the clinical chemistry examinations, a lowering of the triglyceride level was observed at doses of 400 ppm and above. The organ weight determinations and the gross pathological and histopathological examination did not reveal any treatment-related findings. Therefore, the NOAEL in this study was 100 ppm, equal to 7 mg/kg bw/day (Kuhborth *et al.*, 1988a).

In a second study carried out simultaneously to clarify possible carcinogenicity, groups of 50 Wistar rats/sex were given cycloxydim as the sodium salt (purity 93.9%) in the drinking water for 24 months at doses of 0, 100, 400 or 1600 ppm. As in the 18-month study, a reduction in the drinking water consumption and body-weight by up to 18% was observed in both sexes at 1600 ppm, but feed consumption was not impaired in this study. The clinical chemistry examinations revealed a lowering of the triglyceride level in the females at 1600 ppm. The body-weight reduction (both sexes) and lowering of the triglyceride level (females) were also observed at a dose level of 400 ppm. The determination of

organ weights at the end of the study showed a lowering of the absolute and relative liver weights at the high-dose, but there was no corresponding morphological change. No findings related to the test substance administered were obtained in the gross pathological or histopathological examinations. There was no indication of any disturbance of the spontaneous tumour profile of the rat strain used. Under the given test conditions, cycloxydim has no carcinogenic potential in Wistar rats. The NOAEL with regard to clinical or clinical chemistry parameters and organ weight changes was 100 ppm, the same as that of the 18-month toxicity study (Kuhborth *et al.*, 1988b).

Reproduction study

Rats

Investigations of reproductive toxicity were carried out in Wistar rats in a multi-generation study in which 2 litters were used in the first generation and 1 litter in the second generation. Groups of 24 animals per sex were administered cycloxydim as the sodium salt (purity 93.9%) via the drinking water at doses of 0, 100, 400 or 1600 ppm (expressed as acid) for a period of 70 days prior to mating and then throughout gestation and lactation.

The highest dose of 1600 ppm, equal to 150 mg/kg bw/day caused systemic toxicity in the parental animals (reduced food and drinking water consumption and retarded body-weight gain). The number of all pups (live and stillborn) at parturition was slightly reduced and the development of the pups was retarded. Pup mortality was also increased at this dose level. At the mid-dose (400 ppm), there were only indications of systemic toxicity in the parental animals in the form of reduced food and drinking water consumption as well as temporary retardation of body-weight gain. There was no effect on the number of pups or on pup mortality at this dose. Accordingly, the NOAEL was 100 ppm, equal to 10 mg/kg bw/day for parental toxicity and 400 ppm, equal to 38 mg/kg bw/day for reproductive performance (Hellwig *et al.*, 1988b).

Special studies on embryo/fetotoxicity

Rats

Cycloxydim as the sodium salt (purity 93.9%) was administered to 23 or 24 pregnant rats per test group by gavage from days 6 to 15 after mating at doses of 0, 100, 200 or 400 mg/kg bw/day. On day 20 of gestation the dams were sacrificed, and the fetuses were examined after caesarean section. At 100 and 200 mg/kg bw/day no signs of maternal toxicity were observed but body-weight gain and food consumption were reduced at the highest dose level at the beginning of test substance administration compared with the untreated control. The number of implantations and resorptions and the fertility rate were similar to those of the control at every dose level. Fetotoxic effects were observed in the range of

maternal toxicity; they were characterized by reduced fetal body-weight and retardation of ossification of the skeletons of the fetuses. Furthermore, an increased incidence of vertebral column changes in the fetuses, which were classified as anomalies, was detected at the highest dose level. These were mainly changes in thoracic vertebrae, which had a dumbbell-shaped or bipartite appearance. One out of 309 fetuses at 200 mg/kg bw/day and 1/295 fetuses at 400 mg/kg bw/day had anal/tail defects (Hellwig & Hildebrand, 1987a).

In a second study, maternal toxicity after administration of the sodium salt (purity 93.9%) was investigated in more detail. Groups of 25 female Wistar rats were administered cycloxydim at doses of 0, 200, 400, 600 or 800 mg/kg bw/day by gavage from days 6 to 15 of gestation. The dams were sacrificed on day 20 of gestation. After caesarean section the fetuses were evaluated only to a limited extent (weight and sex determinations and gross-pathological examination) since the main aim of the study was to determine the exact maternal toxicity threshold. Pronounced maternal toxicity which, *inter alia*, was manifest in the form of clearly retarded body-weight gain together with reduced food consumption was observed at a dose level of 800 mg/kg bw/day. The general state of health was poor, 5 dams showing vaginal haemorrhages. RBC parameters (haemoglobin content, erythrocyte count, haematocrit) were reduced, and the number of reticulocytes was increased compared with the untreated control indicating a compensatory reaction of the bone marrow. These findings were also obtained at 600 and 400 mg/kg bw/day although they were less pronounced. The NOAEL regarding maternal toxicity was 200 mg/kg bw/day, confirming the result of the first study. Fetal weight was reduced at 600 and 800 mg/kg bw/day and the gross-pathological examination revealed missing or incomplete tail in some cases together with anal atresia in 3 of 270 fetuses (in 3 out of 22 litters) at 800 mg/kg bw/day and in 2 of 296 fetuses (in 2 out of 24 litters) at 600 mg/kg bw/day (Hellwig *et al.*, 1987).

A third study in Wistar rats was carried out to investigate whether the changes in the vertebrae which occurred in the first prenatal study also persisted postnatally. Two groups, each of 60 pregnant females, were given cycloxydim (as the sodium salt, purity 93.9%) by daily oral gavage, between days 6 and 15 of pregnancy at doses of 0 or 400 mg/kg bw/day. Twenty-five dams from each group were sacrificed on day 20 of pregnancy and their fetuses were examined after caesarean section. The remaining dams were allowed to litter and rear their pups. The litters of a further 10 dams from each group were sacrificed on day 7 post-partum and all other litters were sacrificed on day 21 after parturition and examined in detail. In the dams sacrificed on day 20 of gestation there were indications of the beginning of maternal toxicity characterised by retarded body-weight gain and reduced feed consumption. The weight of the fetuses was reduced. Four fetuses (out of 286) from 3/22 litters had tail defects. The incidence of retarded ossification of the skeletons was clearly higher compared with the untreated control. As in the first prenatal toxicity study dumbbell-shaped or bipartite thoracic vertebrae occurred to an increased extent compared with the control. The incidence of the changes in the thoracic vertebrae of the pups

sacrificed 7 or 21 days after birth was clearly reduced compared with the prenatal investigations although these findings, which also occur spontaneously without relation to the test substance, were not completely reversible either in the untreated control or in the animals treated. Furthermore, perinatal mortality was slightly increased at 400 mg/kg bw/day compared to controls. This finding was due to an increased number of dead pups on the day of birth whereas the survival index of the pups sacrificed 7 days after birth remained unaffected (Hellwig & Hildebrand, 1987b).

In addition to the *in vivo* studies, an *in vitro* study was carried out with rat embryos (whole embryo culture technique). Cycloxydim (acid) and the main metabolite (TSO) were investigated in an experiment in which embryos of Wistar rats which were 9.5 days old were incubated in the culture medium with the test substance or solvent control (DMSO) for 48 h. The concentrations were 300 µg/ml for cycloxydim and 150 µg/ml for the TSO metabolite. Concentrations were based on results of *in vivo* teratogenicity studies in the rat and on investigations into the kinetics and metabolism in the same animal species. After incubation, the embryos were evaluated under a stereo-microscope and subsequently examined histopathologically for possible substance-induced changes. No abnormal morphological development was induced either by cycloxydim or by its TSO metabolite in the embryos under the test conditions chosen. These results clearly demonstrate that a direct embryotoxic effect was not seen *in vitro* at this very sensitive development stage of the main organs (Neubert *et al.*, 1987)

Rabbits

Groups of 14 or 15 pregnant Himalayan rabbits were administered cycloxydim as the sodium salt (purity 93.9%) by daily gavage from days 6 to 18 after artificial insemination at doses of 0, 100, 200 or 400 mg/kg bw/day. On day 29 of gestation, the dams were sacrificed and the fetuses were examined in detail after caesarean section. At a dose of 400 mg/kg bw/day, the dams showed a retardation of body-weight gain together with reduced food consumption primarily during the treatment period compared with the untreated control group. This finding was also observed after administration of 200 mg/kg bw/day although it was less pronounced. No signs of maternal toxicity were observed at 100 mg/kg bw/day. There were no indications of an embryotoxic effect of the test substance in the fetuses, except at 400 mg/kg bw/day, where there was a significant reduction in percentage live fetuses and an increase in percentage dead implants. The number of implantations and resorptions, the conception rate and the implantation loss were otherwise unaffected by treatment. Minor inter-group differences remained within the historical control range. The variations, anomalies and retardations that occurred were in the range of historical control data for all test groups (Merkle & Hildebrand, 1985).

In addition to routine X-ray examination, the skeletons of the control and highest dose group were additionally stained to look for abnormalities of the

vertebrae. These investigations did not indicate any substance-induced changes of the vertebrae, as had occurred in rats at a similar dose level (Hellwig, 1986).

Special studies on genotoxicity

A number of genotoxicity tests have been carried out with cycloxydim. The results are summarised in Table 2 (*in vitro*) and Table 3 (*in vivo*).

Special studies on skin and eye irritation and sensitization

The skin irritation potential of cycloxydim (acid) was investigated in rabbits (White Vienna). About 0.5 ml of undiluted test substance was applied to the clipped, intact dorsal skin of 3 animals per sex for 4 h under a semi-occlusive dressing. Very slight erythema in 4/6 animals, within 1 hour of patch removal, which was completely reversible within 2 days, was the only finding that was obtained (Kirsch & Kieczka, 1984c).

The eye irritation potential of cycloxydim (acid) was investigated in rabbits (White Vienna). 0.1 ml of the undiluted test substance was applied to the eyes of 3 male and 3 female animals. Slight conjunctival irritation (redness with lacrimation), which was seen in all animals one hour after treatment, and which was completely reversible within 8 days, was the only finding that was observed. Neither cornea nor iris showed any changes related to the test substance administered (Kirsch & Kieczka, 1984d).

The sensitizing potential of the active ingredient was investigated in guinea-pigs (Pirbright White) in a maximization test in which 10 animals were used in each of the two control groups and 20 animals in the test group. The two challenges carried out after intradermal induction with the test substance did not reveal any skin changes in the animals of the test group indicating that there was no sensitizing potential of cycloxydim under the test conditions chosen (Kirsch & Kieczka, 1985c).

Observations in humans

No information was available.

Table 2: Results of *in vitro* genotoxicity assays on cycloxydim

Test system	Test object	Concentration	Purity	Results	Reference
Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	20-15000 µg/plate (Na salt)	93.9%	Negative	Gelbke & Engelhardt (1985a)
Ames test	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA 1538	20-5000 µg/plate (acid)	NK	Negative	Gelbke & Engelhardt (1984)
Lymphoma forward mutation assay	Mouse L5178Y cells	1.75-20 µg/ml (Na salt)	93.9%	Weak, positive at cytotoxic concentrations	den Boer & Hoom (1985a)
HGPRT forward mutation assay	CHO cells	5-40 mg/ml (Na salt)	93.9%	Negative	Gelbke & Engelhardt (1986)
HGPRT forward mutation assay	CHO cells	0.215-21.5mg/ml (Na salt)	93.9%	Negative	den Boer & Hoom (1985b)
Cytogenetics mutation assay	CHO cells	500-5000 µg/ml (acid)	NK	Weak, positive in absence of activation	Taalman & Hoom (1985a)
Cytogenetics mutation assay	CHO cells	2000-5000 µg/ml (Na salt)	NK	Weak, positive in absence of activation	Taalman & Hoom (1985b)

Table 2 (cont'd)

Test system	Test object	Concentration	Purity	Results	Reference
UDS	Rat hepatocytes	100-2000 $\mu\text{g/ml}$ (acid)	NK	Negative	Cifone & Myhr (1985)
UDS	Rat hepatocytes	0.9-90.6 $\mu\text{g/ml}$ (Na salt)	NK	Negative	Cifone & Brusick (1985)

NK: not known

Table 3: Results of *in vivo* genotoxicity assays on cycloxydim

Test system	Test object	Dose	Purity	Results	Reference
Micronucleus test	NMRI mice	225, 450, 900 mg/kg bw	93.9%	Negative	Gelbke & Engelhardt (1985b)
Micronucleus test	Chinese hamsters	500, 1700, 5000 mg/kg bw	NK	Negative	Taalman & Hoorn (1987)

NK: not known

COMMENTS

Cycloxydim was extensively absorbed after oral administration to rats and almost completely excreted within 5 days of dosing. Elimination proceeded predominantly via the urine (74-86% of applied dose), with lower levels in the faeces (12-25% of the applied dose). Less than 1% of an administered dose was retained in the body. Studies confirmed that enterohepatic circulation occurred in rats.

The metabolism of cycloxydim has been investigated in rats and a biotransformation pathway has been proposed. The major metabolite was the sulfoxide, and the pattern of metabolites was similar in urine, bile and tissue residues.

Cycloxydim has low acute oral toxicity. WHO has classified cycloxydim as unlikely to present acute hazard in normal use (WHO, 1992).

Owing to instability in dietary admixture the sodium salt of cycloxydim was administered in the drinking-water in repeat-dose rodent studies. In dogs, sufficient dietary stability was demonstrated, and dietary administration of cycloxydim was therefore used.

In mice, two 4-week dose range-finding studies were conducted, followed by a 24-month long-term study. In the dose range-finding studies, employing concentrations between 30 and 9000 ppm, reduced food and water intake, and reduced body-weight gain were seen at higher doses, together with increased liver weight and hydropic vacuolar hepatocyte degeneration. The NOAEL was equal to 7 mg/kg bw/day in males and 28 mg/kg bw/day in females, the lower NOAEL in males resulting from liver weight increase in the absence of any histopathological change. In the long-term study, no treatment-related effects were seen, up to the highest dose tested of 32 mg/kg bw/day, although liver weights were not measured.

In rats a 4-week dose range-finding study was followed by a 13-week study, an 18-month toxicity study and a 24-month carcinogenicity study. Findings were generally similar to those observed in mice, the liver being identified as the only target organ of note, although no histopathological changes were seen at doses up to 900 mg/kg bw/day. The NOAELs were 100 mg/kg bw/day over 4 weeks, 25 mg/kg bw/day over 13 weeks and 7 mg/kg bw/day over 18/24 months, based on reduced body-weight gain at 25 mg/kg bw/day and above.

Cycloxydim was not carcinogenic in rats or mice.

In dogs, a 4-week dose range-finding study was followed by a 13-week and a 12-month study. In the dose range-finding study (at doses up to 360 mg/kg bw/day) the liver and the red blood cells were identified as target organs and the

NOAEL was 40 mg/kg bw/day. In the longer-term dog studies, the NOAELs were 50 mg/kg bw/day over 13 weeks and 20 mg/kg bw/day over 52 weeks. Target organs were the liver and red blood cells. A marginal anaemia was seen, along with a compensatory bone marrow response, but no serious treatment-related histopathological effects were noted at 80 or 300 mg/kg bw/day.

In a multi-generation reproduction study in rats, the NOAELs were about 10 mg/kg bw/day for parental toxicity and 38 mg/kg bw/day for reproductive performance, pup mortality being slightly increased at 150 mg/kg bw/day.

Teratogenicity studies have been carried out with cycloxydim in rats and rabbits. In the rat teratogenicity studies the NOAEL for maternal toxicity was 200 mg/kg bw/day. Fetotoxicity was seen at maternally toxic doses, together with findings which may be considered indicative of a teratogenic potential (missing/incomplete tail and anal atresia at 600 and 800 mg/kg bw/day and vertebral anomalies at 400 mg/kg bw/day). Although the vertebral anomalies were not completely reversible post-natally (up to 21 days after birth), *in vitro* embryo culture studies demonstrated that cycloxydim did not show any direct embryotoxic effects. In the rabbit teratogenicity study the NOAEL for maternal toxicity was 100 mg/kg bw/day. There was no indication of fetotoxicity in rabbits, even in the presence of maternal toxicity and no indication of any treatment-related vertebral anomalies, even when a special examination was conducted to look specifically for such changes.

After reviewing the available genotoxicity data, the Meeting concluded that cycloxydim and its sodium salt were not genotoxic.

An ADI was allocated based upon the NOAEL from the long-term study in rats, using a safety factor of 100.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effect

- Mouse: > 240 ppm in the drinking water, equal to > 32 mg/kg bw/day (two-year study)
- Rat: 100 ppm in the drinking-water, equal to 7 mg/kg bw/day (18- and 24-month studies)
- 100 ppm in the drinking-water, equal to 10 mg/kg bw/day (multi-generation study)
- 200 mg/kg bw/day (teratogenicity study, maternal and fetal toxicity)

Dog: 400 ppm in the diet, equal to 20 mg/kg bw/day (one-year study)

Rabbit: 100 mg/kg bw/day (teratogenicity study, maternal toxicity).

Estimate of acceptable daily intake for humans:

0-0.07 mg/kg bw

Studies which will provide information valuable in the continued evaluation of the compound

Observations in humans.

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