CYCLOXYDIM

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Explanation 203
Evaluation for acceptable daily intake 204
1. Biochemical aspects 204
1.1 Absorption, distribution, metabolism and excretion 204
1.2 Biotransformation 210
2. Toxicological studies 213
2.1 Acute toxicity 213
2.2 Short-term studies of toxicity 213
(a) Oral route 216
(b) Dermal route 232
2.3 Long-term studies of toxicity and carcinogenicity 233
2.4 Genotoxicity 240
2.5 Reproductive toxicity 240
(a) Multigeneration studies 240
(b) Developmental toxicity 247
(c) In vitro studies 255
2.6 Special studies 255
(a) Neurotoxicity studies 255
(b) Studies on metabolites/impurities 255
3. Observations in humans 258
3.1 Medical surveillance on manufacturing plant personnel 258
3.2 Direct observation (e.g. clinical cases and poisoning incidents) 259
4. Literature review 259
Comments 259
Toxicological evaluation 262
References 264

Explanation

Cycloxydim is the International Organization for Standardization (ISO)–approved name for (5*RS*)-2-[(*EZ*)-1-(ethoxyimino)butyl]-3-hydroxy-5-[(3*RS*)-thian-3-yl]cyclohex-2-en-1-one (International Union of Pure and Applied Chemistry [IUPAC]). The Chemical Abstracts Service (CAS) chemical name for cycloxydim is 2-[1-(ethoxyimino)butyl]-3-hydroxy-5-(tetrahydro-2H-thiopyran-3-yl)-

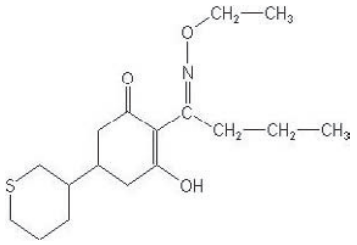
2-cyclohexen-1-one, and the CAS No. is 101205-02-1. Cycloxydim is a cyclohexene oxime herbicide that is used for the control of grass weeds of many agricultural and horticultural broad-leaved crops.

Cycloxydim was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1992, when an acceptable daily intake (ADI) of 0–0.07 mg/kg body weight (bw) was established. Cycloxydim was reviewed by the present Meeting as part of the periodic re-evaluation programme of the Codex Committee on Pesticide Residues. New studies evaluated by the Meeting included studies with repeated percutaneous doses, studies of acute toxicity and genotoxicity with various metabolites, and 28-day and 90-day studies of toxicity in rats given repeated oral doses of metabolites.

Cycloxydim was used in the free acid form in most of the toxicological studies. However, because of chemical instability of the acid in animal feed and because of its low solubility in water, the sodium salt of cycloxydim was used in those studies that required water or feed as vehicle. The name "cycloxydim" refers to the acid form unless otherwise indicated. All the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) guidelines, and certificates of compliance with good laboratory practice (GLP) and quality assurance (QA) were provided.

The chemical structure of cycloxydim is shown in Figure 1.

Figure 1. Chemical structure of cycloxydim



Evaluation for acceptable daily intake

1. Biochemical aspects

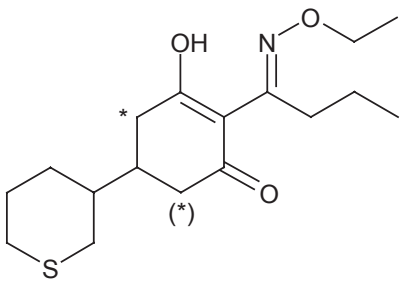
1.1 Absorption, distribution and excretion

Rats

To obtain information on the absorption, distribution and rate and route of excretion of cycloxydim, a study was performed in adult CD rats (body weight ~200 g; aged ~7 weeks [males] and 10 weeks [females]) using [^{14}C]cycloxydim labelled in the 4(6)-position of the cyclohexene ring (Figure 2). The radioactive test substance was sent by BASF to the contract research organization on three different occasions, all with the same batch number. The test substance was purified by the contract research organization ([cyclohexen-^{14}C]cycloxydim [BAS 517H]; batch No. 389211; specific radioactivity 398.9–462.5 MBq/mmol; radiochemical purity >94–100%).

The studies were conducted after oral administration of the free acid and its sodium salt and after intravenous administration of the sodium salt. The free acid was administered at a nominal dose of 10 mg/kg bw (no-effect level) or 300 mg/kg bw (toxic effect level). The sodium salt was administered at a nominal dose equivalent to 10 mg/kg bw as the free acid. In the studies in which excretion via the bile was determined, the animals were anaesthetized, and bile was collected via a catheter for up to 48 h after dosing. Urine and faeces were collected after 24 and 48 h.

Figure 2. Labelling position of the cyclohexene ring



* Denotes position of ^{14}C label

The experiments that were carried out are summarized in Tables 1, 2 and 3.

Table 1. Summary of excretion balance experiments

Parameter	Experimental dose group					
	E0	E1	E2	E3	E4	E5
Purpose	Excretion balance pretest	Excretion balance	Excretion balance	Excretion balance, 14 days unlabelled followed by single labelled dose	Excretion balance	Excretion balance
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	300	10	300	10	10	10
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
No. of animals of each sex	2 males	5 males	5 males	5 males	5 males	5 males
	2 females	5 females	5 females	5 females	5 females	5 females
Duration[a] (h)	120	120	120	120	120	120
Samples	Urine, faeces, carcass, exhaled air	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues	Urine, faeces, tissues

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

Table 2. Summary of biliary excretion experiments

Parameter	Experimental dose group		
	B1	B2	B3
Purpose	Biliary excretion	Biliary excretion	Biliary excretion
Route	Oral	Oral	Oral
Dose (mg/kg bw)	10	300	10
Test substance	Free acid	Free acid	Sodium salt
No. of animals of each sex	3 males	3 males	3 males
	3 females	3 females	3 females
Duration[a] (h)	48	48	48
Samples	Bile, urine, faeces	Bile, urine, faeces	Bile, urine, faeces

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

Table 3. Summary of plasma concentration experiments

Parameter	Experimental dose group					
	P1	P2	P3	P4	P5	P6
Purpose	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics 7 daily doses	Blood/plasma pharmaco-kinetics 7 daily doses	Blood/plasma pharmaco-kinetics	Blood/plasma pharmaco-kinetics
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	10	300	10	300	10	10
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
No. of animals of each sex	5 males 5 females	5 males 5 females	3 males 3 females	3 males 3 females	5 males 5 females	5 males 5 females
Duration[a] (h)	240	240	240	240	240	240
Samples	Blood	Blood	Blood, tissues	Blood	Blood	Blood

From Hawkins et al. (1986)

[a] Maximum time for collection of samples after dosing.

For the excretion balance experiments, the animals were placed in metabolism cages after dosing, and excreta were collected up to 120 h. In the single oral high-dose pretest, the exhaled air was collected in a carbon dioxide trapping system. At the end of the collection period, organs and tissues were prepared for analysis of residual radioactivity.

In the blood/plasma pharmacokinetics studies, blood was withdrawn at the following time points after dosing: 0.25, 0.5, 1, 2, 3, 5, 7, 12 (not group P1), 24, 48, 72, 96, 120, 168 and 240 h. Intravenously dosed rats were additionally bled at 5 min after dosing.

In the tissue distribution study, five male and five female rats were dosed with 10 mg/kg bw of the free acid for 7 days. Pairs of rats (one male and one female) were killed at 5, 12, 24, 72 and 120 h after the final dose. Radioactivity in 18 selected organs/tissues was determined. In addition, five males were treated for 7 days with the free acid and killed at 5, 12, 24, 72 and 120 h after the final dose. These animals were used to prepare whole-body autoradiographs. The excreta, organs and tissues obtained in this study were used for the identification of metabolites.

Urine samples collected during 24 h after the dose from male and female rats from group E2 (single dose 300 mg/kg bw, free acid) were pooled. The samples were adjusted to pH 10 and extracted with dichloromethane. The aqueous fraction was acidified (pH 3) and re-extracted with dichloromethane. Aliquots of the pH 10 and pH 3 extracts and the final aqueous fraction were subjected to thin-layer chromatography (TLC). An aliquot of the pH 3 extract was evaporated and redissolved in the respective high-performance liquid chromatography (HPLC) solvent system. Portions of this solution were subjected to semipreparative HPLC. A single major ultraviolet (UV)-active peak (metabolite I) was detected and further analysed using mass spectrometry. Similarly, aliquots of the pH 10 extract were analysed. Six separate peaks (metabolites II–VII) were collected and subjected to mass spectrometry. A sample of the aqueous fraction was subjected to TLC and HPLC analysis. Urine samples were also subjected to derivatization by means of a peroxidation and methylation reaction.

Bile from a male rat from group B2 (single dose of free acid at 300 mg/kg bw) was incubated overnight with β-glucuronidase. Following incubation, the sample was extracted with dichloromethane at pH 3 and pH 10, as described for urine. The extracts were analysed by TLC using solvent system A. UV detection indicated that the metabolites were present in the pH 10 fraction, and this was subjected to semipreparative HPLC using solvent system Y. Two major radioactive components were

Table 4. Excretion balance after oral administration of [cyclohexene-^{14}C]cycloxydim (free acid) to male and female rats

	Amount excreted (% of administered dose)					
	Dose (mg/kg bw)					
	10		300		10[a]	
	Male	Female	Male	Female	Male	Female
Urine						
- 0–8 h	39.86	28.44	24.06	15.09	30.49	24.85
- 8–24 h	38.94	42.21	52.66	51.61	38.63	39.60
- 24–48 h	3.80	4.87	5.77	13.27	4.06	4.53
- 48–120 h	1.42	2.03	2.35	3.44	2.87	3.14
Cage wash	0.40	0.37	0.63	0.61	0.52	0.70
Subtotal urine and cage wash	84.42	77.91	85.46	84.03	76.57	72.81
Faeces						
- 0–24 h	7.62	9.69	5.97	6.42	13.95	12.07
- 24–48 h	4.76	5.90	4.18	7.26	4.07	5.33
- 48–72 h	0.95	1.85	0.86	1.70	0.74	1.90
- 72–120 h	0.59	0.90	1.07	0.63	0.45	0.79
Subtotal faeces	13.92	18.33	12.06	16.02	19.21	20.10
Gut content	0.08	0.09	0.14	0.11	0.08	0.20
Tissues and carcass	0.31	0.45	0.40	0.59	0.37	0.75
Recovery	98.73	96.78	98.06	100.8	96.23	93.86

From Hawkins et al. (1986)

[a] 14 days of dosing with non-radiolabelled test substance followed by single oral ^{14}C-labelled dose.

designated metabolites VIII and IX. These metabolites were isolated by HPLC as described for urine and submitted to mass spectrometry. Samples of the two isolates were also analysed by TLC in solvent systems A and C, together with extracts of enzyme-treated bile and faeces and reference compounds cycloxydim and BH 517-T2S.

Faeces from male and female rats from group E2 (single dose of free acid at 300 mg/kg bw) and E3 (free acid at 10 mg/kg bw; 14 days non-labelled dose followed by single labelled dose) were used to analyse faecal metabolites. Extracts from male and female animals were analysed separately. For each experiment, faeces collected during 0–24 h and 24–48 h after dosing from each sex were pooled. Aliquots of pooled extracts were then processed, as described for urine, and chromatographed by means of TLC using solvent systems C and D.

Aliquots of liver and kidneys from a male and a female rat of dose group P3 (seven daily doses of free acid at 10 mg/kg bw) were used. Liver and kidney samples were homogenized with methanol. Liver samples were extracted a second time with methanol. Aliquots of the methanol solution were subjected to TLC using solvents A, B and C. Kidney samples were processed and chromatographed as described for the liver using solvent systems A and B. Co-chromatography with reference standards was used for the identification of metabolites.

The results of the excretion balance pretest demonstrated that no radioactivity was eliminated via the expired air. The excretion balance of cycloxydim is shown in Tables 4 and 5.

For the free acid, the results of the investigations indicate that the majority (78–85%) of the administered dose (single high or low) is eliminated via the urine. After a 14-day pretreatment, the

Table 5. Excretion balance after oral and intravenous administration of 10 mg/kg bw of [cyclohexene-^{14}C]cycloxydim (sodium salt) to male and female rats

	Amount excreted (% of administered dose)			
	Oral administration		Intravenous administration	
	Male	Female	Male	Female
Urine				
- 0–8 h	37.36	30.74	47.65	39.61
- 8–24 h	41.60	36.85	17.18	21.68
- 24–48 h	5.03	4.20	2.35	3.70
- 48–120 h	1.58	1.75	1.07	2.23
Cage wash	0.26	0.40	0.30	0.31
Subtotal urine and cage wash	85.83	73.94	68.55	67.53
Faeces				
- 0–24 h	11.76	18.61	12.30	12.18
- 24–48 h	4.59	4.42	3.95	7.76
- 48–72 h	0.90	2.12	1.17	1.94
- 72–120 h	0.42	0.61	0.45	0.72
Subtotal faeces	17.66	25.75	17.87	22.61
Gut content	0.08	0.09	0.06	0.09
Tissues and carcass	0.30	0.49	0.27	0.49
Recovery	103.9	100.3	86.76	90.70

From Hawkins et al. (1986)

amount eliminated via the urine is slightly lower (73–77%). For the cycloxydim sodium salt, the results indicate that the majority (74–86%) of the single oral dose of 10 mg/kg bw is eliminated via the urine, most being excreted within 24 h. A comparison of the excretion balance of the free acid and the sodium salt after oral administration (Table 6) indicates no biologically relevant difference.

The presence of radioactivity in the faeces after intravenous administration (Table 5) indicates that elimination via the bile plays a role. Moreover, the similarities in the excretion balance after oral and intravenous administration of 10 mg/kg bw indicate that both the free acid and the sodium salt of cycloxydim are well absorbed; the bioavailability of the oral dose was approximately 100%.

The results of the bile duct cannulation experiments confirm the notion that biliary excretion plays an important role in the elimination of the test substance. The fact that the amounts of radioactivity found in the faeces in the oral excretion balance studies are far lower than the biliary excretion (Table 6) demonstrates that part of the material excreted via the bile is reabsorbed (enterohepatic circulation) and subsequently eliminated renally.

The pharmacokinetic parameters of the free acid and the sodium salt of cycloxydim at a dose of 10 mg/kg bw were comparable, with the exception that the time to peak concentration in plasma (T_{max}) of the free acid was reached 5 h after oral administration, with peak concentrations (C_{max}) of 5 µg/ml and 6.8 µg/ml for males and females, respectively. In the case of oral administration of the sodium salt, T_{max} was 0.5–1 h, with C_{max} values of 8.4 µg/ml and 9.3 µg/ml for male and female rats, respectively. The C_{max} was similar after single or seven consecutive repeated oral doses of the corresponding concentration of the free acid. The area under the curve (AUC) obtained after the last of seven daily low doses was approximately twice as high as the AUC after a single oral dose, indicating very little accumulation over time at a dose of 10 mg/kg bw. Comparing the AUC obtained after a single low dose with that obtained after a single high dose demonstrates an approximate 60-fold

Table 6. Excretion via the bile after oral administration of [cyclohexene-^{14}C]cycloxydim—free acid and sodium salt—to male and female rats

	Amount excreted (% of administered dose)					
	Free acid				Sodium salt	
	Dose (mg/kg bw)					
	10		300		10	
	Male	Female	Male	Female	Male	Female
Bile						
- 0–24 h	59.89	64.24	50.67	46.28	51.30	63.67
- 24–48 h	1.38	0.98	5.55	8.45	3.5	2.38
Urine						
- 0–24 h	34.28	30.55	31.90	21.03	32.04	32.70
- 0–48 h	36.65	32.09	38.91	23.16	37.24	35.60
Subtotal urine and cage wash (0–48 h)	36.96	32.15	39.20	23.65	37.61	3.78
Faeces						
- 0–24 h	1.99	1.98	0.84	0.95	1.02	1.18
- 24–48 h	2.76	2.42	1.92	1.12	2.06	1.65
Gut content	0.04	0.03	0.13	17.76	0.06	0.04
Tissues and carcass	0.84	0.52	1.36	2.89	0.79	0.56
Recovery	102.0	100.3	98.83	100.2	95.32	104.1

From Hawkins et al. (1986)

Table 7. Pharmacokinetic parameters after oral and intravenous administration of [cyclohexene-^{14}C]cycloxydim—free acid and sodium salt—to male and female rats

Parameter	Experimental dose group					
	P1	P2	P3	P4	P5	P6
Route	Oral	Oral	Oral	Oral	Oral	Intravenous
Dose (mg/kg bw)	10	300	10	300	10	10
No. of doses	Single dose	Single dose	7 daily doses	7 daily doses	Single dose	Single dose
Test substance	Free acid	Free acid	Free acid	Free acid	Sodium salt	Sodium salt
C_{max} (µg/ml)	5.03 (M)	276 (M)	5.5 (M)	239 (M)	8.35 (M)	33.5 (M)
	6.82 (F)	297 (F)	6.8 (F)	263 (F)	9.26 (F)	39.5 (F)
T_{max} (h)	5 (M)	7 (M)	2 (M)	2 (M)	0.5 (M)	5 min (M)
	5 (F)	5 (F)	5 (F)	7 (F)	1.0 (F)	5 min (F)
AUC (µg·h/ml)	69.8 (M)	4731 (M)	130 (M)	6367 (M)	68.1 (M)	63.3 (M)
	111 (F)	5718 (F)	202 (F)	7203 (F)	108 (F)	97.0 (F)

From Hawkins et al. (1986)
F, female; M, male

increase. As the difference between the low and high dose levels was only 30-fold, these data indicate a more than proportional increase of the AUC with dose. The AUC after an intravenous administration of 10 mg/kg bw was nearly identical to the AUC after an oral dose of 10 mg/kg bw, indicating that all of the orally administered radioactivity was absorbed (Table 7).

Table 8. Tissue distribution and residual radioactivity in organs and tissues after seven daily oral treatments of rats with [cyclohexene-^{14}C]cycloxydim free acid at 10 mg/kg bw

Organ/tissue	Concentration (µg/g tissue)					
	Males			Females		
	5 h	24 h	72 h	5 h	24 h	72 h
Liver	8.86	1.19	0.51	15.3	3.17	1.51
Kidney	6.22	1.36	0.48	7.55	2.68	0.85
Thyroid	3.14	1.38	1.48	3.40	1.90	1.73
Intestinal tract	80.0	5.34	1.19	114	22.0	1.73
Adrenal gland	1.85	0.24	<0.15	2.96	0.75	0.28
Lungs	2.08	0.38	0.13	3.35	0.73	0.36
Plasma	4.79	0.70	0.18	7.57	1.44	0.54

From Hawkins et al. (1986)

The amounts of radioactivity found in the organs after seven daily treatments with 10 mg/kg bw in the tissue distribution study are shown in Table 8.

The results of the tissue distribution study indicated that after the last of seven daily doses, radioactivity in the organs rapidly declined over time. At 72 h after the last dose, radioactivity was less than 1 µg/g tissue for almost all organs. The highest amounts of radioactivity were found in the liver and the kidneys (i.e. those organs involved in the excretion of the test substance). Compared with the plasma level, concentrations in the other organs were equal or lower. There was no evidence for bioaccumulation of the test substance.

The results of the whole-body autoradiography confirmed the findings of the tissue distribution studies. Highest amounts of radioactivity were detected in the intestinal tract, liver, kidneys, blood and lungs. There were no organs with unusually high levels of radioactivity.

The study described in this report was conducted in compliance with GLP of the United States Environmental Protection Agency (USEPA), OECD and Japan. A QA statement was attached (Hawkins et al., 1986).

1.2 Biotransformation

The result of the above-described study was used for elucidation of metabolites. The urine samples, faecal extracts, bile and organs were analysed. The analysis indicated that several metabolites were formed. The majority of them could be isolated and identified spectrometrically. As far as possible, the proposed structures for the metabolites were corroborated by comparison with synthetic standards.

Patterns of metabolites in the urine were qualitatively and quantitatively similar after administration of 10 mg/kg bw of either the free acid (Table 9) or the sodium salt (Table 10). At a dose of 300 mg/kg bw (free acid), the qualitative distribution of urinary metabolites was again similar; quantitatively, however, the data indicated that the test substance was less extensively metabolized. The major metabolite in all cases was the sulfoxide of cycloxydim (BH 517-TSO). The next most important metabolite was BH 517-T1SO, resulting from *N*-de-ethoxylation of BH 517-TSO. Other less important metabolites in the urine were BH 517-T1SO$_2$ (sulfone of BH 517-T1SO), BH 517-T2SO (Beckmann rearranged product of the parent sulfoxide) and unchanged parent (cycloxydim). The presence of minor metabolites hydroxylated at the 5-position of the cyclohexene ring of the parent was indicated after oxidation and methylation of a urine sample. Patterns of metabolites in the bile were similar after the administration of either the free acid or the sodium salt of cycloxydim

Table 9. Summary of identified metabolites in urine after single low, single high and multiple low dose administration of [^{14}C]cycloxydim free acid

Metabolite identity	Radioactivity (% of administered dose)					
	Dose group E1 (10 mg/kg bw single oral)		Dose group E2 (300 mg/kg bw single oral)		Dose group E3[a] (10 mg/kg bw multiple oral)	
	Males	Females	Males	Females	Males	Females
Polars	7.5	10.1	3.5	3.4	3.4	8.2
Unknown	1.6	1.9	0.7	0.7	0.7	2.3
A	3.9	4.1	2.4	1.6	2.1	4.7
B	6.6	8.4	3.1	2.9	2.5	B & C combined: 9.3
C	5.1	3.8	2.9	2.5	1.5	
BH 517-T1SO	12.9	9.9	6.5	5.5	5.2	7.7
D	3.1	2.2	4.5	3.8	2.9	2.4
BH 517-T2SO	7.4	5.5	5.1	4.5	3.6	3.8
BH 517-TSO, BH 517-T1SO$_2$	29.9	23.6	46.7	40.5	43.8	24.1
BH 517-TSO$_2$				0.4	1.2	
Cycloxydim	Combined: 0.8	Combined: 1.1	Combined: 1.3	0.5	0.7	Combined: 2.0
Unknown				0.2	0.1	

From Hawkins et al. (1986)

[a] Fourteen non-radiolabelled doses followed by a single ^{14}C-labelled dose.

Table 10. Summary of identified metabolites in urine after single oral and intravenous low dose administration of [^{14}C]cycloxydim sodium salt

Metabolite identity	Radioactivity (% of administered dose)			
	Dose group E4 (10 mg/kg bw single oral)		Dose group E5 (10 mg/kg bw single intravenous)	
	Males	Females	Males	Females
Polars	6.5	10.0	5.7	11.2
Unknown	1.6	3.0	1.2	2.8
A	3.9	4.9	3.9	4.2
B	5.4	5.9	4.5	5.7
C	5.6	4.3	3.7	3.3
BH 517-T1SO	10.5	9.8	8.4	8.1
D	3.6	2.1	2.4	1.6
BH 517-T2SO	6.9	4.5	4.5	5.2
BH 517-TSO, BH 517-T1SO$_2$	33.7	21.4	28.9	18.4
BH 517-TSO$_2$	0.7		0.5	
Cycloxydim	0.1	Combined: 1.6	0.3	Combined: 0.9
Unknown	0.5		0.4	

From Hawkins et al. (1986)

Table 11. Summary of identified metabolites in bile after single oral dose administration of [^{14}C]cycloxydim free acid or sodium salt

Metabolite identity	Radioactivity (% of administered dose)							
	Dose group B (10 mg/kg bw single oral – free acid)				Dose group B (10 mg/kg bw single oral – sodium salt)			
	Males		Females		Males		Females	
	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated	Non-enzyme treated	Enzyme treated
Polars	47.8	5.1	57.4	8.2	51.1	8.5	60.9	10.7
Unknown	1.3	0.7	2.9	0.8	1.3	0.4	1.6	0.5
A	1.7	1.3	2.0	1.1	1.8	1.0	2.3	1.1
B	2.0	0.9	2.2	1.6	1.9	0.5	2.1	1.1
C	3.1	1.3	1.2	1.1	2.2	1.4	2.1	0.9
BH 517-T1SO	1.7	1.0	1.3	1.4	1.4	0.9	1.8	0.7
D	10.6	5.7	7.6	3.8	9.3	6.6	6.1	1.9
BH 517-TSO, BH 517-T1SO$_2$	Combined: 5.4	Combined: 6.9	Combined: 3.5	Combined: 11.5	Combined: 14.5	Combined: 34.3	Combined: 7.5	Combined: 22.9
BH 517-T2SO + E	Combined: 20.3	Combined: 50.2	Combined: 14.2	Combined: 39.2	7.0	13.7	4.6	11.4
E	—	—	—	—	2.3	2.9	3.0	1.0
Unknown	2.7	1.9	4.1	2.3	2.1	—	3.2	1.4
BH 517-T2S	2.7	7.7	3.0	9.3	3.0	10.8	4.0	18.2
Cycloxydim	0.6	17.3	0.8	19.6	2.0	18.8	0.8	28.1
Unknown	0.1	0.1	0.1	0.1	—	0.1	—	0.1

From Hawkins et al. (1986)

(Table 11). Following enzymatic hydrolysis, the major metabolites in the bile corresponded to BH 517-TSO (including BH 517-T1SO$_2$), unchanged parent and BH 517-T2S (*N*-de-ethoxylated and rearranged product of the parent).

Methanol extracts of the liver contained two major components, which corresponded to parent and BH 517-TSO. The pattern of metabolites in the kidneys was similar to that in the urine (Table 12).

The pattern of metabolites in the urine was similar for the free acid and the sodium salt of cycloxydim, and AUC data indicated that elimination was saturable at higher doses. The major metabolite in the urine and bile was the sulfoxide of cycloxydim, BH 517-TSO. Additional metabolites identified were BH 517-T1SO (derived from *N*-de-ethoxylation of BH 517-TSO), BH 517-T1SO$_2$ and BH 517-T2SO. Only small amounts of unchanged parent compound were detected in the urine.

In the faeces, a total of nine different radioactive fractions were detected in the single oral high and low dose groups (free acid). Unchanged parent accounted for 0.7% (males) to 0.9% (females) of the dose in the high dose group and 0.8% (males) to 0.5% (females) in the low dose group. The values for polars were 1.6% (males) to 2.2% (females) (300 mg/kg bw) and 2.2% (males) to 3.2% (females) (10 mg/kg bw). A further component designated "F" accounted for 1.4% (males) to 2.3% (females) (300 mg/kg bw) and 3.3% (males) to 3.7% (females) (10 mg/kg bw). The other components were not designated or identified, but each accounted for less than 1% of the dose.

The metabolic pathway of cycloxydim is shown in Figure 3.

Table 12. Summary of identified metabolites in liver and kidney after seven daily oral low dose administrations of [^{14}C]cycloxydim free acid to rats[a]

Metabolite identity	% of radioactivity in specified organ		
	Liver: 10 mg/kg bw – seven daily doses		Kidney: 10 mg/kg bw – seven daily doses
	Males	Females	Pooled males/females
Polars	7.3	6.4	8.2
Unknown (roi2)	3.6	5.8	3.6
B, C	—	—	7.2
BH 517-T1SO	3.6	5.1	6.1
D	6.0	5.2	5.3
BH 517-TSO	30.2	26.6	—
BH 517-T2SO	—	—	8.8
BH 517-TSO, BH 517-T1SO$_2$	—	—	44.3
Unknown (roi 6)	2.3	1.2	—
G	7.9	5.6	—
Unknown (roi 8)	2.7	1.6	4.0
Cycloxydim	18.6	16.0	3.4
Unknown (roi 10)	<0.01	0.9	0.2

From Hawkins et al. (1986)
roi, region of interest
[a] Liver and kidney components were resolved with TLC solvent A.

The study described in this report was conducted in compliance with GLP of the USEPA, OECD and Japan. A QA statement was attached (Hawkins et al., 1986).

2. Toxicological studies

2.1 Acute toxicity

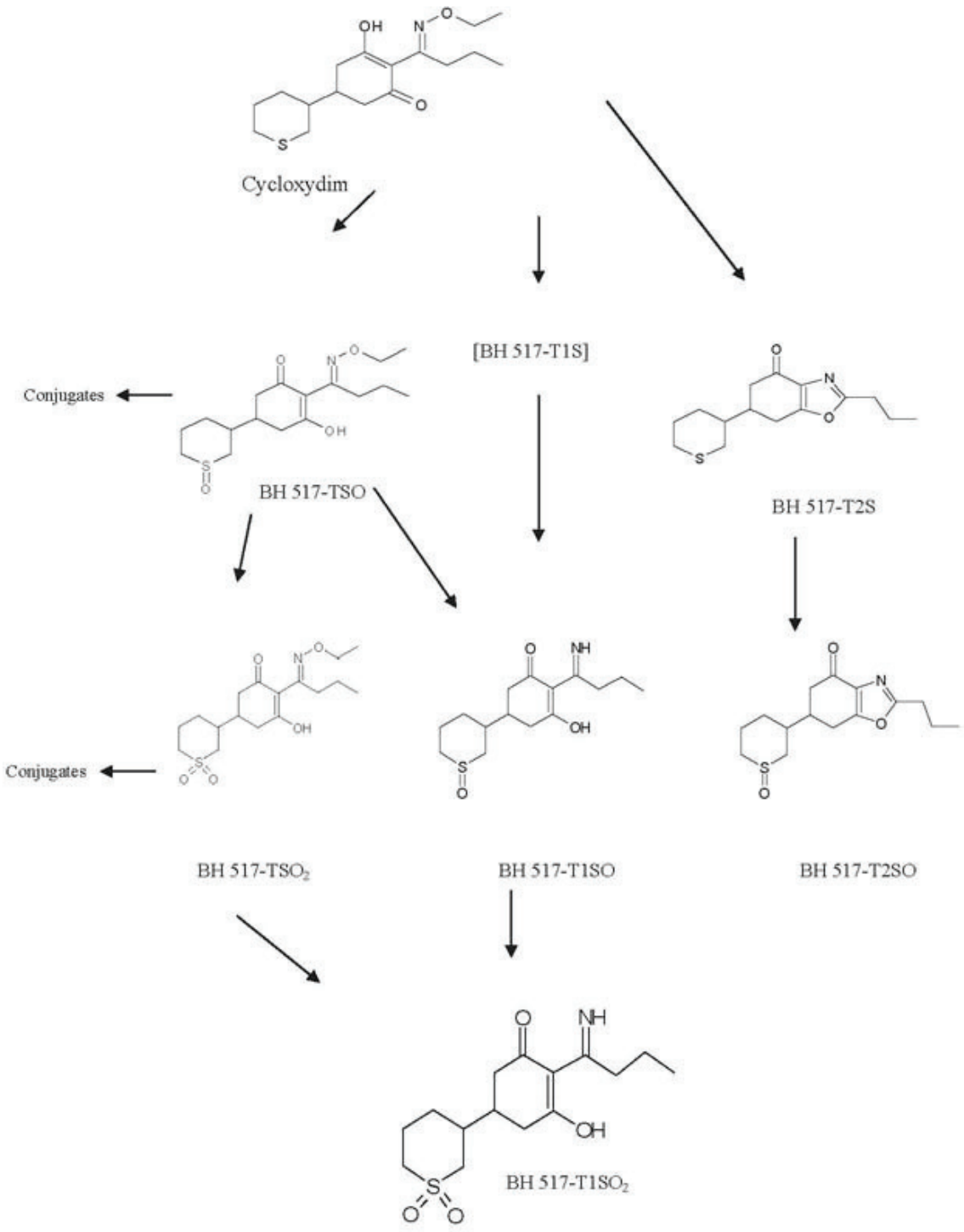
The oral median lethal dose (LD_{50}) of cycloxydim was 3940 mg/kg bw in rats and >5000 mg/kg bw in mice. No specific clinical signs were observed. Macroscopic findings in rats that died after receiving high oral doses by gavage indicated irritation of the gastric mucosa. The dermal LD_{50} in rats was >2000 mg/kg bw, a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When cycloxydim was administered by inhalation, the median lethal concentration (LC_{50}) was >5.28 mg/l of air (4 h exposure). Cycloxydim was not an irritant in a study of skin and eye irritation in rabbits or a skin sensitizer in the guinea-pig maximization test.

The results of the studies on acute toxicity of cycloxydim are presented in Table 13. All the studies were conducted as per OECD guidelines and complied with GLP.

2.2 Short-term studies of toxicity

Short-term studies of oral toxicity in mice, rats and dogs were conducted using cycloxydim sodium salt or cycloxydim free acid. Cycloxydim sodium salt as test substance was applied instead of

Figure 3. The metabolic pathway of cycloxydim



Source: Hawkins et al. (1986)

Table 13. Summary of acute toxicity studies with cycloxydim

Species	Strain	Sex	Route	Batch No.; purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Results	Reference
Mouse	NMRI/ MAN/ WIGA	M + F	Oral	N 64; 94.8	M: >5000 F: >5000 M + F: >5000	—	[a]	Kieczka & Kirsch (1985b)
Rat	Wistar	M + F	Oral	N 11; 92.2	M: 4420 F: 3830 M + F: 3940	—	[b]	Kieczka & Kirsch (1984a)
Rat	Wistar	M + F	Dermal	N 11; 92.2	>2000	—	[c]	Kieczka & Kirsch (1984b)
Rat	Wistar	M + F	Inhalation 4 h (head–nose exposure)	N 64; 94.8	—	>5.28 (liquid aerosol; MMAD 50% 1.6 µm)[d]	[e]	Klimisch et al. (1985)
Rabbit	White Vienna	M + F	Acute dermal irritation/ corrosivity	N 11; 92.2	—	—	Non-irritant	Kieczka & Kirsch (1984c)
Rabbit	White Vienna	M + F	Eye irritation	N 11; 92.2	—	—	Non-irritant	Kieczka & Kirsch (1984d)
Guinea-pig	Pirbright white, Dunkin Hartley HOE DHPK SPF-LAC	F	Skin sensitization effects (guinea-pig maximization)	N 64; 94.8	—	—	Non-sensitizer	Kieczka & Kirsch (1985a)

F, female; M, male; MMAD, mass median aerodynamic diameter

[a] At the highest dose level, mortality was restricted to one male mouse, which died within 1 day after test substance administration. Clinical signs recorded were dyspnoea, apathia, abnormal position, staggering, paresis, absent pain reflex, narcotic-like state, twitching, piloerection, imbalance and poor general state. The onset of signs was observed between 30 min and 1 day after dosing. These signs were observed in animals that survived up to 1 day of the study and were dose dependent starting at 1470 mg/kg bw. At study termination, the surviving animals were free of signs. They can be considered to be unspecific, with no substantial difference between sexes. Macroscopic examination in the mouse that died revealed a yellow-brown discoloration of the liver. No substance-related findings were noted in animals that survived.

[b] Clinical signs recorded were dyspnoea, apathia, abnormal position, staggering, atonia, paresis, tremors, twitching, spastic gait, piloerection, exsiccosis, salivation, lacrimation and poor general state. The onset of signs was observed between 30 min and 5 h after dosing. Signs were observed in animals that survived up to day 7 of the study. They were dose dependent starting at 2150 mg/kg bw. No signs were noted at the lowest dose level of 1210 mg/kg bw. At study termination, the surviving animals were free of signs. The signs can be considered to be unspecific, with no substantial difference between sexes. Macroscopic examination in animals that died revealed bloody ulceration of the glandular stomach and haematinized contents of the intestine in several cases. No substance-related findings were noted in animals that survived.

[c] There were no signs of systemic intoxication, mortalities or local signs of irritation. No macroscopic findings were noted at sacrifice.

[d] MMAD calculated as acid.

[e] There were no mortalities in the study. During exposure to cycloxydim, weak attempts to escape were noted, as well as eyelid closure and shallow and slightly jerky respiration. Later during exposure, respiration was jerky. After exposure, slight piloerection and slightly jerky respiration were noted. No abnormalities apart from slight alopecia on the head were noted on day 6 after exposure. The body weight gain in males and females revealed no substance-related effects. No macroscopic findings were noted at study termination (day 14).

cycloxydim free acid if the free acid could not be used in the feed because of its insufficient stability and could also not be administered in the drinking-water on account of its low solubility.

In rodent short-term toxicity studies, cycloxydim sodium salt was administered via the drinking-water after it was found that the sodium salt was not sufficiently stable when mixed in rodent feed. After only 1 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.

In toxicity studies in dogs, however, dietary administration of cycloxydim sodium salt was possible because fresh test substance feed mixture could be prepared each day shortly before feeding. In addition, sufficient stability could be verified analytically for the short period until the feed had been completely consumed.

In all studies, the feed concentration values (in parts per million [ppm]) and test substance intakes (in mg/kg bw per day) refer to cycloxydim as the acid rather than as its sodium salt. Continuous test substance administration in these studies was verified by regular determination of the consumption of drinking-water containing the test substance (2 times per week).

The results of these studies are characterized by clinicochemical changes associated with changes in water and food consumption and effects on the liver. Effects on erythrocytes were seen only in dogs at high doses. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

(a) Oral route

Mice

In a 4-week range-finding study designed to determine appropriate dose levels for a long-term carcinogenicity study, 10 B6C3F1/Cr IBR mice of each sex per dose were administered cycloxydim as the sodium salt (purity 94.8%; batch No. N 64) via the drinking-water at concentrations of 0, 300, 1000, 3000 and 9000 ppm (equal to 0, 59, 189, 462 and 1008 mg/kg bw per day for males and 0, 63, 218, 591 and 1177 mg/kg bw per day for females, respectively). The test substance intake over the entire study was calculated as the free acid for the purpose of this assessment based on the weekly test substance intake data provided in the report. The mice were 43 days old at the beginning of the administration of the test substance, and the mean body weights were 19.5 g and 18 g for males and females, respectively.

Food consumption and body weight were determined once per week. Water consumption was determined twice per week. The health status of the animals was checked daily. At the end of the test substance administration, blood samples were taken for clinicochemical examinations. All animals were sacrificed after 4 weeks. The organ weights of liver, kidneys, heart, brain, spleen, thymus, testes and adrenals were determined; selected organs were assessed by gross pathology. Subsequently, a histopathological examination of the liver, kidney, stomach, jejunum and lung and of all gross lesions was carried out.

There were no test substance–related mortalities or clinical signs of toxicity in any treatment group. Body weight gain was reduced in the 9000 ppm group. A temporary reduction in body weight was observed in most of the animals of all test groups on day 21 only. As the controls also lost some weight at this time, this isolated change in body weight development is not considered to be test substance related. Food consumption of animals of the 9000 ppm groups was reduced. Water consumption was reduced in males and females of the 9000, 3000 and 1000 ppm groups, as well as in females of the 300 ppm group. The extent of the reduction varied over time; in general, the water consumption reduction was approximately 50% for the 9000 ppm animals, varied between 5% and 35% for the 3000 ppm animals, ranged between 3% and 10% for the 1000 ppm animals and was 13% in the 300 ppm females.

There were no test substance–related haematological changes in any of the treatment groups. Clinicochemical examinations revealed an increase in urea values in 9000 ppm males and females. In addition, a decrease in cholesterol level was seen in 9000 and 3000 ppm animals (Table 14). Owing to the small amount of blood available, it was not possible to evaluate all clinicochemical parameters in all animals. Therefore, for many clinicochemical parameters, the number of available data points per sex and dose varied (occasionally as low as 2 out of 10); hence, the results of the clinicochemical investigation should be viewed with caution.

Table 14. Clinicochemical parameters measured in a range-finding study in mice administered cycloxydim as the sodium salt for 4 weeks

Parameter	Concentration in drinking-water (ppm)				
	0	300	1000	3000	9000
Urea (mmol/l)					
- Males (N^a = 4–8)	5.40	5.16	6.27	6.34	9.55
- Females (N = 3–7)	4.25	10.12	5.52	5.98	11.39
Cholesterol (mmol/l)					
- Males (N = 2–5)	1.78	1.63	1.47	0.85	0.80
- Females (N = 2–4)	1.97	1.23	1.68	0.45	0.53

From Kuehborth et al. (1986a)

[a] N = number of blood samples evaluated for assessment of parameter.

Table 15. Organ weight determinations in a range-finding study in mice administered cycloxydim as the sodium salt for 4 weeks

Parameter	Concentration in drinking-water (ppm)									
	0		300		1000		3000		9000	
Heart weight – males										
- Absolute, g (% of controls)	0.110	(—)	0.107	(97)	0.106	(96)	0.106	(96)	0.092**	(84)
- Relative, % (% of controls)	0.562	(—)	0.561	(100)	0.560	(100)	0.551	(98)	0.533*	(95)
Liver weight – males										
- Absolute, g (% of controls)	0.768	(—)	0.796	(104)	0.810	(105)	0.862	(112)	0.780	(102)
- Relative, % (% of controls)	3.918	(—)	4.176*	(107)	4.264*	(109)	4.491**	(115)	4.512**	(115)
Thymus weight – females										
- Absolute, g (% of controls)	0.0523	(—)	0.0610	(117)	0.0549	(105)	0.0576	(110)	0.0637*	(122)
- Relative, % (% of controls)	0.2889	(—)	0.3506	(121)	0.3006	(104)	0.3352	(116)	0.3943**	(136)

From Kuehborth et al. (1986a)

* $P < 0.05$; ** $P < 0.01$ (Williams t-test)

Organ weight determinations revealed a test substance–related decrease in absolute weights of the kidneys, spleen, testes and brain in 9000 ppm male mice and of the heart, kidney and liver in 9000 ppm females. Because there were no similar changes in relative organ weights, these reductions were considered to be related to the reduction in body weight found at this dose level. Both absolute and relative heart weights were decreased in 9000 ppm male mice only. Relative liver weights were slightly increased in male mice of all dose groups. There was, however, no statistically significant increase in absolute liver weight in males at any dose level. In the high dose group females, the absolute and relative thymus weights were significantly increased (Table 15).

Gross necropsy did not show any test substance–related changes. The histopathological investigations revealed hydropic vacuolar degeneration in hepatocytes of two male 9000 ppm mice. There were no other test substance–related changes observed during the histopathological evaluations.

In conclusion, the administration of cycloxydim sodium salt to mice via drinking-water over a period of 28 days elicited toxic effects in all dose groups. The target organs appear to be the liver and kidney, as can be seen from degeneration of hepatocytes in two males of the high dose group, the increase in relative liver weights in male mice of all dose groups and the increase in urea values in mice of both sexes of the highest dose group. Based on significantly increased relative liver weights

(over 115%) at 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters (enhanced plasma urea level and decreased plasma cholesterol) and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in two animals in the highest dose group, a (limited) NOAEL could be set as 1000 ppm (equal to 189 mg/kg bw per day in male mice and 218 mg/kg bw per day in female mice). A QA statement was attached. The study was conducted as per OECD test guideline 407, with the following deviation: clinicochemical investigations were limited to total bilirubin, urea, potassium chloride, triglycerides, cholesterol and alkaline phosphatase; haematological parameters were not investigated; and histopathological examination was limited to liver, kidneys, stomach, jejunum and lungs (Kuehborth et al., 1986a).

In a second 4-week range-finding study, 10 B6C3F1 mice of each sex per group were administered cycloxydim as the sodium salt (purity 94.8%; batch No. N 64) via the drinking-water at concentrations of 0, 30, 100, 300 and 900 ppm (equal to 0, 7.3, 22.5, 68.3 and 204 mg/kg bw per day for males and 0, 8.8, 28.3, 82.3 and 242 mg/kg bw per day for females, respectively). The test substance intake over the entire study was calculated based on the weekly test substance intake data provided in the report. The mice were 42 days old at the beginning of the administration of the test substance, and the mean weights were 19.1 g and 15.7 g for males and females, respectively.

Food consumption and body weight were determined once a week. Water consumption was determined twice per week. The health of the animals was checked each day. At the end of the administration period, blood samples were taken for a limited clinicochemical examination (activity of lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase). All animals were sacrificed after 4 weeks. The liver weights were determined and assessed by gross pathology. As there were no histopathological changes in the previous mouse study (Kuehborth et al., 1986a) at doses up to 1000 ppm, histopathological examinations were not carried out in this study.

There were no test substance–related mortalities or clinical signs of toxicity in any treatment group. There were no effects on body weight or food consumption. Water consumption was reduced in males and females of the 900 ppm group as well as in females of the 300 ppm group. The extent of the reduction varied over time; in general, the water consumption reduction was approximately 4–9% for the 900 ppm females and 4–7% for the 300 ppm females. In the 900 ppm males, water consumption was reduced by approximately 8% during the first week of the study only. As already discussed above for the previous mouse study (Kuehborth et al., 1986a), because the test substance was administered via the drinking-water, the reduction of water consumption is to be regarded as a palatability effect rather than a specific adverse effect.

There were no test substance–related clinicochemical changes in any of the treatment groups. Organ weight determinations revealed an increase in absolute and relative liver weights in male mice of the 900, 300 and 100 ppm groups. Relative liver weights were increased in 900 ppm females (Table 16).

Gross necropsy did not show any test substance–related changes.

In the previous mouse study (Kuehborth et al., 1986a), there were no histopathological changes at dose levels up to 1000 ppm. From these data, it is inferred that the marginally increased liver weights observed in this study were also without histopathological changes and thus considered to represent adaptive rather than adverse effects. The NOAEL was established at the highest dose level of 900 ppm, because the marginally increased liver weights without histopathological changes were considered to represent adaptive rather than adverse effects. A QA statement was attached. The study was conducted as per OECD test guideline 407, with the following deviations: no histopathology was conducted; and weight determinations were undertaken from limited organs only (Kuehborth et al., 1986b).

With the few available parameters in both studies, an overall NOAEL was set at 1000 ppm (equal to 189 mg/kg bw per day in male mice and 218 mg/kg bw per day in female mice), on the

Table 16. Absolute and relative liver weights in a 4-week range-finding study in mice administered cycloxydim as the sodium salt in the drinking-water

Parameter	Concentration in drinking-water (ppm)									
	0		30		100		300		900	
Liver weight – males										
- Absolute, g (% of control)	0.821	(—)	0.871	(106)	0.910**	(111)	0.887**	(108)	0.923**	(112)
- Relative, % (% of control)	4.019	(—)	4.103	(102)	4.162*	(104)	4.182*	(104)	4.362**	(109)
Liver weight – females										
- Absolute, g (% of control)	0.818	(—)	0.782	(96)	0.773	(94)	0.788	(96)	0.845	(103)
- Relative, % (% of control)	4.461	(—)	4.356	(98)	4.378	(98)	4.438	(99)	4.716**	(106)

From Kuehborth et al. (1986b)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

basis of a significant increase in relative liver weights at concentrations of 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in the first study.

Rats

In a range-finding study, cycloxydim as the sodium salt (purity 92.8%; batch No. N 11) was administered to groups of five male and five female Wistar rats via their drinking-water at concentrations of 0, 300, 1000, 3000 and 9000 ppm (equal to 0, 32, 102, 272 and 683 mg/kg bw per day for males and 0, 35.3, 106, 252 and 678 mg/kg bw per day for females, respectively) for 4 weeks. The rats were 42 days old at the beginning of the administration of the test substance, and the mean weights were 162.56 g and 150.28 g for males and females, respectively.

Food consumption and body weight were determined once a week. Drinking-water consumption was measured twice per week. The health status of the animals was checked daily. Blood samples from five animals of each sex per dose group for clinicochemical and haematological examinations were taken at 12 and 25 days after the beginning of administration. Urinalysis was performed after 22 days of test substance administration. At the end of the 4-week administration period, all animals were fasted for 16 h and then sacrificed by decapitation after they had been anaesthetized with carbon dioxide. Organ weights were determined, and gross pathological as well as histopathological examinations were carried out.

One female of the 9000 ppm group died on day 12 of the study. There were no further mortalities in any of the treatment groups. The general health condition of all males and females of the 9000 ppm group appeared to be temporarily impaired; in two high dose group males, a slight red incrustation of the nose was noted; a further male showed slight redness of the nose. On day 8, ruffled fur was observed in one female, which died 4 days later. None of the symptoms mentioned were observable at the end of the study period.

Body weight gain was reduced in males and females of the 9000 ppm group as well as in 3000 ppm group females (Table 17).

Food consumption of animals of the 9000 ppm group as well as 3000 ppm females was reduced throughout the entire study period. In the males of the 3000 ppm group, a temporary reduction of food consumption occurred during the first week only (Table 18).

Water consumption was reduced in males and females of all dose groups. While the extent of this reduction varied during the 4-week administration period, a dose-related effect on water consumption was obvious.

There were no test substance–related haematological changes in any of the treatment groups. Clinicochemical examinations performed at the end of the study revealed an increase in urea values

Table 17. Body weight at day 28 and body weight gain in a range-finding study in rats administered cycloxydim as the sodium salt in drinking-water

Concentration in drinking-water (ppm)	Males				Females			
	Body weight (day 28)		Body weight gain (days 1–28)		Body weight (day 28)		Body weight gain (days 1–28)	
	g	% of control	g	% of control	g	% of control	g	% of control
0	302.40	—	136.60	—	217.60	—	66.40	—
300	310.00	103	146.60	107	215.60	99	65.80	99
1000	296.80	98	137.20	100	216.20	99	64.20	97
3000	294.20	97	130.20	95	198.20	91	50.40	76
9000	216.60	72	56.60**	41	173.75	80	23.50**	35

From Kuehborth et al. (1986c)
** $P < 0.01$ (Williams *t*-test)

Table 18. Mean food intake during 4-week treatment of rats with cycloxydim as the sodium salt in drinking-water

Concentration in drinking-water (ppm)	Males				Females			
	Days 1–28		Days 1–7		Days 1–28		Days 1–7	
	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control
0	26.07	—	25.49	—	20.22	—	19.57	—
300	26.75	103	25.57	100	19.65	97	19.80	101
1000	25.53	98	24.20	95	19.57	97	19.57	100
3000	25.44	98	23.40	92	17.50	87	16.66	85
9000	17.91	69	13.51	53	14.80	73	9.83	50

From Kuehborth et al. (1986c)

and slightly increased chloride and sodium levels at 9000 ppm in both sexes. At 3000 ppm, a marginal increase in urea concentration was observed in females after 12 days on study, but not after 25 days; this finding was therefore not considered related to treatment. In addition, triglyceride levels were reduced at 9000 ppm in both sexes. A slight increase in cholesterol level in 9000 ppm males was observed on day 12, but not on day 25; therefore, this finding is not considered to be test substance related. A slight decrease in alkaline phosphatase activity observed in high dose group females was not considered to be of toxicological significance (Table 19).

Organ weight determinations revealed a test substance–related decrease in absolute weights of the heart, liver, kidneys, spleen, adrenals and brain in 9000 ppm male rats and of heart and adrenal in 9000 ppm females. Because there was no similar decrease in relative weights, these reductions were considered to be related to the reduction in body weight at this dose level. Similarly, statistically significantly increased relative liver and kidney weights seen at 9000 ppm in males and at 3000 ppm in females were considered to be a consequence of reduced body weights. In females, increases in relative liver and kidney weights at 9000 ppm were judged to be a direct substance-related effect on the organs, because the corresponding absolute organ weights were not decreased and the relative weight increase was higher than the respective decrease in mean terminal body weight (Table 20).

Gross necropsy and histopathological investigations did not show any test substance–related changes.

Table 19. Clinicochemical parameters at day 25 in rats administered cycloxydim as the sodium salt in drinking-water

Parameter	Concentration in drinking-water (ppm)				
	0	300	1000	3000	9000
Urea (mmol/l)					
- Males	6.98	7.06	6.90	7.24	8.19**
- Females	6.97	7.25	7.61	7.76	9.23**
Sodium (mmol/l)					
- Males	141.91	141.24	139.08*	140.71	143.93
- Females	141.25	139.97	141.29	143.81	145.21*
Chloride (mmol/l)					
- Males	98.10	98.15	96.28*	98.88	100.55
- Females	99.93	98.60	98.87	100.54	102.11
Triglycerides (mmol/l)					
- Males	2.16	2.29	1.83	2.50	1.13*
- Females	1.45	1.35	1.63	1.17	1.00
Cholesterol (mmol/l)					
- Males	1.55	1.77	1.72	1.90	1.68
- Females	1.56	1.32	1.66	1.41	1.42
Alkaline phosphatase (µkat/l)					
- Males	10.69	11.71	9.93	10.99	9.74
- Females	7.56	7.32	7.26	7.68	5.34**

From Kuehborth et al. (1986c)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

In view of the above, it can be concluded that the dose level of 9000 ppm resulted in temporary poor general state and a clear reduction in food consumption and body weight development with associated organ weight decreases. Slight reductions in body weight and food intake were also observed in females at 3000 ppm. There was a reduction in water consumption at all dose levels. As the test substance was administered via the drinking-water, the reduction of water consumption is regarded to be a palatability effect rather than a specific adverse effect. At 9000 ppm, clinicochemical changes that were considered treatment related comprised increased urea, sodium and chloride levels in both sexes (all considered secondary to reduced water intake), reduced triglycerides in both sexes and decreased alkaline phosphatase in females. With the exception of increased relative liver and kidney weights in high dose group females, which were considered to represent a direct organ-specific effect of cycloxydim, the other changes in relative organ weights were secondary to reduced body weights. In the absence of any associated clinicochemical or histopathological changes, the increased relative liver and kidney weights in females at 9000 ppm are considered to represent adaptive rather than adverse effects.

The NOAEL was assessed to be 3000 ppm in males (equal to 272 mg/kg bw per day) and 1000 ppm in females (equal to 106 mg/kg bw per day), based on reductions in food consumption and body weight gain at the next higher dose level. Neither the GLP certification nor any QA statement was attached. However, the study was conducted as per OECD test guideline 407 (adopted in 1981), with the following deviations: no functional observational battery (FOB) (not required in the 1981 guideline); no gross examination of large intestines, bone marrow, lymph node, peripheral nerve or spinal cord; and no histopathology of large intestines, spinal cord, bone marrow, heart, trachea, lung,

Table 20. Organ weights in rats administered cycloxydim as the sodium salt in drinking-water

Parameter	Concentration in drinking-water (ppm)									
	0		300		1000		3000		9000	
Terminal body weight, g (% of control)										
- Males	271.66	(—)	281.16	(103)	267.7	(99)	267.46	(98)	200.72**	(74)
- Females	196.52	(—)	193.8	(99)	194.02	(99)	180.76	(92)	162.68**	(83)
Absolute liver weight, g (% of control)										
- Males	9.326	(—)	9.848	(106)	9.296	(100)	9.568	(103)	7.854*	(84)
- Females	6.73	(—)	7.042	(105)	7.12	(106)	6.784	(101)	7.085	(105)
Relative liver weight, % (% of control)										
- Males	3.427	(—)	3.501	(102)	3.471	(101)	3.574	(104)	3.911**	(114)
- Females	3.419	(—)	3.631	(106)	3.667	(107)	3.766*	(110)	4.347**	(127)
Absolute kidney weight, g (% of control)										
- Males	2.162	(—)	2.312	(107)	2.054	(95)	2.310	(107)	1.820**	(84)
- Females	1.554	(—)	1.578	(102)	1.67	(107)	1.596	(103)	1.623	(104)
Relative kidney weight, % (% of control)										
- Males	0.795	(—)	0.823	(104)	0.768	(97)	0.865*	(109)	0.907**	(114)
- Females	0.793	(—)	0.815	(103)	0.862	(109)	0.892	(112)	1.003**	(126)

From Kuehborth et al. (1986c)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

thymus, urinary bladder, prostate/uterus, pituitary gland, brain, skeletal muscle, ovaries, lymph nodes or peripheral nerve (Kuehborth et al., 1986c).

In a 90-day study, cycloxydim (purity 94.8%; batch No. N 64) was administered as the sodium salt to groups of 10 male and 10 female Wistar rats via their drinking-water at dose levels of 0, 30, 100, 300, 900 and 2700 ppm (equal to 0, 2.2, 7.3, 22, 72 and 178 mg/kg bw per day for males and 0, 3.2, 10, 28, 74 and 201 mg/kg bw per day for females, respectively). In order to study the possible reversibility of effects, additional groups, each consisting of 10 males and 10 females, were administered dose levels of 0, 900 and 2700 ppm, respectively, for 3 months and subsequently maintained on test substance–free drinking-water for an additional 6 weeks as recovery groups. At the initiation of the study, the age of the animals was 42 days, and average weights were 192.9 g and 134.7 g for males and females, respectively.

The animals were observed daily for clinical signs and mortalities. A thorough examination, including palpation, was performed once per week. Body weight and feed intake were measured weekly. Drinking-water consumption was determined twice per week. Ophthalmoscopy was performed before the start and at the end of the treatment period and at the end of the recovery period on all animals of the control and high dose groups. Clinicochemical and haematological examinations were performed after approximately 1, 2 and 3 months of test substance administration as well as towards the end of the recovery period. Urinalyses were performed after approximately 3 months of test substance administration and towards the end of the recovery period. Animals were sacrificed on completion of the 3-month treatment period and subjected to a full macroscopic pathological examination. Weights of selected organs were determined, and histopathological investigations were

Table 21. Body weights at the end of the administration and recovery periods in rats administered cycloxydim as the sodium salt

	Body weight (g)					
	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
At end of administration period						
- Males	448.3	450.2	462.1	468.0	439.4	407.0** (90.8)[a]
- Females	258.7	247.2	261.8	250.0	263.0	254.5
At end of recovery period						
- Males	487.1	—	—	—	475.8	475.3
- Females	273.5	—	—	—	275.1	271.2

From Kuehborth et al. (1985)
** $P < 0.01$ (Dunnet's test)
[a] Percentage of control value.

performed. Animals of the recovery groups were also subjected to organ weight determinations as well as to gross pathological and histopathological examinations. Stability and homogeneity of the test substance were verified analytically. The correctness of the concentrations in the drinking-water and the stability in the water were analytically confirmed.

No mortality or clinical signs of toxicity occurred in the study at any dose level. There was a reduction of drinking-water consumption, especially during the earlier phases of treatment, in the high dose group males (−32%) and females (−35%) as well as in the 900 ppm females (−17%). However, towards the end of the administration period, drinking-water consumption in nearly all animals was comparable to that of controls. There were no effects on drinking-water consumption during the recovery period. A slight reduction of food consumption was observed in high dose group males (maximum −11%) throughout the administration period and in high dose group females (maximum −7%) during the initial phases of treatment. Males recovered completely during the treatment-free period. Body weights were reduced in high dose group males throughout the administration period. In high dose group females, there was a slight reduction in body weight development only during the first week of treatment. Thereafter, body weight development in all treated females was comparable to that of controls. There were no test substance–related differences in body weight between test groups in either sex at the end of the recovery period (Table 21).

There were no test substance–related ophthalmological findings in the groups. The haematological examinations and urinalysis did not show test substance–related changes. The clinicochemical investigations revealed a few marginal changes that were considered to be test substance related by the authors of the report (Tables 22 and 23). These are:

- increased alanine aminotransferase activity in 2700 and 900 ppm males and females (only after 1 month of treatment);
- increased creatinine level in 2700 and 900 ppm females;
- increased urea and cholesterol levels in 2700 ppm females (only after 1 month of treatment).

There were no clinicochemical changes in the recovery groups.

Organ weight determinations as well as gross pathological and histopathological examinations did not reveal any test substance–related changes at any dose level. Considering the above, it can be concluded that the high dose level of 2700 ppm resulted in a clear reduction in food consumption and body weight development. At this dose level and in 900 ppm females, there was also a reduction in

Table 22. Clinicochemical changes (after 1 month of treatment)

Parameter	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
Alanine aminotransferase (µkat/l)						
- Males	0.89	0.94	0.93	0.92	0.97**	0.98**
- Females	0.92	0.92	0.99	0.98	1.02*	1.10**
Alkaline phosphatase (µkat/l)						
- Males	8.71	8.80	8.71	8.34	7.82*	7.83*
- Females	6.80	6.46	6.48	6.32	6.41	6.11
Creatinine (µmol/l)						
- Males	55.60	56.57	54.83	53.44	58.34*	56.41
- Females	50.32	50.93	50.31	51.20	53.77**	54.50**
Urea (mmol/l)						
- Males	6.56	6.72	6.61	6.49	6.45	6.52
- Females	7.61	7.98	7.40	7.53	8.05	8.38*
Cholesterol (mmol/l)						
- Males	1.65	1.73	1.56	1.53	1.75	1.69
- Females	1.44	1.37	1.43	1.50	1.60*	1.55

From Kuehborth et al. (1985)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

Table 23. Clinicochemical changes (at the end of the study)

Parameter	Concentration in drinking-water (ppm)					
	0	30	100	300	900	2700
Alanine aminotransferase (µkat/l)						
- Males	1.12	1.06	1.03	1.12	1.00	1.10
- Females	1.10	1.36	1.05	1.27	1.07	1.22*
Alkaline phosphatase (µkat/l)						
- Males	5.25	5.50	5.53	6.18	4.94	5.25
- Females	4.39	4.05	4.36	4.29	4.12	3.75**
Creatinine (µmol/l)						
- Males	53.37	55.79	52.48	57.02	55.56	55.26
- Females	57.83	55.12	55.63	57.85	59.07	59.38
Urea (mmol/l)						
- Males	6.86	7.05	6.83	6.90	6.58	6.79
- Females	7.56	8.11	8.13	8.06	7.76	7.91
Cholesterol (mmol/l)						
- Males	1.64	1.83	1.51	1.46	1.74	1.67
- Females	1.61	1.55	1.62	1.65	1.66	1.87*

From Kuehborth et al. (1985)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

Table 24. Test substance intake (free acid) by dogs administered cycloxydim sodium salt in the diet for 4 weeks

Nominal test substance intake (mg/kg bw per day)	Test substance intake in males (mg/kg bw per day)	Test substance intake in females (mg/kg bw per day)
10	9.9	9.6
40	39.8	39.4
120	118	119
360	356	338

From Hellwig et al. (1985)

water consumption. As the test substance was administered via the drinking-water, the reduction in water consumption is to be regarded as a palatability effect rather than a specific adverse effect.

Based on increases in creatinine, urea and cholesterol in females and increases in alanine aminotransferase activity in both sexes at 900 ppm (corresponding to intakes of 72 mg/kg bw per day in males and 74 mg/kg bw per day in females), the NOAEL was assessed to be 300 ppm (22 mg/kg bw per day in males and 28 mg/kg bw per day in females). GLP compliance certification was attached, and the study was conducted as per OECD test guideline 408 and USEPA guideline 82-1 (Kuehborth et al., 1985).

Dogs

Cycloxydim sodium salt (purity 94.8%; batch No. N 64 [Tox Batch II]) was administered via the diet for 4 weeks to two male and two female Beagle dogs (BASF's own breed) per dose level at concentrations of 0, 300, 1200, 3600 or 10 800 ppm to achieve a daily test substance intake of 0, 10, 40, 120 and 360 mg/kg bw per day (as free acid), respectively. The dietary preparation was made by mixing 350 g of dry dog food with 350 ml of water containing the appropriate concentration of cycloxydim sodium salt. The animals were offered the feed test substance preparation daily for 1 h in the morning. The feed was then removed from the kennel. For estimation of actual food consumption, any feed left over was weighed and subtracted from the amount offered. At the initiation of the study, the age of the animals was 6–8 months, and average weights were 8.9 kg and 8.2 kg for males and females, respectively.

Food consumption and the health status of the animals were checked daily, and body weights were determined once a week. Clinicochemical and haematological examinations were performed 5 days before the start of the administration period and on study days 9 and 24. Urinalyses were performed 6 days before the start of the administration period and on study days 15 and 22.

At the end of the 4-week administration period, all animals were sacrificed. Organ weights were determined, and gross pathological as well as histopathological examinations were carried out. The stability of the test substance was demonstrated for the stock and drinking-water solutions as well as for the formulations of feed/aqueous sodium salt solutions. The stability of the test substance in feed demonstrated an average recovery of more than 90% within the first hour. After 3 h, the recovery ranged between 76.5% and 80.1%. As the dietary preparations were offered to the dogs immediately after preparation and the diets were made available to the dogs only for 1 h, the limited stability is considered not to have affected the intake of unchanged test substance. The correctness of the concentration in the drinking-water was analytically verified, with only a single exception in a single dose group. This is not considered to have had an impact on the results of the study.

The overall mean test substance intake for the 4-week treatment period was calculated based on the weekly test substance intake data provided in the report (Table 24).

There were no mortalities in any of the groups. A single occurrence of vomiting in a high dose group female could be regarded as a possible treatment-related sign of clinical toxicity. Food

Table 25. Haematological and clinicochemical parameters at day 24 in dogs administered cycloxydim sodium salt in the diet

Parameter	Dose level (mg/kg bw per day)				
	0	10	40	120	360
Haemoglobin (mmol/l)					
- Male 1 / Male 2	9.70 / 9.17	7.93 / 8.05	8.55 / 8.27	8.47 / 8.18	7.01 / 8.05
- Female 1 / Female 2	8.91 / 9.53	8.79 / 9.72	10.18 / 9.98	9.14 / 8.88	9.35 / 7.79
Red blood cell count (10^{12}/l)					
- Male 1 / Male 2	7.68 / 7.14	6.26 / 6.37	6.67 / 6.27	6.56 / 6.17	5.27 / 6.06
- Female 1 / Female 2	6.54 / 7.25	6.61 / 7.42	7.67 / 7.51	6.90 / 7.74	7.27 / 6.41
Haematocrit (l/l)					
- Male 1 / Male 2	0.52 / 0.46	0.42 / 0.43	0.45 / 0.43	0.46 / 0.42	0.37 / 0.43
- Female 1 / Female 2	0.47 / 0.51	0.47 / 0.52	0.53 / 0.53	0.48 / 0.47	0.50 / 0.44
Heinz bodies (‰)					
- Male 1 / Male 2	4 / 3	11 / 2	4 / 10	6 / 11	30 / 38
- Female 1 / Female 2	10 / 10	4 / 5	14 / 8	7 / 12	10 / 33
Alkaline phosphatase (µkat/l)					
- Male 1 / Male 2	5.01 / 5.05	5.71 / 6.33	4.90 / 4.16	8.38 / 7.69	10.8 / 16.3
- Female1 / Female 2	3.38 / 5.56	4.22 / 8.42	5.90 / 8.17	4.20 / 6.49	9.00 / 11.1

From Hellwig et al. (1985)

consumption was slightly reduced in the two high dose group females, which is also apparent from the slightly reduced actual test substance intake values compared with the nominal concentration. Body weight gain was not affected in any of the treatment groups. The haematological investigations showed a marginal reduction in the red blood cell count, haematocrit and haemoglobin values in the high dose group males as well as an increased occurrence of Heinz bodies both in males and in one female dog administered 360 mg/kg bw per day. Clinicochemical examinations revealed an increase in alkaline phosphatase activity in males and females of the 360 mg/kg bw per day group as well as in males of the 120 mg/kg bw per day group (Table 25). In addition, the plasma cholinesterase activity was increased in high-dose males; however, this was not considered to represent any toxicologically significant effect and is likely to be an incidental finding.

Organ weight determinations revealed a clear increase in liver weights at 360 mg/kg bw per day and slight liver weight increases in the 40 and 120 mg/kg bw per day groups (Table 26).

Gross necropsy and histopathological investigations did not show any test substance–related changes. In the absence of histopathological liver changes up to the highest dose tested, the increase in relative liver weights is considered to represent an adaptive response rather than an adverse effect.

From the above, it can be concluded that the high dose level of 360 mg/kg bw per day was reasonably well tolerated; there was a single female with vomiting, and there was a slight reduction in food consumption in males. Red blood cell parameters were marginally reduced in high dose group males and in one male of the 120 mg/kg bw per day group. Clinicochemical examinations revealed an increase in alkaline phosphatase activity in males at 120 mg/kg bw per day and in both sexes at 360 mg/kg bw per day. Liver weights were slightly increased at 40 and 120 mg/kg bw per day and clearly elevated at 360 mg/kg bw per day. No evidence of histopathological liver changes was observed up to the highest dose level tested.

The NOAEL in this study is 40 mg/kg bw per day (actual dose) in males and 119 mg/kg bw per day (actual dose) in females, on the basis of effects on the liver. A QA statement was attached (Hellwig et al., 1985).

Table 26. Terminal body and liver weights in dogs administered cycloxydim sodium salt in the diet for 4 weeks

Parameter	Dose level (mg/kg bw per day)									
	0		10		40		120		360	
Terminal body weight, g (% of control)										
- Males	8700	(—)	9150	(105)	10 250	(118)	9900	(114)	9900	(114)
- Females	8450	(—)	8650	(102)	9 600	(114)	9450	(112)	9150	(108)
Absolute liver weight, g (% of control)										
- Males	322.79	(—)	351.00	(109)	407.45	(126)	415.01	(129)	532.74	(165)
- Females	317.62	(—)	341.83	(108)	397.74	(125)	410.49	(129)	464.98	(146)
Relative liver weight, % (% of control)										
- Males	3.713	(—)	3.841	(103)	4.040	(109)	4.196	(113)	5.371	(145)
- Females	3.751	(—)	3.954	(105)	4.143	(110)	4.336	(116)	5.089	(136)

From Hellwig et al. (1985)

In a 90-day study, cycloxydim (purity 94.8%; batch No. N 64) was administered as the sodium salt to groups of four male and four female Beagle dogs via their diet. The dose levels were 0, 60, 300, 1500 and 7500 ppm (equivalent to 0, 2, 10, 50 and 250 mg/kg bw per day). The diet was prepared freshly each day by mixing 350 g of dry dog food with 350 ml of drinking-water containing the appropriate concentration of cycloxydim sodium salt. The animals were offered the feed/test substance preparation daily for 1 h in the morning. The feed was then removed from the kennel. For estimation of actual feed consumption, any feed left over was weighed and subtracted from the amount offered. This procedure was followed in order to ensure sufficient stability of the test substance in the feed preparation. At the initiation of study, the ages of the dogs were 6–9 months, with average weights of 10.4 kg for males and 9.1 kg for females.

The food consumption of the animals was determined daily, and their body weight was measured once a week; the dogs' health was checked each day. Clinicochemical and haematological examinations as well as urinalyses were carried out once prior to the start of the administration period and after approximately 1 month and 3 months of treatment. Ophthalmological examinations were carried out before the beginning of the study and towards the end of the administration period in the animals of the high dose group and the controls. All animals were assessed gross pathologically. The liver, kidneys, testes, thyroids and adrenal glands were weighed. All gross lesions and the livers from all animals under study were histopathologically examined. Furthermore, a comprehensive set of organs from the control, 1500 and 7500 ppm dose groups was subjected to histopathological examination.

There were no mortalities or signs of clinical toxicity in any of the groups. Food consumption and body weight development were not affected at any dose level. The haematological investigations showed, at the high dose level only, a reduction in red blood cell count for both sexes and an increase in platelet count. The number of Heinz bodies was increased in all four high dose group females and in one high dose group male. In high dose group males only, increases in reticulocytes (only at the 1-month sampling time point), polymorphonuclear neutrophilic granulocytes, and mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) values were observed (Table 27).

In clinicochemical examinations, a 2- to 3-fold increase in alkaline phosphatase activity was observed in high dose group males and females. A doubtful (statistically non-significant) increase

Table 27. Haematology results at end of study for dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentrations (ppm)				
	0	60	300	1500	7500
Red blood cells (10^{12}/l)					
- Males	7.64	7.16	7.38	7.63	6.89*
- Females	7.66	7.39	7.81	7.65	7.02**
MCV (fl)					
- Males	68.10	69.58	67.60	68.53	71.78*
- Females	69.42	69.72	69.47	70.50	70.40
MCH (fmol)					
- Males	1.328	1.334	1.317	1.328	1.383*
- Females	1.330	1.336	1.329	1.320	1.351
Reticulocytes (‰)					
- Males (1 month)	3.50	3.00	1.75	2.25	9.25
- Males (3 months)	6.75	4.25	1.00	3.25	5.50
- Females (1 month)	5.0	5.25	2.25	3.25	2.75
- Females (3 months)	0.75	3.25	2.25	4.75	2.67
Heinz bodies (‰)					
- Male No. 1/2/3/4	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	0/0/1/9
- Female No. 1/2/3/4	0/0/0/0	0/0/0/0	0/0/0/0	0/0/0/0	9/7/10/8
Platelets (10^9/l)					
- Males	462.50	442.75	457.25	402.75	554.00*
- Females	426.50	487.00	461.75	392.25	647.25**
Polymorphonuclear neutrophilic granulocytes (10^9/l)					
- Males	4.83	5.56	6.03	6.55	7.97
- Females	6.01	5.68	5.90	6.49	6.31

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)

in the mean alkaline phosphatase activity was seen in males at 1500 ppm, which resulted from a high value of dog no. 1 (7.95 µkat/l); the other three males of the group showed values that were comparable with control levels (range 3.16–3.60 µkat/l). No effects on alkaline phosphatase activity were seen in 1500 ppm females. The albumin concentration was decreased in both sexes at the high dose level only, accompanied by an increased globulin concentration in high dose group females; the total protein content was unaffected by treatment. Furthermore, slightly decreased potassium concentrations were seen in males at 7500 ppm (Table 28).

Organ weight determination revealed a statistically significant increase in absolute and relative liver weights in high-dose males and females, as well as a statistically significant increase in absolute and relative thyroid weights in high-dose males (Table 29).

Gross pathological investigations showed a reddish tinge to the bile for high-dose males and females only. Histopathology revealed an enlargement of hepatocytes in three out of four males and in all females at the high dose level only. There were no other test substance–related changes noted in the histopathological examinations. There were no test substance–related findings at 1500, 300 or 60 ppm.

Table 28. Clinical chemistry results at end of study for dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentration (ppm)									
	0		60		300		1500		7500	
Alkaline phosphatase, µkat/l (% of control)										
- Males	3.03	(—)	3.36	(111)	3.11	(102)	4.83	(159)	9.46**	(312)
- Females	4.64	(—)	3.28	(71)	3.88	(84)	4.54	(98)	10.04**	(216)
Albumin, g/l (% of control)										
- Males	40.20	(—)	37.30	(93)	38.37	(95)	40.12	(100)	35.32*	(88)
- Females	40.45	(—)	40.73	(101)	39.90	(99)	38.27	(95)	34.45**	(85)
Globulin, g/l (% of control)										
- Males	20.93	(—)	20.08	(96)	20.79	(99.33)	22.18	(106)	23.15	(110)
- Females	19.22	(—)	21.09	(110)	20.74	(108)	19.10	(99)	23.77**	(124)
Potassium, mmol/l (% of control)										
- Males	4.07	(—)	4.10	(101)	3.94	(97)	3.81	(94)	3.64*	(89)
- Females	3.92	(—)	3.90	(99.48)	3.99	(101.78)	3.73	(95.15)	3.75	(95.66)

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)

Table 29. Organ weights in dogs administered cycloxydim sodium salt in the diet for 3 months

Parameter	Dietary concentration (ppm)									
	0		60		300		1500		7500	
Absolute liver weight, g (% of control)										
- Males	344	(—)	365	(106)	363	(106)	387	(113)	479**	(139)
- Females	316	(—)	316	(100)	337	(107)	361	(114)	498**	(158)
Relative liver weight, % (% of control)										
- Males	3.01	(—)	3.18	(106)	3.24	(108)	3.36	(112)	4.13**	(137)
- Females	3.07	(—)	3.21	(104)	3.05	(99)	3.29	(107)	4.75**	(155)
Absolute thyroid weight, g (% of control)										
- Males	0.716	(—)	0.774	(108)	0.859	(120)	0.865	(121)	1.060*	(148)
- Females	0.849	(—)	0.674	(79)	0.771	(91)	0.812	(96)	1.079	(127)
Relative thyroid weight, % (% of control)										
- Males	0.0062	(—)	0.0068	(110)	0.0077	(124)	0.0075	(121)	0.009*	(145)
- Females	0.0082	(—)	0.0069	(84)	0.007	(85)	0.0075	(91)	0.010	(127)

From Hellwig et al. (1986)
* $P < 0.05$; ** $P < 0.01$ (Williams *t*-test)

To conclude, the 3-month administration of cycloxydim to dogs via the feed did not result in clinical findings or body weight changes up to the highest dose tested of 7500 ppm. At this dose level, there were signs of an anaemic process with compensatory reactions. The target organ was the liver, with increased weights and hepatocellular hypertrophy; the bile had a reddish colour. Most of the clinicochemical changes were also considered to be related to liver changes. Thyroid weights were also significantly increased in males at 7500 ppm and more than 120% increased at dose levels of 300 and 1500 ppm (not statistically significant); however, no associated histopathological changes of the thyroid were observed. Findings at this dose are considered either not treatment related or not adverse. Slight increases in alkaline phosphatase activity and liver weight (+12%) (for liver) were not statistically significant and not associated with histopathological liver changes. The slight (non-significant) thyroid weight increase was not considered treatment related because it was not reproducible in 4-week or 1-year dog studies; no histopathological changes were seen in dog thyroid. Further, in male dogs, liver weights were increased over 110% at a concentration of 1500 ppm; this effect was not statistically significant, but coincided with increases in alkaline phosphatase activity (159% of control value).

Considering the changes in haematological parameters and liver effects, the NOAEL in this study was 1500 ppm, equal to 50 mg/kg bw per day. It is certified by the laboratory that the test facility was inspected by the competent authority in accordance with the OECD guidelines for national GLP inspection and study audits regarding compliance with the principles of GLP. The study was conducted as per OECD test guideline 409 and USEPA guideline 82-1 (Hellwig et al., 1986).

Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered to six male and six female Beagle dogs per group at dietary dose levels of 0, 400, 1600 and 6400 ppm (equal to 0, 12, 49 and 206 mg/kg bw per day as the acid) for 12 months. The diets were prepared each day by mixing 350 g of pulverized dog food with 350 g of water containing the appropriate amount of the sodium salt of cycloxydim. Food consumption was determined daily, and body weight was determined once a week. The animals' health was checked each day. The dogs were approximately 6–8 months old at the beginning of the study, and the average body weights were 9.7 kg and 9.2 kg for males and females, respectively.

Clinicochemical and haematological examinations as well as urinalyses were carried out prior to the start of treatment and after approximately 3, 6 and 12 months of administration. Ophthalmological examinations were carried out before the start of the study and towards the end of the administration period. All animals were assessed gross pathologically; organ weights were determined, and organs were subsequently subjected to a complete histopathological examination as specified in the OECD guideline.

There were no test substance–related signs of clinical toxicity or mortalities in any group. Food consumption and body weight development were not adversely affected in any treatment group. The haematological investigations (Table 30) demonstrated the following slight changes, which were confined, with two exceptions, to the high dose level and generally observable only at the 3- and 6-month time points:

6400 ppm:

- decreased erythrocytes—males (6 months) and females (3 and 6 months)
- decreased haemoglobin and haematocrit—females (3 and 6 months)
- increased MCH—males (3 months), females (3 and 6 months)
- increased MCV—males (3 months), females (3, 6 and 12 months)
- increased platelets and Heinz bodies—males and females (3, 6 and 12 months).

Table 30. Haematological parameters after 3, 6 and 12 months of dietary administration of cycloxydim sodium salt to dogs

Parameter	3 months				6 months				12 months			
	Dietary concentration (ppm)											
	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
Males												
RBC (10^{12}/l)	6.56	6.23	6.36	6.43	6.61	6.26	6.23	6.19*	7.08	7.12	6.85	6.90
Hb (mmol/l)	9.53	9.12	9.46	9.62	9.75	9.25	9.27	9.35	10.25	10.34	10.06	10.20
Ht (l/l)	0.457	0.434	0.452	0.463	0.467	0.443	0.446	0.451	0.500	0.500	0.485	0.496
MCH (fmol)	1.45	1.46	1.49	1.50*	1.48	1.48	1.49	1.51	1.45	1.45	1.47	1.48
MCV (fl)	69.55	69.52	70.88	71.95*	70.68	70.67	71.43	72.73	70.37	70.23	70.68	71.73
Platelets (10^9/l)	460	516	528	583**	450	450	513*	532	471	464	521	589**
Heinz bodies (‰)	4	8	12	21**	2	3	9**	25**	3	2	5	29**
Females												
RBC (10^{12}/l)	6.84	6.63	6.75	5.90**	6.78	6.64	6.66	5.83*	6.67	6.74	7.04	6.46
Hb (mmol/l)	10.01	9.78	10.03	8.97*	9.96	9.79	9.88	8.89*	9.62	9.68	10.14	9.56
Ht (l/l)	0.477	0.460	0.477	0.430*	0.478	0.469	0.477	0.426*	0.458	0.470	0.499	0.467
MCH (fmol)	1.46	1.48	1.48	1.52*	1.47	1.47	1.48	1.52**	1.45	1.44	1.44	1.48
MCV (fl)	69.63	69.23	70.45	72.70**	70.45	70.58	71.53	72.95*	68.63	69.60	70.72	72.20*
Platelets (10^9/l)	524	452	541	712*	469	467	543	699*	576	464	517	703
Heinz bodies (‰)	3	3	3	24**	3	2	7*	27**	5	9	13**	41**

From Hellwig & Hildebrand (1988)
Hb, haemoglobin; Ht, haematocrit; RBC, red blood cells; * $P < 0.05$; ** $P < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney *u*-test, two-sided)

1600 ppm:

- increased Heinz bodies—males (3 months, marginal effect; 6 months, significant effect), females (6 and 12 months).

The clinicochemical investigation (Table 31) demonstrated the following changes, most of which were already expressed after 3 months of treatment and did not alter during the course of the study:

- increased alkaline phosphatase activity: 6400 ppm (both sexes) and 1600 ppm (males)
- decreased total protein concentration: 6400 ppm (males)
- decreased albumin concentration: 6400 ppm (both sexes) and 1600 ppm (males).

Urinalysis and ophthalmoscopy did not demonstrate test substance–related effects. Organ weight determinations revealed an increase in absolute and relative liver weights in the 6400 ppm males and females, as well as in the 1600 ppm males (Table 32).

Histopathology demonstrated slight or moderate haemosiderosis in the livers of three females of the 6400 ppm group. This effect is considered to have resulted from the transiently increased degradation of erythrocytes induced by the test substance. There were no further test substance–related changes noted during the histopathological investigations. In particular, there were no changes in the liver that could be associated with the increased liver weights.

In view of the above, it can be concluded that a dose level of 6400 ppm induced an anaemic effect in Beagle dogs, which showed signs of reversibility during the last 6 months of treatment.

Table 31. Clinicochemical parameters after 3, 6 and 12 months of dietary administration of cycloxydim sodium salt to dogs

Parameter	3 months				6 months				12 months			
	Dietary concentration (ppm)											
	0	400	1600	6400	0	400	1600	6400	0	400	1600	6400
Males												
AP (µkat/l)	3.73	4.93	6.11**	10.64**	2.96	3.82	4.54*	9.38**	3.03	4.34	5.47**	11.65**
Total protein (g/l)	56.85	54.87	56.77	53.54	59.40	58.24	57.20	54.38*	60.12	58.96	58.10	56.03
Albumin (g/l)	35.76	34.01	33.99	30.90**	39.84	38.71	37.52**	33.77**	39.92	38.09	36.56**	33.84**
Females												
AP (µkat/l)	4.00	4.61	4.90	9.93**	2.91	4.07	3.96	8.99**	5.83	4.70	4.55	9.53
Total protein (g/l)	56.29	57.95	55.95	53.63	57.13	57.33	56.81	55.72	58.57	60.30	60.07	56.83
Albumin (g/l)	34.81	35.17	33.65	30.23**	38.75	39.70	37.71	34.14**	36.73	38.29	37.42	33.98

From Hellwig & Hildebrand (1988)
AP, alkaline phosphatase; * $P < 0.05$; ** $P < 0.02$ (Kruskal-Wallis ANOVA and Mann-Whitney *u*-test, two-sided)

Table 32. Terminal body weights and liver weights in dogs administered cycloxydim sodium salt in the diet for 1 year

Parameter	Dietary concentration (ppm)							
	0		400		1600		6400	
Terminal body weight, kg (% of control)								
- Males	11.92	(—)	12.70	(107)	11.53	(97)	11.53	(97)
- Females	10.87	(—)	11.32	(104)	12.12	(111)	10.63**	(98)
Absolute liver weight, g (% of control)								
- Males	349	(—)	385	(110)	407*	(117)	449**	(129)
- Females	381	(—)	414	(109)	384	(101)	457*	(120)
Relative liver weight, % (% of control)								
- Males	2.94	(—)	3.04	(103)	3.54*	(120)	3.93**	(134)
- Females	3.51	(—)	3.67	(105)	3.17	(90)	4.29**	(122)

From Hellwig & Hildebrand (1988)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test)

Associated with the anaemia, histopathology demonstrated increased haemosiderosis in the livers of females of the 6400 ppm group. Liver weights were increased at this dose level.

The NOAEL in this 12-month dietary study in dogs was 400 ppm (equal to 12 mg/kg bw per day) for males on the basis of effects on erythrocytes and the liver and altered clinicochemical parameters at 1600 ppm. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1988).

(b) Dermal route

Rats

Cycloxydim (purity 92.9%; batch No. WH 16884) was applied to the skin of 10 male and 10 female Wistar rats per dose level on an area corresponding to at least 10% of the body surface for

a period of 6 h/day, 5 days/week, for 4 consecutive weeks. The doses were 0 (vehicle control, olive oil), 60, 300 and 1000 mg/kg bw per day. The administration volume was 2 ml/kg bw per day. The skin was covered by a semiocclusive dressing. After removal of the dressing, the skin was washed with lukewarm water. At the start of the study, the rats were 60 ± 1 days old, and the body weights were in the range of 174.7–215.5 g (group mean 195.8 g) for males and 146.4–192.8 g (group mean 167.9 g) for females.

The animals were examined at least once a day for clinical signs and mortalities. Additional clinical examinations, including detailed examinations of the skin, were carried out daily. Detailed clinical observations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB was performed towards the end of the study. A motor activity measurement was performed on the same day as the FOB. Food consumption and body weight were determined weekly. Clinicochemical and haematological examinations as well as urinalysis were performed at the end of the administration period. Ophthalmological examinations were carried out prior to the start and towards the end of the administration period. All animals were subjected to a gross pathological assessment followed by histopathological examinations.

There were no mortalities or clinical signs of toxicity in the treatment groups. No substance-related effects on food consumption were observed in any treatment group. Food efficiency was statistically significantly decreased in males at 1000 mg/kg bw per day on day 21. A relationship to treatment cannot be excluded with certainty. In females, food efficiency was statistically significantly decreased on day 21 at 60 and 300 mg/kg bw per day, but not at 1000 mg/kg bw per day. Owing to the lack of a dose–response relationship, this was assessed as being incidental and not treatment related. No statistically significant deviations were seen concerning body weight. A statistically significantly lower body weight gain was observed in high dose group males for the 4-week study period (−22% compared with controls). The mean terminal body weight in males at 1000 mg/kg bw per day was decreased by 6% (statistically non-significant) compared with the control group. Body weight change was also statistically significantly decreased in females at 60 and 300 mg/kg bw per day on day 21. However, owing to the lack of a dose–response relationship, this was assessed as being incidental and not treatment related. No statistically significant changes in body weight development were noted in treatment group females when based on the 4-week treatment period, and no effects on terminal body weight were seen that could be considered as substance related (Table 33).

Open field observations, FOB and motor activity determinations did not show test substance–related changes. Clinicochemical and haematological examinations and urinalysis did not reveal test substance–related changes. Gross macroscopic observations and histopathological examinations did not show any treatment-related effect. There were no signs of skin irritation in any of the animals.

The NOAEL for systemic toxicity is 1000 mg/kg bw per day for females (highest dose tested) and 300 mg/kg bw per day for males, based on slight effects on body weight gain and food efficiency at 1000 mg/kg bw per day. Signs of local skin irritation were not observed. The study has been conducted as per GLP and the following guidelines: European Economic Community (EEC) 92/69, OECD 410 and USEPA 870.3200 (Mellert et al., 2001a).

2.3 Long-term studies of toxicity and carcinogenicity

Long-term studies of oral toxicity in mice and rats were conducted using cycloxydim sodium salt or cycloxydim free acid. Cycloxydim sodium salt as test substance was applied instead of cycloxydim free acid if the free acid could not be used in the feed because of its insufficient stability and could also not be administered in the drinking-water on account of its low solubility.

In rodent long-term toxicity studies, cycloxydim sodium salt was administered via the drinking-water after it was found that the sodium salt was not sufficiently stable when mixed in rodent

Table 33. Terminal body weight and body weight gain in rats dermally administered cycloxydim for 4 weeks

Parameter	Dose level (mg/kg bw per day)							
	0		60		300		1000	
Males								
Food efficiency (day 21) (% of control)	10.6	(—)	8.9	(84)	9.3	(88)	7.1*	(67)
Body weight change (days 0–21), g (% of control)	65.6	(—)	60.6	(92)	70.2	(107)	54.0	(82)
Body weight change (days 0–27), g (% of control)	66.7	(—)	60.9	(91)	69.9	(105)	52.2*	(78)
Terminal body weight, g (% of control)	262.4	(—)	256.3	(98)	266.8	(102)	247.3	(94)
Females								
Food efficiency (day 21) (% of control)	11.9	(—)	5.1*	(43)	3.6**	(30)	7.7	(65)
Body weight change (days 0–21), g (% of control)	41.5	(—)	31.2*	(75)	31.9*	(77)	36.9	(89)
Body weight change (days 0–27), g (% of control)	41.6	(—)	37.9	(91)	36.0	(87)	40.5	(97)
Terminal body weight, g (% of control)	211.8	(—)	204.4	(97)	203.7	(96)	207.7	(98)

From Mellert et al. (2001a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided)

feed. After only 1 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.

In all studies, the feed concentration values (in ppm) and test substance intakes (in mg/kg bw per day) refer to cycloxydim as the acid rather than as the sodium salt. Continuous test substance administration in these studies was verified by regular determination of the consumption of drinking-water containing the test substance (2 times per week).

The results of these studies are characterized by clinicochemical changes, associated with changes in water and food consumption, and effects on the liver. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

Mice

Cycloxydim (purity 93.9%; batch No. N 88) was administered as the sodium salt to 50 male and 50 female B6C3F1 mice per dose level for 24 months. The concentrations in the drinking-water were 10, 20, 60 and 240 ppm (equal to 1.3, 3.0, 8.4 and 32 mg/kg bw per day as free acid). Dose levels were selected based on the results of a 4-week feeding study in mice, in which increased liver weights were observed at concentrations of 100 ppm and above. Two groups of untreated controls were maintained in parallel for comparison, each with 50 males and 50 females. At the beginning of the administration of the test substance, the mice were 49 days old, and their mean body weights were 23 g and 20 g for males and females, respectively.

Drinking-water consumption and body weight were determined once a week. Food consumption was not measured. The animals' health was checked at least daily. Detailed clinical examinations were performed once a week. At the end of the administration period, differential blood counts were determined in all surviving animals of the control and high dose groups. Differential blood counts were also determined in all animals that died during the study. After 24 months of administration,

all animals were assessed by gross pathology and histopathology. Organ weights of selected organs were determined.

There were neither test substance–related increased mortalities nor clinical signs of toxicity. Body weight and body weight gain were not affected at any dose level. There were no effects on drinking-water consumption. There were no test substance–related changes found in the examination of the differential blood counts at any dose level. There were no test substance–related changes in organ weights. Gross pathological and histopathological examinations did not show test substance–related effects at any dose level in any dose group. There was no test substance–related increase in the incidence of any neoplasia in any of the satellite groups.

As there were no test substance–related changes at any dose level, the NOAEL in this study was 240 ppm, equal to 32 mg/kg bw per day for males and females (i.e. the highest dose tested). The study was not adequate for the evaluation of carcinogenicity, as the doses delivered were not sufficiently high; the highest dose used was much less than the NOAEL of 1000 ppm identified in the dose range–finding studies. Therefore, it can be concluded that cycloxydim had not been adequately tested in mice. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988b).

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was administered for 18 months in the drinking-water as the sodium salt to 20 male and 20 female Wistar rats per dose level in a chronic toxicity study. The dose levels in the drinking-water were 0, 100, 400, 1600 and 2700 ppm (equal to 0, 7, 28, 103 and 171 mg/kg bw per day). The rats were 42 days old when substance administration began, and the average body weights were 197 g and 150 g for males and females, respectively.

Food consumption was determined once a week for the first 14 weeks. Thereafter, it was determined at 3-month intervals. Body weights and drinking-water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week. Ophthalmological examinations were carried out before the start of the study and after 3, 6, 12 and 18 months of administration in the animals of the control and high dose groups. Clinicochemical and haematological examinations as well as urinalyses were carried out after about 3, 6, 12 and 18 months of administration. After 18 months of administration, all animals were assessed by gross pathology and histopathology. Organ weights of selected organs were determined.

There were no test substance–related mortalities or signs of clinical toxicity in any of the treatment groups. Food consumption was reduced by approximately 8–9% throughout the study in both sexes at 2700 ppm and in males at 1600 ppm compared with the control group. Food consumption in the 400 and 100 ppm treatment groups was comparable to the amount consumed by the controls. Drinking-water consumption was reduced in males and females of the 2700 ppm and 1600 ppm groups. In males, the reduction in drinking-water consumption was more pronounced during the early phases of the study (2 months: 2700 ppm, 80% and 76% of control level for males and females, respectively; 1600 ppm, 81.5% and 87.5% for males and females, respectively; 18 months: 2700 ppm, 94.5% and 74% for males and females, respectively; 1600 ppm, 94.5% and 80.2% for males and females, respectively).

After 18 months of treatment, body weights (Table 34) and body weight gains (Table 35) were reduced in males and females of the 2700 ppm, 1600 ppm and 400 ppm groups compared with the respective control values.

There were no haematological changes that could be related to the administration of the test substance. Clinicochemical examinations revealed a reduction in triglycerides in females of the 2700 ppm, 1600 ppm and 400 ppm groups. This reduction was noted in all of the blood samples taken after 3, 6, 12 and 18 months. The statistically significant decrease in triglycerides noted in females at 100 ppm after 18 months of treatment was considered to have resulted from an exceptionally

Table 34. Body weight after 18 months (day 546) in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Male body weight		Female body weight	
	g	% of control	g	% of control
0	749.9	—	399.0	—
100	711.3	95	380.4	95
400	678.7*	91	357.3*	90
1600	617.9**	82	319.7**	80
2700	589.5**	79	314.1**	79

From Kuehborth et al. (1988c)
* $P < 0.05$; ** $P < 0.01$ (ANOVA + Dunnett's tests)

Table 35. Body weight gain within 18 months (days 0–546) in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Male body weight gain		Female body weight gain	
	g	% of control	g	% of control
0	553.5	—	247.9	—
100	513.6	93	230.2	93
400	482.2	87	207.1	84
1600	422.2	76	169.5	68
2700	393.2	71	163.9	66

From Kuehborth et al. (1988c)

high control group value. As no corresponding decrease in triglyceride concentration was found at 100 ppm in the 24-month carcinogenicity study (Kuehborth et al., 1988a), a substance-related effect at 100 ppm was excluded. In males, a statistically significant decrease in triglyceride concentration was observed only in the 1600 ppm dose group after 12 and 18 months of treatment (Table 36). This effect was due to decreased triglyceride values in individual animals. In the absence of a dose–response relationship, it was concluded that triglycerides were not affected by treatment in males.

Urinalysis did not reveal any test substance–related changes. There were no ophthalmological changes related to the administration of the test substance. Organ weight determinations revealed no test substance–related changes. Gross pathological examinations did not show test substance–related effects at any dose level. In the liver, bile duct proliferation was more frequently recorded in treated males at 1600 and 2700 ppm than in controls, but the incidence was within the historical control range for this age-associated lesion. The incidence across the increasing dose groups was 20% (control), 20%, 15%, 35% and 40% in males and 0%, 5%, 5%, 10% and 0% in females. The relative number of males with basophilic foci in liver tissue was 10% (control), 5%, 35%, 10% and 10%, indicating no dose–response relationship for the occurrence of basophilic foci. Microscopic findings in other organs were found to be not treatment related.

In view of the above, it can be concluded that the administration of cycloxydim via the drinking-water resulted in a reduction of body weight gain in both sexes at concentrations of 400 ppm and above. Drinking-water consumption was reduced in the 1600 ppm and 2700 ppm groups. In females, there was a reduction in triglycerides at concentrations of 400 ppm and higher.

The NOAEL in this study was 100 ppm, equal to 7 mg/kg bw per day, in males and females based on a statistically significant reduction in body weight, body weight gain and triglyceride

Table 36. Triglyceride concentrations at 3, 6, 12 and 18 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Triglyceride concentration							
	3 months		6 months		12 months		18 months	
	mmol/l	% of control	mmol/l	% of control	mmol/l	% of control	mmol/l	% of control
Males								
0	3.67	—	4.90	—	5.24	—	6.28	—
100	3.95	108	5.52	113	6.15	117	5.82	93
400	4.29	117	5.52	113	5.20	99	6.04	96
1600	2.75	75	3.24	66	2.92**	56	3.75*	60
2700	3.45	94	3.99	81	4.05	77	5.03	80
Females								
0	3.74	—	5.27	—	5.89	—	7.82	—
100	3.45	92	4.16	79	5.06	86	5.13**	66
400	2.18*	58	3.30*	63	3.05**	52	4.59**	59
1600	2.68	72	2.73**	52	3.40**	58	4.24**	54
2700	2.02*	54	2.71**	51	2.77**	47	2.59**	33

From Kuehborth et al. (1988c)
* $P < 0.05$; ** $P < 0.01$ (*t*-test)

concentrations at a drinking-water concentration of 400 ppm, equal to 28 mg/kg bw per day. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988c).

In a 2-year study, cycloxydim (purity 93.9%; batch No. N 88) was administered in the drinking-water as the sodium salt to 50 male and 50 female Wistar rats per dose level. The treatment levels in the drinking-water were 100, 400 and 1600 ppm (equal to 6.4, 26.4 and 99.2 mg/kg bw per day as free acid). The controls consisted of 100 males and 100 females. The rats were 42 days old when the test substance administration was initiated, and the body weights of the rats were 196 g and 155 g for males and females, respectively.

Food consumption was not determined; however, as there were no effects in the chronic study in rats (Kuehborth et al., 1988c), this does not affect the quality of the study. Body weight and drinking-water consumption were determined once per week for the entire administration period. The animals' health was checked each day. Detailed clinical examinations were performed once a week. Clinicochemical and haematological examinations as well as urinalysis were carried out on 10 animals of each sex per dose group towards the end of the administration period. In addition, creatinine, triglycerides and cholesterol levels were determined for all surviving animals at the end of the treatment period. After 24 months of administration, all animals were assessed for gross pathology and histopathology. Organ weights of selected organs were determined.

There were no test substance–related mortalities or signs of clinical toxicity in any of the treatment groups. Drinking-water consumption was reduced in males and females of the 1600 ppm group. In females, the extent of the reduction of drinking-water consumption was slightly more pronounced during the early phases of the study (Table 37).

Body weight gain was reduced in males and females of the 1600 ppm group. At a concentration of 400 ppm, the reduction in body weight was statistically significant only during the first year of treatment (Table 38).

Table 37. Water consumption by rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Water consumption											
	0–3 months		0–6 months		0–9 months		0–12 months		0–18 months		0–24 months	
	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control	g/day	% of control
Males												
0	28.1	—	28.4	—	28.2	—	28.4	—	29.9	—	32.3	—
100	27.6	98	28.1	99	27.7	98	27.8	98	29.0	97	31.4	97
400	27.2	97	27.7	97	27.4	97	27.6	97	29.0	97	31.2	97
1600	25.4	90	26.2	92	25.7	91	25.3	89	27.0	90	29.4	91
Females												
0	23.6	—	25.5	—	26.1	—	27.1	—	29.2	—	31.1	—
100	23.4	99	24.8	98	25.5	98	26.5	98	29.0	99	30.9	99
400	22.8	97	24.9	98	25.4	97	26.0	96	28.9	99	30.9	99
1600	18.3	77	20.0	79	20.6	79	21.2	78	23.9	82	26.0	83

From Kuehborth et al. (1988a)

Table 38. Body weight after 12 and 24 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Body weight at 12 months				Body weight at 24 months			
	Males		Females		Males		Females	
	g	% of control	g	% of control	g	% of control	g	% of control
0	725.1	—	410.3	—	836.5	—	495.4	—
100	713.6	98	402.1	98	809.2	97	511.1	103
400	692.3	95	373.6**	91	828.9	99	457.4	92
1600	645.9**	89	339.3**	83	771.3	92	405.5**	82

From Kuehborth et al. (1988a)
** $P < 0.01$ (ANOVA + Dunnett's test)

There were no haematological changes that could be related to the administration of the test substance. Clinicochemical examinations revealed a reduction in triglyceride concentrations in females of the 1600 ppm and 400 ppm groups. Statistical significance was not attained owing to the high standard deviation in the control group. In the second determination, the reduction in triglyceride concentrations was confirmed, although, again, statistical significance was not attained (Table 39).

Urinalysis did not reveal any test substance–related changes. Organ weight determinations revealed reduced absolute liver weights in the 1600 ppm females. Relative liver weights were significantly decreased in high dose group females when based on brain weight, which was similar between treatment groups, but relative liver weights were increased in the same group when related to body weight, indicating that the absolute liver weight decrease was a result of the overall reduction in body weight rather than an organ-specific effect (Table 40).

Gross pathological and histopathological examinations did not show test substance–related effects at any dose level. In the liver, however, bile duct proliferation was more frequently recorded in treated males than in controls. The incidence across the groups 0 (control), 100, 400 and 1600 ppm

Table 39. Triglyceride concentrations at 24 months in rats administered cycloxydim sodium salt in the drinking-water

Concentration in drinking-water (ppm)	Triglyceride concentration			
	Males		Females	
	mmol/l	% of control	mmol/l	% of control
0	4.39	—	11.17	—
100	4.82	110	13.73	123
400	7.59	173	9.51	85
1600	6.79	155	5.24	47

From Kuehborth et al. (1988a)
Statistical evaluation: ANOVA + Dunnett's test

Table 40. Absolute and relative liver weights in rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Body weight		Absolute liver weight		Liver weight relative to body weight		Liver weight relative to brain weight	
	g	% of control	g	% of control	%	% of control	%	% of control
Males								
0	836.5	—	22.33	—	2.67	—	1028	—
100	809.2	97	21.37	96	2.64	99	986	96
400	828.9	99	21.08	94	2.54	95	991	96
1600	771.3	92	21.90	98	2.84	106	1020	99
Females								
0	495.4	—	15.31	—	3.09	—	754	—
100	511.1	103	15.52	101	3.04	98	757	100
400	457.4	92	14.07	92	3.08	100	712	94
1600	405.5**	82	13.52**	88	3.33	108	673*	89

From Kuehborth et al. (1988a)
* $P < 0.05$; ** $P < 0.01$ (Dunnett's test, two-sided); no statistics available for liver weight relative to body weight

was 2% (control), 8%, 16% and 24% in males and 8% (control), 12%, 4% and 12% in females. The number of males with bile duct proliferation exhibited a statistically significant positive trend with respect to dose rate ($Z = 4.04$, one-tailed $P < 0.001$), but the maximum incidence of bile duct proliferation in the high dose group males of the 24-month study (24%) was similar to the control group incidence in the 18-month study (20%) and below the average range of historical control data for this age-associated lesion. In females, no statistically positive trend was evident. In addition, foci or areas of various types of hepatocellular alteration were more frequently recorded in males than in females. The relative proportion of males with basophilic foci was 7% (control), 22%, 28% and 28%. The frequency of the basophilic foci did not show a dose–response relationship. Both bile duct proliferation and basophilic foci in male rats exhibited a statistically significant positive trend with respect to dose rate, but the incidences were within the historical control range (Table 41).

Cycloxydim did not have carcinogenic properties.

The administration of cycloxydim via the drinking-water resulted in a reduction in body weight at concentrations of 400 ppm and 1600 ppm. Drinking-water consumption was reduced in the 1600 ppm group. In females, there was a reduction in triglyceride levels at concentrations of 400 and 1600 ppm. The NOAEL was 100 ppm, equal to 7 mg/kg bw per day, on the basis of a reduction in

Table 41. Histopathological changes in rats administered cycloxydim sodium salt in the drinking-water for 2 years

Concentration in drinking-water (ppm)	Incidence[a]				Historical data: bile duct proliferation		Historical data: basophilic foci[b]	
	Bile duct proliferation		Basophilic foci					
	18 months	24 months	18 months	24 months				
Males								
0	4/20 (20)	2/100 (2)	2/20 (10)	7/100 (7)	Time frame	1983–1992	Time frame	1983– 1992
100	4/20 (20)	4/50 (8)	1/20 (5)	11/50 (22)	Average	27%	Average	13.9%
400	3/20 (15)	8/50 (16)	7/20 (35)	14/50 (28)	Minimum	0%	Minimum	0%
1600	7/20 (35)	12/50 (24)	2/20 (10)	14/50 (28)	Maximum	65%	Maximum	65%
2700	8/20 (40)	—	2/20 (10)	—	Studies	21	Studies	21
Females								
0	0/20 (0)	8/100 (8)	5/20 (25)	8/100 (8)	Time frame	1983–1992	Time frame	1983–1992
100	1/20 (5)	6/50 (12)	0/20 (0)	5/50 (10)	Average	11%	Average	17%
400	1/20 (5)	2/50 (4)	3/20 (15)	8/50 (16)	Minimum	0%	Minimum	0%
1600	2/20 (10)	6/50 (12)	1/20 (5)	3/50 (6)	Maximum	75%	Maximum	56%
2700	0/20 (0)	—	0/20 (0)	—	Studies	21	Studies	21

From Kuehborth et al. (1988a)

[a] No. of occurrences/no. of animals examined; percentage given in parentheses.

[b] In early studies at the test facility, different liver foci types (i.e. eosinophilic, basophilic, clear cell, mixed cell) were subsumed under the diagnostic term "foci of hepatocellular alteration". No differential diagnosis for different foci types was made; therefore, foci subsets, including basophilic foci, were not listed in early studies. This is considered to be the reason for the apparent time-dependent shift in control group incidences (zero incidences in early studies, e.g. 1981–1983, and moderate to high incidences in later studies, e.g. 1988–1993), which is the consequence of the gradual introduction of foci type–specific quantification of hepatocellular foci by the test facility pathologists in the mid to late 1980s.

body weight and a reduction in concentrations of triglycerides in rats given drinking-water containing cycloxydim at concentrations of 400 ppm and above. Cycloxydim was not carcinogenic to rats. The study complied with GLP, and a QA statement was attached (Kuehborth et al., 1988a).

2.4 Genotoxicity

Cycloxydim was tested for genotoxicity in an adequate range of assays of 11 genotoxicity studies, of which 9 are in vitro studies and 2 are in vivo studies (Table 42). Cycloxydim acid and the sodium salt gave negative results throughout, except at cytotoxic concentrations in studies of chromosomal aberrations in vitro. Most of the studies complied with GLP, and QA statements were attached.

On the basis of these studies, it is concluded that cycloxydim is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was administered to groups of 24 male and 24 female Wistar rats (F_0 parental generation) as the sodium salt via the drinking-water at concentrations of 0, 100, 400 and 1600 ppm. At the beginning of the substance administration, the age of F_0 generation rats was 55 days, and the mean weights were 246.6 g and 168.0 g for males and females, respectively.

Table 42. Results of genotoxicity studies with cycloxydim

Test system	Test compound Strain/species	Concentration	Purity (%)	Result	Reference
In vitro					
Gene mutation Bacterial cells Ames test	Cycloxydim sodium salt *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 Standard plate technique	0, 20, 100, 500, 2000, 5000 µg/plate (1st experiment) 0, 60, 300, 1500, 7500, 15 000 µg/plate (2nd experiment)	93.9	Negative with S9 mix Negative without S9 mix	Engelhardt & Gelbke (1985b)
Gene mutation Bacterial cells Ames test	Cycloxydim (acid) *S. typhimurium* TA98, TA100, TA1535, TA1537 Standard plate technique	0, 20, 100, 500, 2500, 5000 µg/plate	92.2	Negative with S9 mix Negative without S9 mix	Engelhardt & Gelbke (1983)
Gene mutation Mammalian cells	Cycloxydim sodium salt CHO cells (*HPRT* locus)	5, 10, 15, 21, 28, 35, 40 mg/ml	93.9	Negative with S9 mix Negative without S9 mix	den Boer & Hoorn (1985)
Gene mutation Mammalian cells	Cycloxydim sodium salt CHO cells (*HPRT* locus)	Ranged from 0.215 to 21.5 mg/ml (1st experiment), 0.1 to 4.64 mg/ml (2nd experiment) and 0.215 to 4.64 mg/ml (3rd experiment)	93.9	Negative with S9 mix Negative without S9 mix	Jaeckh & Gelbke (1986)
Gene mutation/ chromosomal aberration Mammalian cells	Cycloxydim sodium salt Mouse lymphoma forward mutation assay L5178Y Tk+/− cells (*TK* locus)	Ranged from 1.75 to 20 mg/ml with and without metabolic activation (1st experiment) 5–12.5 mg/ml in the 2nd experiment (with metabolic activation only)	93.9	Weakly positive in the presence of cytotoxicity with S9 mix Negative without S9 mix	den Boer (1985)
Chromosomal aberration Mammalian cells	Cycloxydim sodium salt CHO cells	2000, 3333, 4000, 5000 µg/ml	93.9	Negative with S9 mix Weakly positive in the presence of cytotoxicity without S9 mix	Taalman (1985a)
Chromosomal aberration Mammalian cells	Cycloxydim (acid) CHO cells	500, 1666.6, 5000 µg/ml 16.6, 50, 166.6, 500, 1666.6 µg/ml	93.9	Negative with S9 mix Weakly positive in the presence of cytotoxicity without S9 mix	Taalman (1985b)
Unscheduled DNA synthesis	Cycloxydim sodium salt Rat primary hepatocytes	0.906, 1.81, 3.63, 9.06, 18.1, 36.3, 90.6 µg/ml	93.9	Negative	Cifone & Brusick (1985)
Unscheduled DNA synthesis	Cycloxydim (acid) Rat primary hepatocytes	100, 250, 375, 500, 750, 1000, 1500, 2000 µg/ml	93.9	Negative	Cifone & Myhr (1985)
In vivo					
Micronucleus test	Cycloxydim sodium salt NMRI mice	Oral (gavage, one application): 0, 225, 450, 900 mg/kg bw	93.9	Negative	Engelhardt & Gelbke (1985a)
Chromosomal aberration test	Cycloxydim sodium salt Chinese hamster	Oral (gavage, one application): 0, 500, 1700, 5000 mg/kg bw	36.56	Negative	Taalman (1987)

CHO, Chinese hamster ovary; S9, 9000 × *g* rat liver supernatant

A stock solution of the test substance was prepared by dissolving cycloxydim in 2 N NaOH. The end concentrations were achieved by dilution of the stock solution with drinking-water. The pH of the drinking-water solutions was adjusted to a neutral range (at a pH of between 7 and 8.3) with 0.5 N HCl solution.

The doses were selected based on the results of a 4-week study and a 3-month study and the preliminary results of a chronic study in the same rat strain and using the same route of administration (drinking-water studies with the sodium salt). At least 70 days after the beginning of treatment, one female was mated with one male overnight for a maximum period of 3 weeks to produce the F_1 litter. The F_0 generation was remated at least 10 days after the last weaning to produce a second litter. From the F_{1a} pups, 24 males and 24 females per dose group were selected as the F_1 parent generation to produce the F_2 generation (one litter). The F_1 generation parental animals received the test substance at least 98 days before mating (Table 43).

The examination of parental animals included monitoring for clinical symptoms/mortalities, food consumption, body weight development, mating and reproductive performance. Pathological examination was performed by gross inspection as well as extensive histopathological examination, with special attention to the organs of the reproductive system. Pups were sexed and monitored with respect to their developmental stages and behaviour in certain tests. All pups were examined macroscopically at necropsy (external and organ findings); stillborn pups and pups that died intercurrently were additionally examined for any skeletal findings.

Sufficient stability of the test substance was demonstrated for the duration of the study (93.9% at the start and 90.1% at the last determination), and its homogeneity was proven. The stability of the aqueous sodium salt solution as well as the correctness of intended concentrations (dose levels) were confirmed by analysis. The test substance intake is given in Table 44.

There were no clinical signs or mortalities at any dose level in parental animals treated with cycloxydim sodium salt that could be attributed to the test compound. Effects on food consumption, water consumption and body weight development are summarized in Table 45.

At the high dose level of 1600 ppm, the pattern of effects observed during the study differed between the F_0 and F_1 parents:

Table 43. Study design: two-generation drinking-water study in Wistar rats

Parameter	Concentration in drinking-water (ppm)			
	0 (control)	100	400	1600
F_0 generation – parent animals	Treatment period at day 55 postpartum for at least 70 days prior to mating to produce F_{1a} and F_{1b} pups until sacrifice. Infertile animals were remated with a fertile partner.			
- No. of males	24	24	24	24
- No. of females	24	24	24	24
F_1 generation – parent animals	Treatment at least 98 days prior to mating to produce F_2 pups (only one litter) until sacrifice. Infertile animals were remated with a fertile partner.			
- No. of males	24	24	24	24
- No. of females	24	24	24	24
$F_{1a/b}$ and F_{2a} generation pups	Reared until day 4 postpartum, when four males and four females were selected for further rearing until day 21 postpartum. The remainder were sacrificed and examined macroscopically (external and organs). Stillborn pups or pups that died as well as pups showing remarkable findings during rearing or abnormalities during macroscopic inspection were examined according to the method of Wilson (head) and Kimmel (modified) or X-rayed to examine the skeleton.			

From Hellwig et al. (1988)

Table 44. Test substance intake in a two-generation drinking-water study in Wistar rats

Parameter	Test substance intake (mg/kg bw per day)		
	Concentration in drinking-water (ppm)		
	100	400	1600
F_0 generation animals			
Premating period (days 0–70), males	7.46	30.66	113.26
Premating period (days 0–70), females	9.81	38.12	128.65
Average intake males/females	8.64	34.39	120.96
F_{1a} litter gestation period (days 0–20), females	10.59	43.37	149.09
F_{1a} litter lactation period (days 0–21), females	21.23	85.31	253.15
F_{1b} litter gestation period (days 0–20), females	10.19	37.85	120.82
F_{1b} litter lactation period (days 0–21), females	23.93	83.99	242.99
F_1 generation animals			
Premating period (days 0–98), males	9.68	38.19	132.18
Premating period (days 0–98), females	11.93	44.11	142.06
Average intake males/females	10.81	41.15	137.12
F_{2a} litter gestation period (days 0–20), females	10.36	42.38	149.90
F_{2a} litter lactation period (days 0–21), females	26.58	107.54	368.19

From Hellwig et al. (1988)

F_0 generation:

- reduced food consumption in females during gestation and lactation;
- reduced water consumption in females throughout the study, most pronounced during lactation of the F_{1a} and F_{1b} litters (65% and 56% of control value, respectively);
- reduced body weight and/or body weight gain in females, statistically significant during gestation and lactation.

F_1 generation:

- reduced food consumption in males and females during the premating period and in females during lactation;
- reduced water consumption in males during the premating period and in females throughout the study; more pronounced during the premating than during the lactation period (56% versus 77% of control value, respectively);
- reduced body weight and/or body weight gain in males and females, statistically significant decrease in females during lactation.

At the middle dose level of 400 ppm, these effects were less pronounced:

- water consumption was slightly reduced in F_0 dams during F_{1b} lactation (86%) and in F_1 females during F_{2a} premating (82% of control value);
- body weight and body weight gain were temporarily reduced in F_0 females during gestation and especially during lactation, whereas F_1 female body weight development appeared to have been marginally affected during the premating period.

Table 45. Effects on food consumption, water intake and body weight gain in a two-generation drinking-water study in Wistar rats

Parameter	Effects (% of control)								
	F_0 generation → F_{1a} litter			F_0 generation → F_{1b} litter			F_1 generation → F_{2a} litter		
	Concentration in drinking-water (ppm)								
	100	400	1600	100	400	1600	100	400	1600
Premating period (F_0: days 0–70; F_1: days 0–98)									
Food consumption[a]									
- Males	102	101	99	NA	NA	NA	98	98	87
- Females	100	98	96	NA	NA	NA	98	94	86
Water consumption[a]									
- Males	102	103	95	NA	NA	NA	99	96	71
- Females	103	100	81	NA	NA	NA	94	82	56
Body weight gain									
- Males	102	96	95	NA	NA	NA	96	142	85
- Females	98	96	85	NA	NA	NA	95	94	83
Gestation period (days 0–20 p.c.) females									
Food consumption[a]	96	93	90	95	91	87	96	96	93
Water consumption[a]	100	102	83	99	90	67	93	92	72
Body weight gain	96	91	77**	93	85**	73**	104	102	92
Lactation period (days 0–21 p.p.) females									
Food consumption[a]	86	90	76	90	87	71	99	100	86
Water consumption[a]	94	93	65	102	86	56	101	100	77
Body weight gain	76	62*	23**	106	91	78	127	146	111
Body weight (day 21 p.p.)	97*	95**	88**	98	95**	87**	100	99	88**

From Hellwig et al. (1988)
NA, not applicable; p.c., postcoitum; p.p., postpartum; * $P < 0.05$; ** $P < 0.01$ (Williams test)
[a] No statistical analysis performed.

No effects on food consumption, drinking-water uptake and body weight or body weight gain were noted at the low dose of 100 ppm that were considered treatment related.

There were no effects on reproductive function, such as mating performance, pregnancy rate, mean duration of pregnancy or littering/lactation of the offspring. The relatively low fertility index in the F_{1a} litter at 1600 ppm must be regarded as incidental, as all F_0 parents (sires and dams) proved to be fertile at the F_{1b} mating. In addition, no such findings were noted for the F_1 parental animals and the F_{2a} mating.

Treatment-related findings in pups (Table 46) were confined to the highest dose group of 1600 ppm. On the day of birth (day 0 postpartum [p.p.]), the mean number of live pups per litter was slightly reduced in all high dose group litters (F_{1a}, −6%, $P < 0.01$; F_{1b}, −10%, not statistically significant [n.s.]; and F_{2a}, −9%, n.s.). For the F_{1a} litters only, the reduction in the number of live pups per litter was accompanied by an increased mean number of dead pups per litter (0.84 versus 0.09, $P < 0.01$); however, the percentage of stillborn F_{1b} and F_{2a} pups of the high dose group was lower when compared with the respective control groups. The viability index appeared to be slightly reduced in the case of the high dose group F_{1b} pups, but was of a similar range in the high dose group F_{1a} and F_{2a} pups when compared with the control group indices. The lactation index was clearly decreased with regard to the F_{1a} and F_{1b} pups compared with the control, but not in the case of the F_{2a} pups, for which

Table 46. Pup findings in a two-generation study in Wistar rats (F_{1a}/F_{1b} and F_{2a} pups)

	Concentration in drinking-water (ppm)							
	0 (control)		100		400		1600	
No. of live pups per litter, day 0 p.p. (% of control)								
F_{1a} litter	12.65	(—)	12.26	(97)	11.70	(92)	11.89††	(94)
F_{1b} litter	14.74	(—)	14.29	(97)	13.54	(92)	13.22	(90)
F_{2a} litter	12.74	(—)	13.52	(106)	13.76	(108)	11.64	(91)
Viability index, day 4 versus day 0 p.p. (% of control)								
F_{1a} litter	95.94	(—)	88.75	(93)	94.98	(99)	90.58	(94)
F_{1b} litter	94.55	(—)	94.97	(100)	93.62	(99)	85.77	(91)
F_{2a} litter	94.10	(—)	94.33	(100)	94.12	(100)	92.77	(99)
Lactation index, day 21 versus day 4 p.p. (% of control)								
F_{1a} litter	87.63	(—)	86.83	(99)	87.51	(100)	70.74	(81)
F_{1b} litter	80.39	(—)	88.88	(111)	83.17	(103)	62.31†	(78)
F_{2a} litter	84.10	(—)	98.34	(117)	97.91	(116)	92.90	(110)
Pup body weight males, g (% of control)								
F_{1a} litter								
- Day 0	6.12	(—)	6.03	(99)	6.13	(100)	5.95	(97)
- Day 21	44.08	(—)	44.82	(102)	44.65	(101)	39.84	(90)
F_{1b} litter								
- Day 0	5.97	(—)	5.92	(99)	6.07	(102)	5.66**	(95)
- Day 21	41.09	(—)	39.73	(97)	38.58	(94)	33.85**	(82)
F_{2a} litter								
- Day 0	6.11	(—)	5.85	(96)	5.89	(96)	5.89	(96)
- Day 21	46.84	(—)	42.69	(91)	42.07*	(90)	41.65*	(89)
Pup body weight females, g (% of control)								
F_{1a} litter								
- Day 0	5.74	(—)	5.75	(100)	5.72	(100)	5.67	(99)
- Day 21	41.95	(—)	40.81	(97)	42.08	(100)	36.23*	(86)
F_{1b} litter								
- Day 0	5.67	(—)	5.58	(98)	5.71	(101)	5.30**	(93)
- Day 21	39.14	(—)	38.10	(97)	37.04	(95)	34.08**	(87)
F_{2a} litter								
- Day 0	5.77	(—)	5.63	(98)	5.56	(96)	5.64	(98)
- Day 21	46.21	(—)	41.11*	(89)	39.79*	(86)	41.86*	(91)
Litter size, day 21 p.p. (% of control)								
F_{1a} litter	10.65	(—)	10.00	(94)	9.91	(93)	8.16	(77)
F_{1b} litter	11.35	(—)	12.46	(110)	11.00	(97)	7.39	(65)
F_{2a} litter	10.39	(—)	12.87	(124)	12.95	(125)	10.09	(97)
Litter weight, day 21 p.p. (% of control)								
F_{1a} litter	469.5	(—)	448.2	(95)	442.5	(94)	325.1	(69)
F_{1b} litter	466.4	(—)	495.0	(106)	424.4	(91)	250.2	(54)
F_{2a} litter	483.3	(—)	539.3	(112)	530.0	(110)	421.3	(87)

From Hellwig et al. (1988)

* $P < 0.05$ / ** $P < 0.01$ (Williams trend test); † $P < 0.05$ / †† $P < 0.01$ (Krauth test)

the highest incidence of intercurrent deaths (i.e. lowest lactation index) was established in the control group. Pup weight and growth were reduced or retarded for all generations (F_{1a}, F_{1b} and F_{2a}).

A lower mean pup weight was also observed in F_{2a} pups at dose levels of 100 and 400 ppm. These reductions in mean pup weight should not be considered test substance related, but can be explained by the higher number of pups that died during rearing in the F_{2a} controls. Thus, dams of the 100 and 400 ppm test substance groups had to nurse more pups in comparison with the control group. The increased litter sizes at 100 and 400 ppm correspond to a 10% higher mean litter weight, however, causing a reduced weight development in the groups with high litter size.

In F_{1a} and F_{1b} pups of high dose group dams, morphological development appeared to be retarded: ear unfolding and opening were delayed in the F_{1b} litter, and eye opening was delayed in both F_{1a} and F_{1b} litters. In addition, an increased number of female F_{1a} pups failed in the gripping (holding) reflex test. These effects on morphological and behavioural development are considered to be related to the growth retardation noted for these pups.

There was no indication of any adverse effects on development in F_{2a} pups up to the highest dose tested.

With respect to external, internal and skeletal examination, there were no treatment-related effects on F_{1a}, F_{1b} or F_{2a} pups at any dose level.

In order to assess the effects on F_0 offspring during the lactation period, the quality and extent of maternal toxicity have to be taken into consideration. Besides decreased food consumption and resulting decreased body weight gain, the notably low consumption of drinking-water during gestation and lactation most probably resulted in decreased nutritional status of the F_{1a} and F_{1b} pups. The reduced water intake of the high dose group dams is considered to stem from the decreased palatability of the drinking-water in which the test substance was administered. Dams of the F_{1b} pups (in which the adverse effects observed were most remarkable) also showed the lowest water intake (56% of the control value). It is highly probable that less mothers' milk was available to pups of the high dose group F_0 dams compared with the other treatment group pups. A reduction in the milk quantity can be explained by the reduced water intake of dams (due to unfavourable palatability). This conclusion is substantiated by the fact that adverse effects of F_{2a} pups, whose dams consumed a considerably higher amount of drinking-water than did F_0 dams, were either non-existent or far less pronounced than in F_{1a} and F_{1b} pups. A direct effect of cycloxydim on milk quantity is highly unlikely. In conclusion, the growth retardation of high dose group pups during the lactation period is most probably related to a reduced water intake of the dams and not to a direct test substance–related effect on milk quality or quantity.

In view of the above findings and analysis, it can be concluded that cycloxydim administered as the sodium salt via the drinking-water to rats in a two-generation study caused clear signs of toxicity, such as reduced food consumption and depression of mean body weights and body weight gain in parental animals at the high dose level of 1600 ppm. In addition, a pronounced reduction in drinking-water consumption was observed at 1600 ppm. The number of live pups from high dose group F_{1a}, F_{1b} and F_{2a} litters was reduced at the day of birth, which, only in the case of the F_{1a} pups, was also associated with a slightly increased number of dead pups on day 0 p.p. Furthermore, the lactation index was decreased for F_{1a} and F_{1b} pups (not for F_{2a} pups) at 1600 ppm. High dose group pups from all generations showed reduced weight and retarded growth rate; morphological development (time of ear and eye opening) as well as gripping reflex test were impaired to some degree only in the case of F_1 pups. No adverse effects on morphological or behavioural development were seen in F_{2a} pups. Effects on high dose group F_{1a} and F_{1b} pups observed during the lactation period (increased mortality, delayed growth and development) were most probably a consequence of drastically reduced water intake by the F_0 dams (as low as 56% of the control level), which must have had an impact on milk quantity and hence resulted in malnutrition of their pups during lactation. The reduced water intake was considered to be related to unfavourable palatability of the drinking-water in which the test sub-

stance was offered to the dams. At 400 ppm, female parental animals were affected with respect to marginally reduced food and water consumption as well as reduced body weights and/or body weight gains during some parts of the study. No effects were noted for the pups at this dose level. A dose of 100 ppm did not show any findings at all. The fertility after administration of the test substance was not affected at any of the doses tested.

The NOAEL for offspring toxicity was 400 ppm, equal to 38 mg/kg bw per day, on the basis of reduced survival, reduced growth and developmental retardation in pups at 1600 ppm, equal to 129 mg/kg bw per day, the highest dose tested. Reproductive toxicity was not affected by treatment at drinking-water concentrations of up to 1600 ppm. The NOAEL for parental toxicity was 100 ppm, equal to 9.7 mg/kg bw per day, on the basis of reductions in food consumption, body weight and body weight gain in dams at 400 ppm. The study complied with GLP, and a QA statement was attached (Hellwig et al., 1988).

(b) Developmental toxicity

Rats

Cycloxydim (purity 93.9%; batch No. N 88) was prepared as a sodium salt and examined for its prenatal toxicity in Wistar rats. The dams (25 per test group) were treated from day 6 through day 15 postcoitum (p.c.) with daily cycloxydim doses of 0 (control treated with the vehicle, double-distilled water), 100, 200 and 400 mg/kg bw per day by gavage at a constant dosing volume of 10 ml/kg bw per day.

The animals were observed for food consumption and body weight gain regularly throughout the study period. Their state of health was checked daily. On day 20 p.c., all females were sacrificed and assessed by gross pathology. After caesarean section, reproductive parameters such as numbers of implantations, resorptions and corpora lutea were determined, and preimplantation and postimplantation loss and conception rate were calculated. The fetuses were dissected from the uterus, sexed and weighed. About two thirds of the fetuses were subjected to assessment of the skeleton; about one third of the fetuses were assessed for visceral abnormalities. The fetuses intended for assessment of the skeleton were also eviscerated, and the organs were assessed (after being fixed in ethanol).

Maternal data are compiled in Table 47. There were no clinical signs or mortalities noted in treatment group dams. Food consumption was slightly reduced at the onset of dosing (days 7 and 8), gaining statistical significance (about 8% absolute level) at the high dose level of 400 mg/kg bw per day and at the middle and high dose levels (4% and 8%, respectively) when compared on a milligram per kilogram body weight basis. At the end of the treatment period, the body weight gain at the high dose level was 13% below the control (days 13–15, no statistical significance). The assessment of the difference between the body weights of the dams at the end (without uterus) and at the beginning of the study showed a decrease of 9% at 400 mg/kg bw per day, which was statistically significant compared with control values. The slight, statistically significant decrease in food consumption at 200 mg/kg bw per day is considered incidental, because it did not correspond with changes in the net body weight change.

Evaluation of reproductive parameters following caesarean section (Table 48) revealed no treatment-related effects on uterus or placental weights, number of corpora lutea, incidence of live or dead implantations, incidence of early, intermediate or late resorptions, preimplantation or postimplantation loss, or incidence of live or dead fetuses. At the 400 mg/kg bw per day level, a statistically significant reduction in the mean fetal weight (especially in the male fetuses: −5.2%; overall: −4.3%) was observed. No significant effects on fetal weight were observed at 200 or 100 mg/kg bw per day.

The assessment of external abnormalities did not reveal any substance-induced increase compared with the control, even at the highest dose level. The incidence of skeletal changes was not increased up to a dose of 200 mg/kg bw per day. At a dose level of 400 mg/kg bw per day, however, a

Table 47. Maternal data: first prenatal toxicity gavage study in Wistar rats

	Effects on food consumption and body weight gain							
	Dose (mg/kg bw per day)							
	0		100		200		400	
	g	% of control	g	% of control	g	% of control	g	% of control
Food consumption								
- Absolute, days 7–8	47.6	—	48.0	101	45.8	96	43.8**	92
- Relative,[a] days 7–8	196.0	—	194.8	99	187.9*	96	179.8**	92
Body weight gain								
- Days 6–15	43.35	—	41.82	96	39.83	92	39.96[n.s.]	92
- Days 13–15	11.04	—	10.13	92	10.67	97	9.61[n.s.]	87
Corrected body weight gain[b]	65.1	—	69.2	106	63.1	97	59.5*	91

From Hellwig & Hildebrand (1987a)
* $P < 0.05$; ** $P < 0.01$ (Williams test); n.s., not statistically significant
[a] g/kg bw.
[b] Net weight change during the study.

Table 48. Mean fetal weight in first prenatal toxicity gavage study in Wistar rats

	Mean fetal weight							
	Dose (mg/kg bw per day)							
	0		100		200		400	
	g	% of control	g	% of control	g	% of control	g	% of control
Males	3.85	—	3.82	99.2	3.74	97.1	3.65**	94.8
Females	3.66	—	3.66	100	3.57	97.5	3.50*	95.6
Males and females	3.74	—	3.76	101	3.64	97.3	3.58*	95.7

From Hellwig & Hildebrand (1987a)
* $P < 0.05$; ** $P < 0.01$ (Williams test)

pronounced increase in changes of the vertebral column and the sternebrae was observed. Statistically significantly increased numbers of fetuses per litter with retardations of the skeleton were observed. Although these findings are also seen in the control, their increased incidence is clearly attributable to the test substance administration at 400 mg/kg bw per day and is in line with the reduced fetal body weight at this dose level. Statistically significant increases in the number of anomalies compared with the untreated control were found. The majority of anomalies were found mainly in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage (Table 49).

Keeping in view the above facts, it can be concluded that cycloxydim when given as the sodium salt in aqueous solution from day 6 through day 15 p.c. to pregnant rats via gavage caused no effects on the maternal or fetal organism at 100 or 200 mg/kg bw per day. At the top dose of 400 mg/kg bw per day, maternal toxicity was evident with respect to a slight, statistically significant reduction in food consumption during onset of dosing (days 7–8). Body weight gain at the end of the treatment period (day 20) was 13% lower (no statistical significance) than control, as was the corrected body weight gain (9%; statistically significant). In fetuses, reduced fetal weight was observed at 400 mg/kg bw per day, which correlated with an increase in skeletal anomalies,

Table 49. Incidence of skeletal anomalies in fetuses in the prenatal rat study

Skeletal anomalies	Fetal incidence[a]			
	Dose level (mg/kg bw per day)			
	0	100	200	400
"Dumbbell"-shaped ossification centres, thoracic region	5/197 (2.5)	3/174 (4.6)	2/205 (4.4)	60/199 (30)
"Bipartite"-shaped ossification centres, thoracic region	0/197 (0.0)	4/174 (2.3)	2/205 (1.0)	7/199 (3.5)
"Bipartite" ossification centres of the xyphoid process	2/197 (1.0)	3/174 (1.7)	5/205 (2.4)	8/199 (4.0)
Insufficient cartilaginification of the 13th rib (ossification centres present)	21/197 (11)	15/174 (8.6)	23/205 (11)	33/199 (17)
"Bipartite" ossification centres of the vertebral bodies in the thoracic region (without "cones" of cartilage)	0/197 (0.0)	0/174 (0.0)	0/205 (0.0)	4/199 (2.0)
Incomplete ossification of the sternebrae (cartilage present)	10/197 (5.1)	14/174 (8.0)	31/205 (15)	66/199 (33)

From Hellwig & Hildebrand (1987a)
[a] No. of fetuses with anomalies/no. of fetuses examined; percentage given in parentheses.

both gaining statistical significance at this dose level. Skeletal anomalies were found mainly in the thoracic part of the vertebral column and consisted of dumbbell-shaped or bipartite vertebral bodies with involvement of the cartilage.

The NOAEL for maternal toxicity and embryo/fetotoxicity was 200 mg/kg bw per day. Increased numbers of fetuses per litter with retardations and a statistically significant increase in the frequency of anomalies of the vertebral column and the sternebrae with involvement of the cartilage and incomplete ossification were observed. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987a).

A supplementary study in rats was undertaken to determine the dose of cycloxydim that elicits maternally toxic effects in pregnant animals. Investigation of the fetal toxicity was confined to assessment of resorption rates and to fetal weight determinations. Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered to 25 Wistar rats per group from day 6 through day 15 p.c. with doses of 0 (control treated with the vehicle, double-distilled water), 200, 400, 600 and 800 mg/kg bw per day by gavage and at a constant dosing volume of 10 ml/kg bw per day. Half of the total daily dose was given twice a day at an interval of at least 4 h to avoid test substance flocculation and a change in pH, which would have occurred if 600 or 800 mg/kg bw per day would have been given in one dose daily.

The dams were observed for food consumption and body weight gain regularly throughout the study period. Their health was checked daily. Haematology (leukocytes, erythrocytes, haemoglobin, haematocrit, mean cell volume, mean haemoglobin content per erythrocyte, mean corpuscular haemoglobin concentration, platelets) and clinicochemical parameters (enzymes: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase; blood chemistry: sodium, potassium, chloride, inorganic phosphate, calcium, urea, creatinine, glucose, total bilirubin, total protein, albumin, globulins, triglycerides, cholesterol, iron, copper, zinc, manganese) were determined on days 16 and 20 of the study in 10 animals to better understand maternal toxicity at these dose levels in pregnant rats. On day 20 p.c., all females were sacrificed and assessed by gross pathology. Reproductive parameters after caesarean section, such as numbers of implantations, resorptions and corpora lutea, were determined, and preimplantation and postimplantation loss and conception rate were calculated.

The fetuses were dissected from the uterus, sexed, weighed and examined externally. After fixation, they were examined macroscopically. No further fetal examinations were performed.

No mortalities were observed in any treatment group. One dam at 600 mg/kg bw per day temporarily showed reduced nutritional state, piloerection and intermittent respiration. At 800 mg/kg bw per day, two dams showed vaginal haemorrhages, and three further dams were found to have fur smeared with urine.

On days 7–8, there was a (substantially dose-dependent) reduction in food consumption in all of the treated groups. Whereas the marginal effect at the 200 mg/kg bw per day dose level was assessed as incidental in the absence of effects on body weight, the reduced feed intake at 400 mg/kg bw per day may have been test substance related, as it was associated with body weight loss at the same time point of investigation. The effect on food consumption at 600 and 800 mg/kg bw per day persisted throughout the treatment period. Compared with control values, a statistically significant decrease in body weight (mainly $P < 0.01$) was noted at the 600 and 800 mg/kg bw per day dose levels, whereas there was no statistical significance or biological relevance at 200 or 400 mg/kg bw per day throughout treatment. Body weight gain was affected on days 6–8 at 400, 600 and 800 mg/kg bw per day ($P < 0.01$). At later intervals, body weight gain was affected only in some rats at dose levels of 600 and 800 mg/kg bw per day, and overall body weight gain was reduced at 600 and 800 mg/kg bw per day (83.9% and 84.4%, respectively; $P < 0.01$). The corrected body weight gain was statistically significantly below the respective control value at 400 mg/kg bw per day ($P < 0.05$) and higher doses ($P < 0.01$).

Some clinicochemical parameters appeared to have been affected by treatment. The increase in creatinine values at the high dose level is in line with the toxicological profile of cycloxydim and has also been observed in other toxicological studies. It is unclear whether the increase in reticulocytes observed at the second examination point on day 20 should be interpreted as a compensatory effect of the bone marrow due to blood sampling at day 16 p.c. or as a consequence of the slightly reduced haemoglobin, erythrocyte and haematocrit values observed at the first sampling. The fact that other affected parameters could not be reproduced at the second sampling could be the consequence of the short time between both sampling points (4 days). Another possible explanation given by the study director is the fact that food consumption increased after substance administration had been stopped. Selected organ weights measured (absolute and relative liver, kidney and adrenal weights) showed no difference compared with the untreated control. Assessment of embryo/fetal mortality revealed no decrease in the number of live fetuses and no increase in prenatal mortality. Some macroscopic findings, such as absent/filiform tail, anal atresia and one skin defect, were observed in a very small number of fetuses at 600 mg/kg bw per day (two fetuses in two litters examined) or 800 mg/kg bw per day (four fetuses in three litters examined). Fetal weight was statistically significantly decreased at 600 and 800 mg/kg bw per day.

In view of the above, it is concluded that increased creatinine values at 800 mg/kg bw per day, decreased inorganic phosphate levels at 400–800 mg/kg bw per day and a weak effect on red blood cells (reduced haemoglobin, erythrocyte and haematocrit values) at doses of 400 mg/kg bw per day and above were indicative of maternal toxicity. Clinical findings (vaginal haemorrhages, fur smeared with urine) in a few animals at 800 mg/kg bw per day were also indicative of maternal toxicity.

A full-scale examination of the fetuses was not the scope of this study, and therefore fetuses were not examined for internal or skeletal changes. Nevertheless, there is no indication that the NOAEL for parameters examined is lower than that in the first study. Fetal weights were reduced at 600 and 800 mg/kg bw per day with statistical significance. The types of external findings at the 600 and 800 mg/kg bw per day dose levels (filiform tail with caudal vertebrae absent, anal atresia, dorsal skin defect, absent tail) have also been observed in the historical control.

The NOAEL for maternal toxicity was established at 200 mg/kg bw per day. A NOAEL for embryo/fetotoxicity was not derived owing to the limited scope of examinations performed. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987c).

A second supplementary study in rats was undertaken to investigate the persistence of the changes to vertebral bodies observed in the prenatal toxicity study at high dose levels of cycloxydim (i.e. 600 and 800 mg/kg bw per day) observed in the above study. Cycloxydim (purity 93.9%; batch No. N 88) as the sodium salt was administered by gavage to pregnant rats at a dose level of 400 mg/kg bw per day from day 6 through day 15 p.c. The effects noted in dams for the prenatal segment were comparable with those noted in the previous prenatal toxicity study conducted in the same rat strain and described above (Hellwig & Hildebrand, 1987a). The maternal body weight gain was clearly reduced during the first days of treatment, and body weights were slightly reduced during the rest of gestation. Corrected body weight was decreased by 9%. The pattern of findings at caesarean section (decreased fetal weight) and skeletal findings (marked increase in dumbbell-shaped or bipartite vertebral bodies and retarded skeletal ossification) also corresponded to the results of the main prenatal toxicity study in rats. In the postnatal segment, the same pattern of maternal effects was noted. Perinatal mortality (deaths on days 0–1) was slightly increased in pups from dams sacrificed on day 21 p.p., but not in pups from dams killed on day 7 p.p., although treatment was identical. It is noteworthy that the incidence of specific skeletal "anomalies" is reduced by two thirds in a comparison of the total fetal incidence (day 20 p.c.) with the pup incidence approximately 3 weeks later (day 21 p.p.).

As this study was designed to compare the effect level for skeletal anomalies from the late fetal stage through pup development until day 21 p.p., no NOAEL determination has been taken into consideration for this study. The study complied with GLP, and a QA statement was attached (Hellwig & Hildebrand, 1987b).

Rabbits

This study was undertaken for ascertaining the prenatal toxicity of cycloxydim in Himalayan rabbits (strain Chhb:HM) in accordance with the USEPA/Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines, OECD test guideline 414 and GLP of USEPA/FIFRA (Merkle & Hildebrand, 1985). Subsequently, however, an amendment to the study was done. Instead of discarding the skeleton of rabbits after evaluation of X-ray pictures, the skeletons of all rabbit fetuses in the highest dose group (400 mg/kg bw per day) and individual fetuses in the control group were stained according to the method of Kimmel for comparison and assessed (Hellwig, 1986).

The sodium salt of cycloxydim (purity 93.9%; batch No. N 88) in aqueous medium was administered to 15 female Himalayan rabbits (strain Chhb:HM) per test group by gavage from days 6 through 18 postinsemination (p.i.). Doses selected were 0 (control), 100, 200 and 400 mg/kg bw per day in a constant volume of 10 ml/kg bw per day. The control group was administered the vehicle (distilled water) only. Body weights and food consumption were monitored throughout the study. The animals were examined twice daily for mortality and clinical symptoms. All surviving animals were sacrificed on day 29 p.i., and the fetuses were delivered by caesarean section. Postmortem examinations included gross macroscopic examinations of all internal organs. Reproductive parameters after caesarean section, such as uterine weight and numbers of corpora lutea, implantations, resorptions and live/dead fetuses, were determined. Fetuses were sexed, weighed and further examined for any external, soft tissue and skeletal findings. In addition to X-ray analysis, the fetuses from the 400 mg/kg bw per day dose groups and individual fetuses from the control group were stained according to the method of Kimmel and assessed, comparable to the developmental studies in rats (Hellwig et al., 1988), in order to determine whether the vertebral body effects observed in rats also occur in rabbits using similar examination techniques.

One dam from the low dose group and one dam from the high dose group died shortly before the 8th and 10th days of treatment, respectively. As both deaths occurred during handling prior to dosing of the test substance, an influence of the test substance can be ruled out. Clinical signs were confined to one case of premature birth at 200 mg/kg bw per day and one case of abortion by a doe dosed with 400 mg/kg bw per day. Findings of premature birth and abortion are common in this

Table 50. Findings at caesarean section in a developmental toxicity study in rabbits administered cycloxydim sodium salt

Parameter	Dose level (mg/kg bw per day)				Historical control data[a]			
	0	100	200	400	A	B	C	D
Uterine weights (g)	341.86	322.92	343.23	284.58*	ND	ND	ND	ND
No. of implantations per pregnant animal	7.21	7.31	6.92	6.75	4.94	5.57	6.31	5.47
% viable implantations per pregnant animal	90.94	87.50	87.67	81.53†	68.75	90.00	91.01	83.01
% resorptions per pregnant animal	9.06	12.50	12.33	18.47†	31.25	10.00	9.00	17.00
- No. of early resorptions	9	10	9	8	ND	ND	ND	ND
- No. of intermediate resorptions	0	0	0	4	ND	ND	ND	ND
- No. of late resorptions	1	2	2	2	ND	ND	ND	ND
- No. of dead fetuses	0	0	0	1	ND	ND	ND	ND
Mean fetal weight (g)	39.3	37.3	41.2	36.5	ND	ND	ND	ND

From Merkle & Hildebrand (1985)

ND, not determined; * $P < 0.05$ (Williams test); † $P < 0.05$ (Krauth asymptotic test)

[a] Historical control data compiled for Chbb:HM Himalayan rabbit; vehicles used: A, carboxymethyl cellulose; B, 0.5% NaCl percutaneously, C, inhalation; D: oil.

rabbit strain; as there was only one case of each in the 200 and 400 mg/kg bw per day groups, these findings were not considered substance related.

At 400 mg/kg bw per day, food consumption was statistically significantly reduced from day 7 to day 21 p.i. ($P < 0.01$, Williams test), especially towards the end of test substance administration during days 15–18 p.i., when food consumption was reduced by more than 56% compared with control feed intakes. Food consumption of high dose group rabbits increased after the end of the administration period and was even higher than in controls during days 22–29 p.i. During the administration period, the dams continuously lost weight, amounting to a total of 100 g from days 6 to 18 p.i. (−4% of the initial body weight), whereas control rabbits gained weight by about 69 g (+3% of the initial body weight) during gestation days 6–18. Statistically significant reductions in body weight were observed on days 16–21 p.i. ($P < 0.05$, Williams test); thereafter, high dose group rabbits gained body weight from days 21 to 29 p.i. (i.e. after the end of treatment). The mean net body weight gain ($[bw_{end}$ − uterine weight] − bw_{start}) at 400 mg/kg bw per day was −120 g compared with −68.5 g for control group dams; statistical significance was not attained for this difference.

At 200 mg/kg bw per day, mean daily feed intakes were transiently reduced during days 12–16 p.i. (77–80% of control intakes, statistically not significant) but were similar to control levels for the other time points of investigation. Body weight gain was reduced on days 11–14 p.i. (6.86 g versus 42.36 g; $P < 0.05$, Williams test) and increased on days 21–23 p.i. (36.14 versus 10.29 g; $P < 0.05$, Williams test). No other statistically significant effects on body weight or body weight gain were observed during the study. The corrected body weight gain was comparable to control levels.

At 100 mg/kg bw per day, there were no effects observed that were suggestive of maternal toxicity.

At the high dose level, uterine weights were decreased by 17% compared with control values. The decreased uterine weight was considered to be the consequence of a slight increase in the number of dead implantations in combination with a slight decrease in the number of viable fetuses. As can be seen from the historical control data, the percentage of resorptions varies to a great extent. As there was no meaningful difference concerning the individual stages of resorptions between the treated groups and the control group, this slight effect was considered to be of a spontaneous nature. No other parameters were affected at caesarean section (Table 50).

Table 51. Total incidence of anomalies in a development toxicity study in rabbits

Parameter	Incidence[a]				Historical control data[b]		
	Dose level (mg/kg bw per day)						
	0	100	200	400	Mean	Minimum (%)	Maximum (%)
Total anomalies							
- Fetal incidence	58/91(64)	67/83 (81)	70/79 (89)	62/66 (94)			
- Litter incidence	13/14 (92.86)	13/13 (100)	13/13 (100)	12/12 (100)			
- % affected fetuses per litter	*63.20*	*80.13*	*87.40**	*92.98***			
Fetal external anomalies ("caesarean section")							
- Fetal incidence	0/91 (0)	2/83 (2)	1/79 (1.3)	8/66 (12)	24/1552 (1.5)	0.0	5.3
- Litter incidence	0/14 (0.0)	2/13 (15.4)	1/13 (7.7)	4/12 (33.3)	18/247 (7.3)	0.0	15.4
- % affected fetuses per litter	*0.0*	*3.53*	*1.10*	*9.62*	1.4	0.0	4.1
Soft tissue ("organ") anomalies							
- Fetal incidence	58/91 (64)	65/83 (78)	69/79 (87)	57/66 (86)	434/1552 (28.0)	12.1	58.7
- Litter incidence	13/14 (92.9)	13/13 (100)	13/13 (100)	12/12 (100)	182/247 (73.7)	46.2	100
- % affected fetuses per litter	*63.20*	*77.75*	*86.30**	*85.24*	28.6	11.3	58.0
Fetal skeletal anomalies							
- Fetal incidence	3/91 (3)	4/83 (5)	1/79 (1.3)	10/66 (15)	181/1552 (11.7)	4.1	19.8
- Litter incidence	2/14 (14.3)	3/13 (23.1)	1/13 (7.69)	7/12 (58.3)	117/247 (47.4)	14.3	80.0
- % affected fetuses per litter	*3.06*	*4.33*	*0.96*	*16.79*	12.0	4.1	24.7

From Merkle & Hildebrand (1985)

* $P < 0.05$; ** $P < 0.01$ (Krauth test, asymptotic)

[a] No. of fetuses (litters) with anomalies/no. of fetuses (litters) examined; percentage is in parentheses.

[b] Historical control data: 20 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986 and 1993.

The total incidence of anomalies and the incidence of specific anomalies may be seen in Table 51 and Table 52, respectively. From these tables, it can be seen that an increased incidence of affected fetuses per litter was established at 200 and 400 mg/kg bw per day. The specific anomalies are separated origin of carotids (also termed carotid branching abnormality), pseudoankylosis, asymmetrical sternebrae and fused sternebrae.

At the maternally toxic dose of 400 mg/kg bw per day, the percentage of viable implantations per dam was significantly decreased. The percentage of resorptions and skeletal anomalies was also increased (still within the historical control range, but possibly treatment related). The incidence of pseudoankylosis (flexure of paw, a common external abnormality) was increased over controls and was outside the historical control range, suggesting a substance-induced effect, at 400 mg/kg bw per day. A statistically significantly increased incidence of total anomalies (on a percentage affected fetuses per litter basis, not on a litter basis) at 200 and 400 mg/kg bw per day was mainly due to an increased occurrence of the strain-specific soft tissue abnormality "separated origin of carotids". The reason for these higher incidences is unclear, but the anomalies are nevertheless likely to be of a spontaneous origin. Owing to the very frequent occurrence of this abnormality, the increased incidence in the treatment groups is considered to be marginal.

Table 52. Incidence of specific anomalies in a developmental toxicity study in rabbits

Anomalies	Incidence[a]				Historical control data[b]		
	Dose (mg/kg bw per day)						
	0	100	200	400	Mean	Minimum (%)	Maximum (%)
External anomalies detected at caesarean section							
Pseudoankylosis							
- Fetal incidence	0/91 (0)	2/83 (2.4)	1/79 (1.3)	8/66 (12)	24/1552 (1.5)	0.0	5.3
- Litter incidence	0/14 (0)	2/13 (15.4)	1/13 (7.7)	4/12 (33.3)	18/247 (7.3)	0.0	15.4
Meningocele							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	1/66 (1.5)			
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	1/12 (8.3)			
Scoliosis							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	1/66 (1.5)			
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	1/12 (8.3)			
Soft tissue anomalies							
Separated origin of the carotids							
- Fetal incidence	58/91 (64)	65/83 (78)	69/79 (87)	57/66 (86)	315/1552 (20.3)	5.3	58.7
- Litter incidence	13/14 (93)	13/13 (100)	13/13 (100)	12/12 (100)	145/247 (58.7)	15.4	93.3
Gallbladder agenesis							
- Fetal incidence	1/91 (1.1)	1/83 (1.2)	2/79 (2.53)	2/66 (3)	14/1552 (0.9)	0.0	4.7
- Litter incidence	1/14 (7.14)	1/13 (7.69)	2/13 (15.38)	1/12 (8.33)	10/247 (4)	0.0	15.4
Skeletal anomalies							
Asymmetrical sternebra(e)[c]							
- Fetal incidence	3/91 (3.3)	3/83 (3.6)	1/79 (1.3)	5/66 (7.6)	38/1552 (2.4)	0.0	8.1
- Litter incidence	2/14 (14.3)	2/13 (15.4)	1/13 (7.7)	4/12 (33)	35/247 (14.2)	0.0	38.5
Fused sternebra(e)							
- Fetal incidence	0/91 (0)	0/83 (0)	0/79 (0)	4/66 (6.1)	66/1552 (4.3)	0.0	8.8
- Litter incidence	0/14 (0)	0/13 (0)	0/13 (0)	3/12 (25)	46/247 (18.6)	0.0	38.5

From Merkle & Hildebrand (1985)

[a] No. of fetuses (litters) with effect/no. of fetuses (litters) examined; percentage given in parentheses.

[b] Historical control data: 20 prenatal toxicity studies with Himalayan rabbits (supplier: Dr. K. Thomae) of the test facility started between 1986 and 1993.

[c] Asymmetrical sternebrae are now summarized under the term "sternebra(e) with irregular shape".

The NOAEL for maternal toxicity was 100 mg/kg bw per day, based on transiently reduced mean daily food intakes during days 12–16 p.i. and reduced body weight gain on days 11–14 p.i. The maternal toxicity observed at doses of 200 and 400 mg/kg bw per day occurred late in the study, indicating that repeated dosing over several days was required to elicit the effect. At 400 mg/kg bw per day, the percentage of viable implantations per dam was decreased, and the incidence of several skeletal anomalies (e.g. asymmetrical sternebrae and fused sternebrae) was increased above the range for the historical controls. The NOAEL for embryo/fetotoxicity was 200 mg/kg bw per day. The study complied with GLP, and a QA statement was attached (Merkle & Hildebrand, 1985).

(c) In vitro studies

Rats

An in vitro study was performed to further elucidate if the anomalies on the skeleton observed in the in vivo rat studies at a dose level of 400 mg/kg bw per day could also be induced without contribution of the maternal organism and to exclude maternal toxic effects. For this purpose, rat embryos were exposed directly to cycloxydim at a concentration of 300 µg/ml and to the main metabolite BH 517-TSO (purity not reported; batch No. L28/145) at a concentration of 150 µg/ml. Based on evaluation of toxicokinetic data, the chosen cycloxydim concentration was considered to correspond to peak serum levels that result from oral gavage treatment at 400 mg/kg bw per day (oral dose at which the skeletal changes of the thoracic vertebra were observed). For the main metabolite, the peak serum concentration was assumed to be less than half of the cycloxydim peak serum level.

The rat embryos aged 9.5 days were exposed for 48 h to the culture medium, with addition of dimethyl sulfoxide (vehicle control was used to dissolve the test compounds), cycloxydim or the BH 517-TSO metabolite. At least 30 embryos were used for the three subexperiments carried out on each tested compound. The following parameters were examined: yolk sac diameter, crown–rump length, number of somite pairs, protein content (micrograms per embryo), developmental score according to Klug and embryos showing abnormal development (macroscopic and histological evaluation).

Following 48 h of in vitro exposure of fetuses to BH 517-TSO at 150 µg/ml, no evidence of abnormal development was detectable using various parameters, including histology. Fetal exposure to cycloxydim at 300 µg/ml resulted in a slight, statistically significant growth retardation (evident as reduced protein content and a very slightly reduced crown–rump length that led to a very slightly reduced overall score). These changes can be interpreted as unspecific signs of cytotoxicity. It is noteworthy that such alterations are also observed with any test compound if the concentration is sufficiently high. However, no abnormal morphogenetic differentiation could be observed when the embryos were examined with a stereomicroscope. The extensive histological investigations also revealed no evidence for a substance-related interference with morphogenetic differentiation. Some necrosis observed was within the physiological range of developing embryonic tissues.

In view of the above, it can be concluded that no abnormal morphogenetic differentiation was observed in vitro using whole embryo culture technique in 9.5-day-old Wistar rat embryos when they were exposed to concentrations of cycloxydim or the BH 517-TSO metabolite at 300 µg/ml and 150 µg/ml, respectively. These serum concentrations are expected after oral dosing of 400 mg/kg bw per day of cycloxydim based on toxicokinetic data. In particular, no signs of abnormal development could be noted at the developing somites that will give rise to the vertebrae. A slight growth retardation of embryos exposed to cycloxydim was considered to be an expression of unspecific cytotoxicity. The study was not undertaken according to any approved guideline or GLP (Neubert, 1987b).

2.6 Special studies

(a) Neurotoxicity studies

Studies on the potential neurotoxicity of cycloxydim have not been carried out. However, there were no clinical signs that would suggest a neurotoxic potential in any of the species tested, even at very high dose levels. In addition, standard histopathological examinations of neuronal tissue gave no indication of a test substance–related effect.

(b) Studies on metabolites/impurities

Different studies have been undertaken for four compounds that are either present as impurities in technical cycloxydim or present as plant metabolites and do not figure in animal metabolism. These are:

Table 53. In vitro genotoxicity assays with BH 517-TSO, a cycloxydim metabolite/impurity

End-point	Test object	Concentration	Purity (%)	Result	Reference
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	0 (solvent control), 22.5, 111, 555, 2775 and 5500 µg/plate (0.1 ml of each concentration per plate)	90.8	Negative (with and without metabolic activation)	Engelhardt & Leibold (2004)[a]

[a] Complied with GLP.

Table 54. Acute toxicity of BH 517-5-OH-TSO, a cycloxydim metabolite

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Effects	Reference
Rat	Wistar	Male and female	Oral	>2000	No clinical findings	Gamer & Hoffmann (2001)[a]

[a] Complied with GLP.

- *BH 517-TSO*—This has been identified as an impurity in technical cycloxydim batches and as a metabolite in plants, animals, soil and water.
- *BH 517-5-OH-TSO*—The relevant residues in plant agricultural commodities include the 5-hydroxylated metabolite species, which has not been detected in animal matrices in appreciable amounts.
- *BH 517-TGSO*—This cycloxydim metabolite is a substituted glutaric acid derivative of cycloxydim and has been identified as a plant metabolite in straw of soybean and maize, in maize forage and, at a very low level, in sugar beet roots.
- *BH 517-$TGSO_2$*—This cycloxydim metabolite is a substituted glutaric acid derivative of cycloxydim and has been identified as a plant metabolite in straw of soybean and maize, in maize forage and, at a very low level, in sugar beet roots.

BH 517-TSO

BH 517-TSO was tested for genotoxicity in vitro, with negative results (Table 53).

BH 517-5-OH-TSO

BH 517-5-OH-TSO is of low acute oral toxicity in rats; no mortality or clinical symptoms were observed at the limit dose of 2000 mg/kg bw (Table 54).

Cycloxydim metabolite BH 517-5-OH-TSO (purity 90.3%; batch No. 01743-66) was tested in a 3-month dietary study in rats. This study was undertaken in order to establish a NO(A)EL, as this metabolite to which humans could potentially be exposed has not been identified in the relevant toxicity studies with the active substance. According to the European Commission draft guidance document on relevant metabolites, "A NOAEL of 50 mg/kg body weight per day is considered sufficient for a hazard approach". This principle was adopted in this study. Thus, BH 517-5-OH-TSO was administered via the diet to 10 male and 10 female Wistar rats per group at dose levels of 0 and 50 mg/kg bw per day for 3 months. The animals were observed daily for clinical symptoms and mortalities. An FOB and measurement of motor activity were carried out towards the end of the observation period. Ophthalmological examinations were carried out prior to the start and towards the end of the administration period. Body weight and food consumption were measured weekly. Clinicochemical and haematological examinations as well as urinalysis were performed on all animals at the end of the administration period. All animals were subjected to a full macroscopic and histopathological examination. Weights of 11 selected organs were determined.

Table 55. In vitro genotoxicity of BH 517-5-OH-TSO, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	References
Salmonella typhimurium/*Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	22.2–5550 µg/plate (1st experiment) 20–5000 µg/plate (2nd experiment)	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2000)[a]
In vitro gene mutation test	Chinese hamster ovary (CHO-K1) cells	225–3600 µg/ml	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2001a)[a]
In vitro chromosomal aberration assay	V79 cells	1st experiment 0, 900, 1800 and 3600 µg/ml (4 h exposure time, 18 h harvest time, with and without S9 mix) 2nd experiment (for confirmation) 0, 900, 1800 and 3600 µg/ml (18 h exposure time and 28 h harvest time, without S9 mix) 0 and 3600 µg/ml (18 h exposure time and 28 h harvest time, without S9 mix) 0, 900, 1800 and 3600 µg/ml (4 h exposure time, 28 h harvest time, with S9 mix)	90.3	Negative (with and without metabolic activation)	Engelhardt & Hoffmann (2001b)[a]

[a] Complied with GLP.

Table 56. In vitro genotoxicity assays with BH 517-TGSO, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	Reference
Salmonella typhimurium/*Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	21.2, 106, 530, 2650 and 5300 µg/plate in standard plate test, and 331.3, 662.5, 1325, 2650 and 5300 µg/plate in preincubation assay	94.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008a)[a]

[a] Complied with GLP.

As no abnormalities were found in the parameters examined, the NOAEL of BH 517-5-OH-TSO in this study is 50 mg/kg bw per day (i.e. the highest dose tested). The study complied with GLP, and a QA statement was attached (Mellert et al., 2001b).

BH 517-5-OH-TSO was tested for genotoxicity in vitro, with negative results (Table 55).

BH 517-TGSO

BH 517-TGSO was tested for genotoxicity in vitro, with negative results (Table 56).

BH 517-TGSO$_2$

BH 517-TGSO$_2$ (Reg. No. 356102; purity 80%; lot/batch No. TP08/152/1/1) was administered to groups of five male and five female Wistar (Crl:WI(Han)) rats in the drinking-water at concentrations of 0, 1000, 3000 and 6000 ppm (equal to 0, 81.6, 240.4 and 440.5 mg/kg bw per day for males

Table 57. In vitro genotoxicity assays with BH 517-TGSO$_2$, a cycloxydim metabolite

End-point	Test object	Concentration	Purity (%)	Result	References
Salmonella typhimurium/ *Escherichia coli* reverse mutation assay	Ames test (standard plate and preincubation test) *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 uvrA	0.4–5000 µg/plate in standard plate test and 0.4–2500 µg/plate in preincubation assay	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008c)[a]
In vitro forward mutation assay in mammalian cells (HPRT test)	CHO-K1 cells	168.8–3300 µg/ml	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008b)[a]
In vitro chromosomal aberration assay in mammalian cells	V79 cells	Up to 3300 µg/ml (10 mmol/l)	80.0	Negative (with and without metabolic activation)	Schulz & Landsiedel (2008d)[a]

[a] Complied with GLP.

and 0, 88.7, 293.1 and 560.9 mg/kg bw per day for females, respectively) for a period of 4 weeks. The rats were 42 ± 1 days old at the start of administration, and body weights at dosing were 184.8–188.2 g (male rats) and 145.9–154.3 g (female rats). Food consumption, water consumption and body weights were determined weekly. The animals were examined for signs of toxicity or mortality at least once a day. Detailed clinical examinations in an open field were conducted prior to the start of the administration period and weekly thereafter. An FOB and measurement of motor activity were carried out after about 4 weeks of treatment in males and females. Clinicochemical and haematological examinations as well as urinalyses were performed towards the end of the administration period. All animals were assessed by gross pathology, followed by histopathological examinations.

The oral administration of BH 517-TGSO$_2$ to Wistar rats in drinking-water over a period of 4 weeks did not cause any signs of toxicity. Therefore, the NOAEL for BH 517-TGSO$_2$ under the conditions of the present study was 6000 ppm, equal to 440.5 mg/kg bw per day in males and 560.9 mg/kg bw per day in females. The study complied with GLP, and a QA statement was attached (Kaspers et al., 2008).

BH 517-TGSO$_2$ was tested for genotoxicity in vitro, with negative results (Table 57).

3. Observations in humans

No reports of adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to cycloxydim were available, except for three cases of eye irritation that occurred during production/filling of an old formulation, “Focus Ultra”.

3.1 Medical surveillance on manufacturing plant personnel

The BASF personnel who were handling cycloxydim in formulation were surveyed by regular medical examinations. This surveillance programme was not aimed specifically at detecting cycloxydim-related symptoms or diseases. Thus, it was not possible to evaluate a causal association between the compound and any specific medical effect.

3.2 Direct observation (e.g. clinical cases and poisoning incidents)

No poisoning incidents were reported. Three cases of eye irritation occurred during the production/filling of "Focus Ultra" (BAS 517 22 H), on 7 February 1997, 9 October 1998 and 15 June 2001. Meanwhile, BAS 517 22 H has been replaced by the new Focus Ultra formulation "BAS 517 24 H", for which there is no evidence of eye irritation from animal studies or from case reports.

However, no observations regarding health effects after exposure of the general public are known to the manufacturer.

4. Literature review

A search in literature databases for published toxicological data on cycloxydim was performed on 15 June 2009 via the online database platform STN (Karlsruhe), which included CAS No. and synonyms of cycloxydim as search parameters. Databases searched via STN were AGRICOLA, ESBIOBASE, HEALSAFE, TOXCENTER, CABA, EMBAL, LIFESCI, CSNB, EMBASE and NTIS.

Based on the results of the literature search, only one publication of potential relevance was identified and therefore retrieved for a detailed review.

In order to establish in vitro tools to study mammalian acetyl coenzyme A (CoA) carboxylase (ACC) activity, Seng et al. (2003) investigated the ability of different members of the cyclohexanedione class of herbicides, including cycloxydim, to inhibit ACC. ACC plays an important role in lipid metabolism. For this reason, ACC came into the focus for therapeutic intervention in metabolic diseases. The monocot chloroplast enzyme is the selective target of cyclohexanedione herbicides. In contrast to other publications, where it is known that cyclohexanedione does not inhibit mammalian ACC activity, Seng et al. (2003) reported a competitive inhibition of rat heart ACC activity. Seng et al. (2003) measured ACC activity from homogenized rat hearts in vitro by the carboxylation of acetyl CoA to form malonyl CoA. Beside cycloxydim, the cyclohexanedione herbicides alloxydim, clethodim, sethoxydim, trepraloxydim and tralkoxydim were tested. With the exception of alloxydim, all investigated cyclohexanedione herbicides were found to inhibit ACC with similar potency (median inhibitory concentration [IC_{50}] values ranging between 18 and 40 nmol/l); alloxydim was much less potent. The results were plotted poorly, and the IC_{50} values for each tested cyclohexanedione cannot be read from the graph. The IC_{50} values were given only as a summarized range for all tested cyclohexanediones and not in detail. Thus, the poor presentation of the results does not allow any firm conclusions to be drawn (Seng et al., 2003).

Comments

Biochemical aspects

Both the free acid and the sodium salt of cycloxydim are well absorbed; bioavailability was approximately 100%. The results of excretion balance studies indicated that most (74–86%) of a single oral dose of the sodium salt of cycloxydim at 10 mg/kg bw per day is eliminated via the urine, most being excreted within 24 h. Biliary excretion (50–65% of the administered dose) and enterohepatic circulation play an important role in the elimination of cycloxydim. The highest concentrations of radiolabel were found in the liver and the kidneys. Quantities of radiolabel in all organs rapidly declined over time. There was no evidence for bioaccumulation of cycloxydim. The pattern of metabolites in the urine was similar for the free acid and the sodium salt of cycloxydim, and AUC data indicated that elimination was saturable at higher doses. The major metabolite in the urine and bile was the sulfoxide of cycloxydim, BH 517-TSO. Additional metabolites identified were BH 517-

T1SO (derived from *N*-de-ethoxylation of BH 517-TSO), BH 517-T1SO$_2$ and BH 517-T2SO. Only small amounts of unchanged parent compound were detected in the urine.

Toxicological data

Cycloxydim is of low acute toxicity when administered orally, dermally or by inhalation.

The oral LD_{50} of cycloxydim was 3940 mg/kg bw in rats and >5000 mg/kg bw in mice. No specific clinical signs were observed. Macroscopic findings in rats that died after receiving high oral doses by gavage indicated irritation of the gastric mucosa. The dermal LD_{50} in rats was >2000 mg/kg bw, a dose of 2000 mg/kg bw causing neither mortality nor systemic toxicity. No local skin reaction was observed at the application site. When cycloxydim is administered by inhalation, the LC_{50} is >5.28 mg/l of air (4 h exposure). Cycloxydim was not an irritant in a study of ocular and dermal irritation in rabbits or a dermal sensitizer in the Magnusson & Kligman maximization test in guinea-pigs.

Short-term and long-term studies of oral toxicity in mice, rats and dogs were conducted using cycloxydim sodium salt or cycloxydim free acid. In all the studies described below, the dose or dietary concentration of the test substance is expressed as cycloxydim free acid rather than as its sodium salt.

The results of these studies are characterized by clinicochemical changes associated with changes in water and food consumption and effects on the liver. Effects on erythrocytes were seen only in dogs at high doses. Where the test substance was administered in the drinking-water, the reduction in water consumption is regarded to be a palatability effect rather than a specific adverse effect.

With the few available parameters measured in two 4-week range-finding studies in mice, an overall NOAEL was set at 1000 ppm, equal to 189 mg/kg bw per day, on the basis of a significant increase in relative liver weights at concentrations of 3000 ppm and 9000 ppm in combination with altered clinicochemical parameters and the occurrence of hydropic vacuolar parenchymal degeneration of hepatocytes in the first study.

In rats, a 90-day study of oral toxicity indicated that the target organs were the kidney and liver on the basis of increases in concentrations of creatinine, urea and cholesterol in females and increases in the activity of alanine aminotransferase in males and females at 900 ppm. The NOAEL was 300 ppm, equal to 22 mg/kg bw per day.

In the 4-week study of oral toxicity in dogs, the NOAEL was 40 mg/kg bw per day in males on the basis of effects on the liver. The results of a 3-month study of oral toxicity in dogs showed changes in haematological parameters and liver effects, with a NOAEL of 1500 ppm, equal to 50 mg/kg bw per day. In a 1-year study of toxicity in dogs, the NOAEL was 400 ppm, equal to 12 mg/kg bw per day, on the basis of effects on erythrocytes and the liver and altered clinicochemical parameters.

The 2-year study of carcinogenicity in mice did not demonstrate any substance-related change at any drinking-water concentration, and the NOAEL was 240 ppm, equal to 32 mg/kg bw per day (i.e. the highest dose tested). The study was not adequate for the evaluation of carcinogenicity, as the doses delivered were not sufficiently high; the highest dose used was much less than the NOAEL of 1000 ppm identified in the dose range–finding studies.

In an 18-month study in rats, there was a statistically significant reduction in body weight, body weight gain and triglyceride concentrations at drinking-water concentrations of 400 ppm and above, with a NOAEL of 100 ppm, equal to 7 mg/kg bw per day. In a 2-year study of carcinogenicity in rats, administration of drinking-water containing cycloxydim at concentrations of 400 ppm and 1600 ppm resulted in a reduction in body weight. Consumption of drinking-water was reduced in the group at 1600 ppm. In female rats, there was a reduction in concentrations of triglycerides. The NOAEL was 100 ppm, equal to 7 mg/kg bw per day, on the basis of a reduction in body weights and a reduction in concentrations of triglycerides in rats given drinking-water containing cycloxydim at concentrations of 400 ppm and above.

The Meeting concluded that cycloxydim was not carcinogenic in rats but had not been adequately tested in mice.

Cycloxydim was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Cycloxydim acid and the sodium salt gave negative results throughout, except at cytotoxic concentrations in studies of chromosomal aberration in vitro.

The Meeting concluded that cycloxydim is unlikely to be genotoxic.

Although the carcinogenicity study in mice was not adequate, it was still possible to reach a conclusion on carcinogenicity to humans, in view of the lack of genotoxicity and the absence of carcinogenicity in rats. The Meeting concluded that cycloxydim is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study in rats, the NOAEL for offspring toxicity was 400 ppm, equal to 38 mg/kg bw per day, on the basis of reduced survival, reduced growth and developmental retardation in pups at 1600 ppm, equal to 129 mg/kg bw per day, the highest dose tested. Reproductive toxicity was not affected by treatment at drinking-water concentrations of up to 1600 ppm. The NOAEL for parental toxicity was 100 ppm, equal to 9.7 mg/kg bw per day, on the basis of reductions in food consumption, body weight and body weight gain in dams at 400 ppm.

Studies of developmental toxicity have been carried out in rats and rabbits. In the study of developmental toxicity in rats, the NOAEL for maternal toxicity and embryo/fetotoxicity was 200 mg/kg bw per day. Increased numbers of fetuses/litters with retardations and a statistically significant increase in the frequency of anomalies of the vertebral column and the sternebrae with involvement of the cartilage and incomplete ossification were observed. Maternal toxicity and fetal effects were also observed in two subsequent supplementary studies. In the study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day. The maternal toxicity observed at doses of 200 and 400 mg/kg bw per day occurred late in the study, indicating that repeated dosing over several days was required to elicit the effect. At 400 mg/kg bw per day, the percentage of viable implantations per dam was decreased and the incidence of several skeletal anomalies (e.g. asymmetrical sternebrae and fused sternebrae) was increased above the range for the historical controls. The NOAEL for embryo/fetotoxicity was 200 mg/kg bw per day. The Meeting concluded that cycloxydim causes maternal toxicity that occurred at a late stage during the study. The dose that caused maternal toxicity also caused embryo/fetotoxicity. The Meeting concluded that cycloxydim was not teratogenic.

Some toxicological studies and studies of genotoxicity have been undertaken for four compounds that either are present as impurities in technical cycloxydim or are metabolites in plants and not in animals.

BH 517-5-OH-TSO is of low acute oral toxicity in rats; no mortality or clinical symptoms were observed at the limit dose of 2000 mg/kg bw. Repeated exposure to diets containing BH 517-5-OH-TSO for 90 days did not cause any adverse effects at a dose of 50 mg/kg bw per day. In a 28-day study in rats, the NOAEL for BH 517-$TGSO_2$ was greater than 440.5 mg/kg bw per day, the highest dose tested.

BH 517-TSO, BH 517-5-OH-TSO, BH 517-TGSO and BH 517-$TGSO_2$ were tested for genotoxicity in vitro. All gave negative results.

No reports of adverse health effects or poisoning in manufacturing plant personnel or in operators and workers exposed to cycloxydim were available except for three cases of eye irritation that occurred during production/filling of an old formulation, “Focus Ultra”; after replacement of this formulation by a new formulation, no more such cases have occurred.

The Meeting concluded that the existing database on cycloxydim was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.07 mg/kg bw based on the NOAEL of 7 mg/kg bw per day identified on the basis of a reduction in body weights and a reduction in serum triglycerides at concentrations of 400 ppm and above in the long-term dietary study in rats and using a safety factor of 100.

An acute reference dose (ARfD) of 2 mg/kg bw was established for women of childbearing age, based on a NOAEL of 200 mg/kg bw per day identified on the basis of certain skeletal anomalies at 400 mg/kg bw per day in the studies of developmental toxicity in rats and rabbits, and with a safety factor of 100. The Meeting could not exclude the possibility that these skeletal anomalies were the result of a single exposure.

The Meeting concluded that the establishment of an ARfD for the general population was not necessary on the basis of the low acute toxicity of cycloxydim, the lack of evidence for any acute neurotoxicity and absence of any other toxicologically relevant effect that might be attributable to a single dose.

Levels relevant to risk assessment

Species	Study	Effects	NOAEL	LOAEL
Mouse	Two-year study of carcinogenicity[a]	Carcinogenicity	240 ppm, equal to 32 mg/kg bw per day[b]	—
Rat	18-month study of toxicity[a]	Toxicity	100 ppm, equal to 7 mg/kg bw per day	400 ppm, equal to 28 mg/kg bw per day
	Two-year study of toxicity[a]	Carcinogenicity	1600 ppm, equal to 99 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Offspring toxicity	400 ppm, equal to 38 mg/kg bw per day	1600 ppm, equal to 129 mg/kg bw per day
		Reproductive toxicity	1600 ppm, equal to 129 mg/kg bw per day[b]	—
		Parental toxicity	100 ppm, equal to 9.7 mg/kg bw per day	400 ppm, equal to 38 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	200 mg/kg bw per day	400 mg/kg bw per day
		Embryo/fetotoxicity	200 mg/kg bw per day	400 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
		Embryo/fetotoxicity	200 mg/kg bw per day	400 mg/kg bw per day
Dog	One-year study of toxicity[d]	Toxicity	400 ppm, equal to 12 mg/kg bw per day	1600 ppm, equal to 49 mg/kg bw per day

[a] Administration in drinking-water.
[b] Highest dose tested.
[c] Gavage administration.
[d] Dietary administration.

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate of acute reference dose

2 mg/kg bw for women of childbearing age

Unnecessary for the general population

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other observational studies of human exposure

Critical end-points for setting guidance values for exposure to cycloxydim

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and almost completely absorbed (>90%) within 24 h
Distribution	Widely distributed; highest concentrations in liver and kidney
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	About 78–85% of the administered dose is eliminated via the urine within 5 days. Faeces contained approximately 12–25%; enterohepatic recirculation occurred.
Metabolism in animals	Extensive. The major metabolite was the sulfoxide (TSO)
Toxicologically significant compounds (animals, plants and the environment)	Cycloxydim
Acute toxicity	
Rat, LD_{50}, oral	3940 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>5.28 mg/l air
Rabbit, dermal irritation	Not an irritant
Rabbit, ocular irritation	Not an irritant
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body weight and liver
Lowest relevant oral NOAEL	1000 ppm (189 mg/kg bw per day) (4-week study in mice) 300 ppm (22 mg/kg bw per day) (3-month study in rats) 400 ppm (12 mg/kg bw per day) (1-year study in dogs)
Lowest relevant dermal NOAEL	300 mg/kg bw per day (28-day study in rats)
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight
Lowest relevant NOAEL	100 ppm (7 mg/kg bw per day) (rats)
Carcinogenicity	No carcinogenic potential
Reproductive toxicity	
Reproduction target/critical effect	Reduced survival growth and development in pups at parentally toxic doses
Lowest relevant reproductive NOAEL	400 ppm (38 mg/kg bw per day)
Developmental target/critical effect	Increase in the number of skeletal anomalies at maternally toxic doses
Lowest relevant developmental NOAEL	200 mg/kg bw per day (rats and rabbits)

Neurotoxicity/delayed neurotoxicity

No data; no concerns raised by other studies

Medical data

No significant health effects were reported among manufacturing personnel.

Summary

	Value	Study	Safety factor
ADI	0–0.07 mg/kg bw	Rat, 2-year study	100
ARfD[a]	2 mg/kg bw	Rat and rabbit; study of developmental toxicity	100

[a] For women of childbearing age, unnecessary for the general population.

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