CYHEXATIN

First draft prepared by
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Explanation

Cyhexatin (tricyclohexyltin hydroxide) is an organotin compound that is used as an agricultural acaricide and is chemically related to azocyclotin (tri(cyclohexyl)-1*H*-1,2,4-triazole-1-yltin) (see azocyclotin, this volume). Cyhexatin and 1,2,4-triazole are formed from the breakdown of azocyclotin. The systemic toxicological properties of cyhexatin and azocyclotin are similar, but azocyclotin may have additional properties attributable to the formation of 1,2,4-triazole.

Toxicological data on cyhexatin were reviewed by the JMPR in 1970, 1973, 1977, 1978, 1980, 1981, 1988, 1989, 1991 and 1994. Azocyclotin was evaluated by the JMPR in 1974, 1981, 1989 and 1991. The Meeting in 1991 considered that the acceptable daily intake (ADI) for cyhexatin should also cover exposure to azocyclotin. In 1994, an ADI of 0–0.007 mg/kg bw was

established based on a no-observed-adverse-effect level (NOAEL) of 0.7 mg/kg bw per day for reduced pup survival and decreased pup body-weight gain during lactation in a multigeneration study in rats.

Azocyclotin and cyhexatin were considered by the present Meeting as part of the Codex Committee on Pesticide Residues periodic review programme.

Several new studies that complied with good laboratory practice (GLP) with cyhexatin were evaluated that had not been previously available, including investigations of absorption, distribution, metabolism and excretion, short-term studies of toxicity, tests for genotoxicity, and a long-term study of combined toxicity/carcinogenicity incorporating a neurotoxicity phase.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

The transdermal absorption of cyhexatin is slow. When ICR mice were given uniformly radiolabelled [^{14}C]cyhexatin at a dose of 1 mg/kg bw applied to a 1.2 cm^2 area of shaved skin, there was a mean dermal penetration of 0.7% of the absorbed radioactivity after 1 h, 1.4% after 6 h and 5.5% after 24 h. At 1, 6 and 24 h after dosing, respectively, 5.4%, 5.8% and 3.0% of the absorbed dose was found in the liver, 1.8%, 1.6% and 1.1% in the kidneys, 0.2%, 0.2% and 0.07% in fat, 1.9%, 1.1% and 0.3% in blood, 33%, 35% and 26% in the carcass, and 55%, 56% and 69% had been collected in excreta (Grissom et al., 1985).

Rats

In a study of oral absorption, which complied with GLP, Sprague-Dawley rats were given micronized cyhexatin (purity, 96%) as a single gavage dose at 3 mg/kg bw in aqueous carboxymethylcellulose. Animals in the control group received aqueous carboxymethylcellulase only. Blood samples were taken at pairs of treated rats at 0.5, 1, 13, 4, 8 and 24 h after dosing and from controls at 24 h. Two rats each from the control and treated groups were placed in metabolism cages for 24 h for collection of urine and faeces. The peak mean concentration of tin in blood (C_{max}) was 18.8 µg/l, which was achieved at 3 h after dosing (Barrow, 1991d).

Similar experiments looking at the pharmacokinetics of micronized (Barrow, 1991e) and non-micronized cyhextin (Barrow, 1991f) in groups of five female Sprague-Dawley rats showed that the C_{max} was higher (tin, 13.8 µg/l) in the rats given micronized cyhexatin than in those given non-micronized cyhexatin (tin, 8.89 µg/l) and the C_{max} was reached at a later time-point (4 h rather than 3 h).

In a study of oral absorption, which complied with GLP, two groups of male Fischer rats were given uniformly radiolabelled [^{14}C]cyhexatin (purity, 94.5%) as a single gavage dose at 5 mg/kg bw in 0.5% aqueous Methocel. One group of three rats was killed at 24 h after dosing and urine and faeces were collected along with a selection of organs and tissues. A group of four rats was assigned to a balance portion of the study, in which urine, faeces and expired air were collected at 12 h intervals for 72 h and thereafter at 24 h intervals until 120 h after dosing. After 24 h, 2.96% of the administered radioactivity was recovered in the urine, 24.2% in faeces, 3.94% in tissues/organs plus carcass and 58.2% in the gastrointestinal tract and its contents. After 120 h, 10.9% was recovered in the urine, 74.3% in faeces, 1.38% in tissues/organs plus carcass, 0.213%

in the full gastrointestinal tract and 0.261% in expired air. Only 12.5% of the radioactivity of the oral dose was absorbed in 120 h (Domoradzki et al., 1988).

Two Wistar rats (sex not stated) were each given a gelatin capsule containing radiolabelled [^{119}Sn]cyhexatin (purity, 95.8%) as a single oral dose at 25 mg/kg bw. Urine and faeces were collected over the following 10 days. Most (75% and 85%) of the radioactivity was recovered in excreta over the first 96 h and > 99% was recovered after 10 days. Almost all of the radioactivity was in the faeces, with just 2–3% being in the urine (Smith & Fischer, 1970).

Wistar rats (males and females) were given diets containing radiolabelled [^{119}Sn]cyhexatin (purity, 95.8%) at a concentration of 100 ppm for 90 days. Groups of three rats were killed after 0, 2, 5, 8, 15, 40, 60 and 90 days of treatment and at 0, 2, 5, 10, 20, 40, 80 and 115 days after the end of treatment. The amount of radioactivity was measured in various tissues and organs. Levels of radioactivity equivalent to tin concentrations of 0.1–0.8 ppm were detected in all organs and tissues, with the lowest concentrations being found in blood and fat and the highest in kidneys. After withdrawal of treatment, the residues gradually decreased, with the rate of decrease being relatively slow in muscle and brain. After 80 days, the residues of tin were < 0.20 ppm in all the organs. The half-lives for removal of radioactivity from the organs and tissues ranged from 80 to 115 days. Muscle was analysed for metabolites by thin-layer chromatography, with cyhexatin, and dicyclohexyltin being detected along with traces of monocyclohexylstannoic acid and inorganic tin (Smith & Fischer, 1970).

In a pharmacokinetics study that complied with GLP, groups of five female Sprague-Dawley rats were given micronized cyhexatin (purity, 96%) as intravenous doses at 0.5 mg/kg bw in ethanol. Blood concentrations of tin returned to pretreatment levels after 6 h (Woehrle, 1991a).

A GLP-compliant investigation of the metabolites in bile was performed in Sprague-Dawley rats that had been cannulated in the stomach and bile duct. Uniformly radiolabelled [^{14}C]cyhexatin (purity, 96%) was given by oral gavage or via the stomach cannula to groups of two to four rats of each sex at doses of 3 or 30 mg/kg bw in 1% aqueous methylcellulose. Bile and urine were collected at intervals for the 96 h after dosing. The amounts of radioactivity in bile, urine, faeces and the carcass were measured. The results for the 96 h samples are summarized in Table 1. They show that, although most of the administered doses was found in the faeces, little of the faecal radioactivity was attributable to biliary excretion. Most of the doses that were administered orally passed through the gastrointestinal system without being absorbed (Caldwell, 2001).

Table 1. Mean amount of radioactivity at 96 h (percentage of administered dose) in rats given radiolabelled cyhexatin by oral gavage or via the stomach cannula

Medium	Dose (mg/kg bw)			
	3		30	
	Males	Females	Males	Females
Bile	5.01	9.49	3.38	6.30
Urine (+ cage wash)	1.64	3.72	0.76	1.70
Faeces	91.75	74.12	81.92	82.63
Carcass	0.88	2.34	0.26	0.99

From Caldwell (2001)

The absorption, distribution, metabolism and excretion of cyhexatin was studied in rats in a GLP-compliant study that conformed to OECD methodological guideline 417 (OECD, 1984). Groups of four Wistar rats of each sex were given either [^{14}C]cyhexatin (site of the radiolabel not stated) as single doses at 3 or 30 mg/kg bw, or unlabelled cyhexatin (purity, 95.85%) as 10 daily doses at 1.5 mg/kg bw per day followed by [^{14}C]cyhexatin as a single dose at 1.5 mg/kg bw. The doses were given by gavage in a solution in 1% aqueous methylcellulose. Faeces and urine were collected throughout the study. The animals in the three groups (3 mg/kg bw, 30 mg/kg bw and 10 × 1.5 mg/kg bw per day) were killed at 96, 120 and 96 h after the last dose, respectively. The radioactivity in urine, faeces, and a selection of organs and tissues were measured. Metabolites were extracted and characterized by thin-layer chromatography, HPLC and LC-MS. Group mean proportions of 5.2–6.0% of the administered dose was recovered in the urine and 61.3–97.4% in the faeces. There was no relationship between these proportions and the dose or duration of dosing. Most of the excretion took place between 8 and 48 h after dosing (Frieling, 2003).

The blood kinetics of cyhexatin in rats was studied in a GLP-compliant study that conformed to OECD methodological guideline 417 (OECD, 1984). Groups of three male and three female Wistar rats were given [^{14}C]cyhexatin (site of the radiolabel not stated) as a single dose at 3 or 30 mg/kg bw or unlabelled cyhexatin (purity, 95.85%) as 10 daily doses at 1.5 mg/kg bw per day followed by [^{14}C]cyhexatin as a single dose at 1.5 mg/kg bw. The doses were given by gavage as a solution in 1% aqueous methylcellulose. Blood and urine were collected throughout the study. The animals in the three groups (3 mg/kg bw, 30 mg/kg bw and 10 × 1.5 mg/kg bw per day) were killed at 72, 96 and 72 h after the last dose, respectively. The radioactivity in blood, urine and a selection of organs and tissues were measured. Group means of 5.2–6.6% of the radioactivity was recovered in the urine. About 10% of the orally administered doses were absorbed into the blood. There was no relationship between the proportions excreted in urine or the absorbed into the bloodstream and the dose or duration of dosing. The kinetic parameters for the different groups are summarized in Table 2. The results seemed to suggest a difference between males and females in the kinetics of cyhexatin, with a higher bioavailability and lower clearance efficiency in females (Frieling, 2003).

A series of three experiments investigated the pharmacokinetics of (i) single oral doses of non-micronized cyhexatin at 3 mg/kg bw; (ii) single oral doses of micronized cyhexatin at 3 mg/kg bw; and (iii) single intravenous doses of micronized cyhexatin at 0.5 mg/kg bw. Blood samples and excreta were collected from groups of five female rats (strain not stated) at various times up to 24 h after dosing for analysis for tin concentrations. The blood kinetics results are summarized in Table 3. Intravenous doses of cyhexatin were rapidly distributed to the tissues, with negligible urinary excretion (< 1%) and 34.5% of the administered dose being excreted in the faeces in the first 24 h. After oral dosing, the blood concentrations were much less than with intra-

Table 2. Kinetic parameters for ^{14}C in rats given oral doses of [^{14}C]cyhexatin

Parameter	Low dose (3 mg/kg bw)		High dose (30 mg/kg bw)		Repeated doses (1.5 mg/kg bw per day)	
	Males	Females	Males	Females	Males	Females
C_{max} (μg/g)	0.047	0.059	0.343	0.288	0.030	0.071
T_{max} (h)	8	12	4	72	12	12
Half-life (h)	22.28	38.03	21.67	78.01	13.99	23.06
AUC_{0-t} (μg.h/g)	1.41	1.95	21.99	23.23	0.84	2.40
$AUC_{0-\infty}$ (μg.h/g)	1.66	2.61	26.03	49.36	0.87	2.74
k (h^{-1})	0.03	0.02	0.03	0.01	0.05	0.03

From Frieling (2003)

Table 3. Blood kinetics for tin in rats given oral doses of cyhexatin

Parameter	Oral non-micronized cyhexatin	Oral micronized cyhexatin	Intravenous micronized cyhexatin
AUC (μg.h/l)	20	46	635
Bioavailability (%)	0.53	1.2	—
Mean absorbed dose (mg/kg bw)	0.016	0.036	0.5
Blood half-life (h)	1.16	1.55	3.35
Clearance (l/min.kg)	—	—	0.00403
V_d (l/kg)	—	—	1.174
C_{max} (μg/l)	4.56	8.1	2020
T_{max} (h)	2	2.5	—
R^2 (%)	94	79	89

From Salmona & Gagliardi (1991a)

venous dosing: 450 and 250 times less for non-micronized and micronized forms, respectively. Urinary excretion over the 24 h following oral dosing was < 1% of the administered dose and the amount of tin recovered in faeces was much greater than the amount absorbed from the gut, indicating the minimal contribution of biliary excretion to the amount voided in faeces. The micronized form was more readily absorbed from the gut than the non-micronized cyhexatin (Salmona & Gagliardi, 1991a).

Rabbits

Dermal and oral absorption of micronized cyhexatin (purity, 96%) were investigated a GLP-compliant experiment in female New Zealand White rabbits. Doses of cyhexatin at 0 or 3 mg/kg bw in carboxymethylcellulose (5 ppm) were administered either by oral gavage or by application to shaved skin. Blood samples were taken from pairs of rabbits from each group at 0.5, 1, 13, 4, 8 and 24 h after dosing. Further pairs of control and of treated animals were kept in metabolism cages for 24 h for collection of urine and faeces. The C_{max} values for tin concentrations in the blood were 119 μg/l for oral treatment and 20 μg/l for dermal treatment. In both cases the C_{max} was reached at 3 h after treatment (Barrow, 1991a).

The experiment in rabbits treated dermally was repeated in two other GLP-compliant experiments using non-micronized (Barrow, 1991b) and micronized (Barrow, 1991c) cyhexatin in groups of four female New Zealand White rabbits and using several time points for collecting excreta. In these experiments, C_{max} concentrations of 10.88 (non-micronized) and 11.14 μg/l (micronized) were reached at 8 h after dosing. Peak concentrations of both urine and faeces were achieved at 32 (non-micronized) and 24 h (micronized) after dosing. Blood concentrations returned to control levels by 46–56 h.

The rabbit oral dosing experiment was repeated in two other GLP-compliant experiments using non-micronized (Barrow, 1991g) and micronized (Barrow, 1991h) cyhexatin in groups of four female New Zealand White rabbits. In these experiments, C_{max} concentrations of 13.98 (non-micronized) and 18.73 μg/l (micronized) were reached at 4 h after dosing. Blood concentrations returned to control levels at 32 h after dosing.

The results of this series of experiments in rabbits showed that there was much less absorption of cyhexatin by the dermal route than by the oral route. The micronized form of cyhexatin appeared to be absorbed to a slightly greater degree than the non-micronized form (Barrow, 1991a–h).

In a GLP-compliant study of pharmacokinetics, groups of five female New Zealand White rabbits were given intravenous doses of micronized cyhexatin (purity, 96%) at 0.5 mg/kg bw in ethanol. Blood concentrations of tin returned to pretreatment levels after 6 h (Woehrle, 1991b)

A series of three experiments were performed in rabbits (strain not stated). Micronized or non-micronized cyhexatin was administered by the oral or percutaneous route as a single dose at 3 mg/kg bw. Micronized cyhexatin was administered intravenously at 0.5 or 3 mg/kg bw. Samples of blood and excreta were collected for analysis for tin for up to 54 h after treatment. All the rabbits at 3 mg/kg bw per day administered by intravenous injection died within 4 h, so no results are available for this group. The blood kinetics results are summarized in Table 4. In all cases, urinary excretion was low (< 1% of the administered dose). Faecal concentrations of tin were only greater than controls in the animals dosed orally (mainly because of unabsorbed test material). After intravenous dosing, distribution to the tissues was rapid, but the low levels of urinary and biliary excretion indicated that clearance from the tissues was slow. Absorption and bioavailability of cyhexatin following oral or percutaneous dosing was limited, with no clear difference between the micronized and non-micronized forms (Salmona & Gagliardi, 1991b).

Pregnant rabbits

The oral absorption of cyhexatin in pregnant rabbits was investigated. Cyhexatin was administered by oral gavage or by dermal application to the clipped skin of groups of six pregnant New Zealand White rabbits on days 6–19 of gestation. The doses used for both routes of application were 0, 0.1 and 1.0 mg/kg bw per day in aqueous methylcellulose (0.5% for oral dosing; 2% for topical dosing). On days 6 and 19 only, the dose administered was prepared using [^{14}C]cyhexatin that was radiolabelled at the 1-*N* position on each hexyl ring. Groups of animals were killed at 1 and 10 h after dosing and maternal blood, fetuses, placenta and amniotic fluid were analysed for radioactivity. Blood samples taken on day 6 of gestation after the first dose of [^{14}C]cyhexatin were also analysed. Peak blood concentrations after oral treatment were approximately ten times those achieved with the same doses applied to the skin. Radioactivity equivalent to mean concentrations of cyhexatin-equivalents of 34 ng/ml, 7.6 ng/ml, 14 ng/g and 20 ng/g were found in blood, amniotic fluid, placenta and fetuses at 1 h after dosing and concentrations of 14 ng/ml, 4.2 ng/ml, 20 ng/g and 44 ng/g at 24 h after dosing. It was noted that cyhexatin and/or its metabolites can cross the placental barrier (Bailey et al., 1992).

Table 4. Blood kinetics for tin in rabbits treated with cyhexatin

Parameter	Oral		Percutaneous		Intravenous
	Non-micronized	Micronized	Non-micronized	Micronized	Micronized
Estimated AUC (µg.h/l)	157	102	159	135	279
Measured AUC (µg.h/l)	154	129	128	115	—
Bioavailability (%)	9.2	7.7	7.7	6.9	—
Mean absorbed dose (mg/kg bw)	0.279	0.231	0.231	0.207	0.5
Blood half-life (h)	9.12	2.32	21.7	14.1	2.31
Clearance (l/min.kg)	—	—	—	—	0.0092
V_d (l/kg)	—	—	—	—	1.837
C_{max} (µg/l)	8.1	11.5	3.37	4.3	316.4
T_{max} (h)	5.5	3.5	11.7	9.1	—
R^2 (%)	89	77	69	76	97

From Salmona & Gagliardi (1991b)

The distribution of cyhexatin in the pregnant rabbit was investigated in a GLP-compliant study. Groups of 18 pregnant New Zealand White rabbits were given cyhexatin (purity, 96%) at a dose of 0 or 3 mg/kg bw per day by oral gavage on days 6–18 of gestation. Blood samples were taken at 2, 3, 4 and 24 h after dosing. Six animals from each group were killed 24 h after the last dose and concentrations of tin were measured in tissues and organs. The rest of the animals were killed for analysis 7 days later. The tin in the blood returned to control levels by 24 h after dosing. Immediately after the treatment period, raised concentrations of tin were seen in the kidneys and the liver, but only the concentrations in the kidney were statistically significantly different ($p < 0.05$) from those of controls. No increase was seen in the brain. Elevated concentrations of tin were seen in fetuses, amniotic fluid and placenta. Concentrations of tin in all samples returned to control levels by the end of the 7-day recovery period (Salmona & Gagliardi, 1991c; Woehrle, 1992).

Guinea-pigs

Two guinea-pigs (strain and sex not stated) were each given a gelatin capsule containing radiolabelled [^{119}Sn]cyhexatin (purity, 95.8%) as a single oral dose at 2 mg/animal. The animals were killed at 24 and 48 h after dosing, and bile was collected. The biliary excretion of radioactivity was almost zero. Insufficient bile was collected to allow any chemical analyses to be performed (Smith & Fischer, 1970).

1.2 Biotransformation

The tissues collected in the study by Frieling (2003) were analysed. Metabolites were extracted and characterized by thin-layer chromatography, HPLC and LC-MS. Seven polar metabolites were separated from the urine, but could not be identified. No unaltered cyhexatin was detected in the urine and dicyclohexyltin oxide (DCTO), monocyclohexylstannic acid (MCTA) and cyclohexanol were not present. The radioactivity in the faeces was characterized as cyhexatin (62%), DCTO (3%), cyclohexanol (8%), unidentified extractables (16%) and unextractables (10%). It was suggested that the metabolites in faeces were mostly produced from the bacterial breakdown of cyhexatin.

A series of experiments were performed in vitro and in vivo to investigate the metabolism of cyclohexyltin compounds, including cyhexatin (Kimmel et al., 1979). The experiments used [^{14}C]cyhexatin (site of radiolabel not stated) that had been manufactured in the laboratory.

In an investigation of microsomal metabolism in vitro, [^{14}C]cyhexatin was metabolized by microsomes derived from the livers of male rats (strain not stated). The products of the metabolism were separated by thin-layer chromatography. It was shown that the metabolism of cyhexatin required the presence of both microsomes and NADPH. On average, 64% of the cyhexatin remained unchanged, 3.6% was found as destannylation products, 8.0% as hydroxyl products (with 2-OH being in greatest amounts followed by 3-OH and then 4-OH), 17.3% as unknown polar compounds, 3.2% as unknown apolar compounds and 4.9% as bound compounds.

Experiments in vivo were performed in male Swiss-Webster mice, male Sprague-Dawley rats, male Hartley guinea-pigs and male rabbits (strain not stated) given [^{14}C]cyhexatin as a single oral dose at respective concentrations of 1.35, 0.84, 0.64 and 0.32 mg/kg bw. The animals were placed in metabolism cages for collection of excreta during 72 h. The faeces were then examined for radioactivity and the metabolites were separated by thin-layer chromatography. In all four species, most of the administered radioactivity (52–73%) remained in the form of [^{14}C]cyhexatin. Hydroxy-derivatives and destannylation products were identified, but as it is known that absorption of cyhexatin is poor and little biliary excretion occurs, it is not clear whether the substances identified were products of mammalian metabolism or break-down products of unabsorbed material.

Studies are also available on the metabolism of azocyclotin in rats (summarized in the chapter on azocyclotin). Cyhexatin is a major metabolite of azocylotin. As such, the data on the metabolism azocyclotin are relevant to cyhexatin. Studies show that the first step in the metabolism of azocyclotin is the release of a triazole moiety to give cyhexatin. This hydrolysis can occur spontaneously in aqueous solution. The subsequent metabolism of cyhexatin appeared to involve both the removal of hexyl moieties and oxidation. Hexyl moieties were split off (presumably as cyclohexanol) to give dicyclohexyltin oxide and monocyclohexylstannoic acid. A variety of unidentified polar substances that were separated from faeces and urine of rats treated with azocyclotin were thought to be the products of oxidation of cyhexatin, cyclohexanol, dicyclohexyltin oxide and monocyclohexylstannoic acid. This proposed metabolic pathway is illustrated in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity are summarized in Table 5. Nearly all of the studies were performed in accordance with GLP. The exception was Dickhaus & Heisler (1982), which was performed before the introduction of GLP. The conduct of the studies was broadly in line with the appropriate OECD testing guidelines: 401, 402 and 403 (OECD, 1981a, 1987a and 1987b).

By the oral route, cyhexatin was of moderate acute toxicity to rats. There was no consistent difference between the toxicities of micronized and non-micronized forms of cyhexatin. The acute dermal toxicity of cyhexatin was very low in rats and in rabbits. When administered by inhalation, cyhexatin was of high acute toxicity in rats.

In the studies of acute toxicity after administration orally or by inhalation, signs of toxicity shown by the affected animals included piloerection, hunched posture, waddling gait, bulging eyes, lethargy, decreased respiratory rate, ptosis and ungroomed appearance. In the studies of exposure by inhalation, the rats that died and several of the survivors showed pulmonary haemorrhage, pulmonary oedema and lung damage.

(b) Dermal and ocular irritation, and dermal sensitization

Cyhexatin (purity not specified) was shown to be irritant to skin in a non-GLP study in New Zealand White rabbits. Various doses of cyhexatin (1%, 10% and 100% concentrations in an unidentified diluent) were applied on gauze to clipped dorsal skin (one abraded site and one unabraded site) and left under occlusive dressing for 24 h. The reactions at the sites of application were assessed when the dressing was removed and at 72 h and 7 days later. All concentrations of cyhexatin caused skin reactions that included erythema and oedema at abraded and non-abraded sites at the initial measurement. The reactions were still evident 72 h later. The Meeting concluded that cyhexatin could cause skin irritancy (Dickhaus & Heisler, 1981a).

The potential to cause irritancy to the eye was investigated in a non-GLP study in New Zealand White rabbits. The study did not conform to the recommendations of OECD methodological guideline 405 (OECD, 2002). Ocular instillations of 0.1 g of cyhexatin (purity, 97.3%) were given to each of the eyes of three New Zealand White rabbits, with the right eye being washed after 30 s while the left eye remained unwashed. Observations of the condition of the eyes were made at 24 h, 48 h and 7 and 14 days after the instillation. For the first 7 days, there was severe conjunctival inflammation, moderate corneal damage and slight iritis in both eyes.

Figure 1. Proposed metabolic pathway of azocyclotin and cyhexatin (tricyclohexyltin hydroxide) in rats

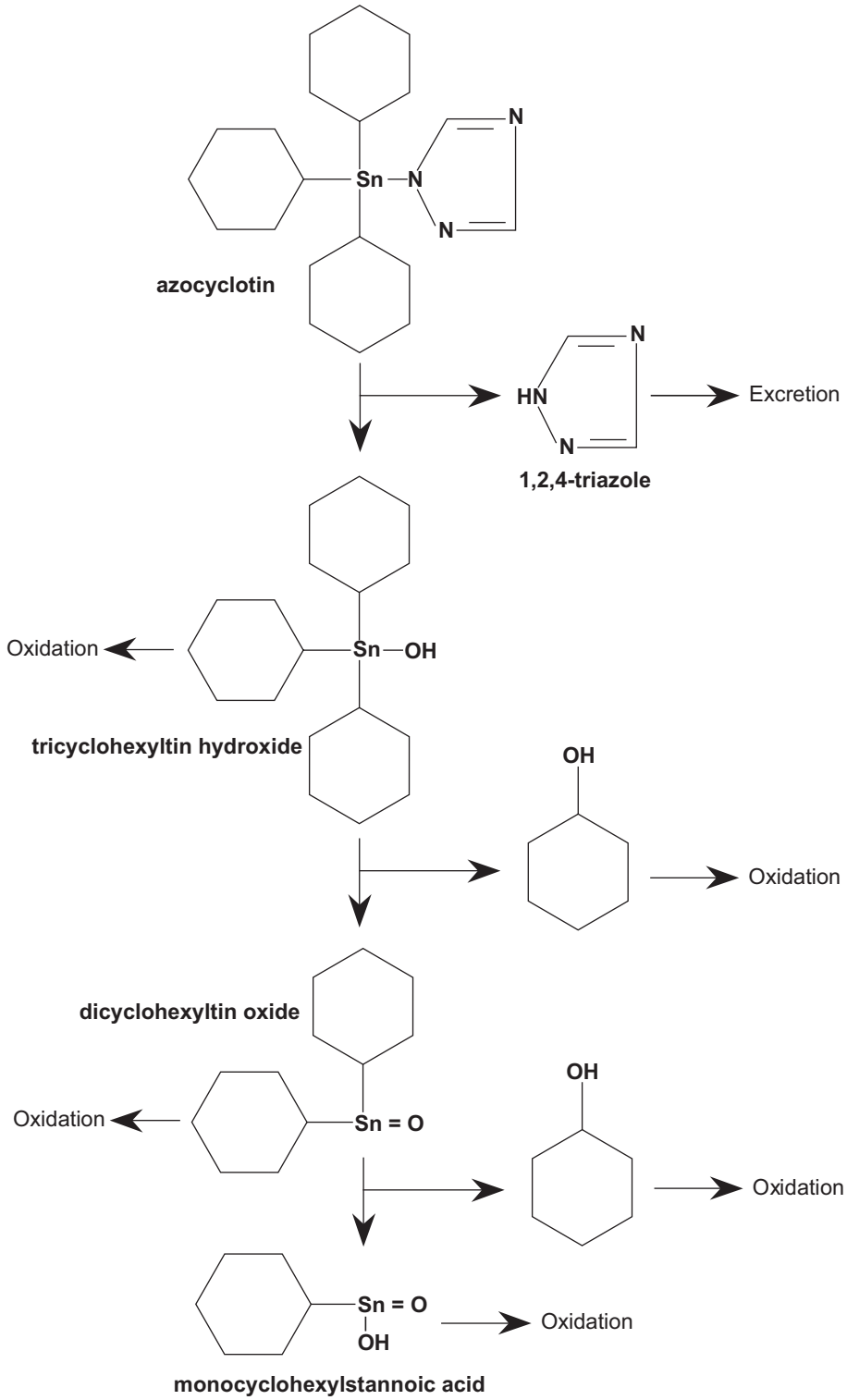

Table 5. Acute toxicity of cyhexatin

Species	Strain	Route	Purity (%) and form	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Reference
Rat	SD	Oral	96, non-micronized	Male, 599 Female, 654	Denton (1993a)
Rat	SD	Oral	95–95, non-micronized	Male, 425 Female, 274	Longobardi 1994a)
Rat	SD	Oral	96, micronized	Male, 501 Female, 265	Denton (1993b)
Rat	SD	Oral	95–95, micronized	Male, 407 Female, 411	Longobardi 1994b)
Rat	Wistar	Dermal	93.5	Male, 7600 Female, 3600	Dickhaus & Heisler (1982)
Rabbit	NZW	Dermal	95.5	> 2000	Jeffrey et al. (1986)
Rat	SD	Inhalation (4-h nose-only exposure)	Purity not stated.; mass mean diameter of particles = 4.0–5.2 μm	Both sexes, 0.02	McDonald (1989)
Rat	SD	Inhalation (4-h nose-only exposure)	Purity not stated; 75–93% of particles were of equivalent aerodynamic diameter of < 6 μm	Both sexes, 0.016	Cracknell (1993)
Rat	Fischer F344	Inhalation (4-h nose-only exposure)	96.7 Mass median aerodynamic diameter of particles = 3.8–6.4 μm	Male, 0.02 Female, 0.04	Nistchke et al. (1987)

SD, Sprague-Dawley; NZW, New Zealand White

At 14 days, there were no longer any corneal or iridal changes visible, but there was still slight conjuntival inflammation in both eyes. The Meeting concluded that cyhexatin could cause severe ocular irritancy (Rampy & Keller, 1973).

In a non-GLP study, groups of six New Zealand White rabbits of unspecified sex had 0.1 g of different dilutions of technical cyhexatin (purity not known) instilled into their right eye. The dilutions were of strengths 1%, 10% and 100% cyhexatin, but no details were given of the medium in which the cyhexatin was diluted. The left eyes were left untreated for control purposes. The eyes were observed for 7 days. The study appeared to have been performed to a protocol that was broadly in line with OECD methodological guideline 405 (OECD, 2002). The treatment with 100% cyhexatin caused conjuntival irritation, with redness, chemosis and secretion beginning on the first day of treatment. Opacity developed on the second day and the animals of the 100% group were killed at this point. The 10% cyhexatin caused conjunctival irritation (moderate redness, chemosis, half-closed eyelids and eye watering) from day 1, but this cleared up after 4 days. The 1% cyhexatin caused slight conjunctival redness from day 2 until day 4. As the undiluted cyhexatin caused severe ocular lesions that required euthanasia to be performed, cyhexatin was regarded as a severe irritant to eyes (Dickhaus & Heisler, 1981b).

Technical-grade cyhexatin (purity not specified) was tested for skin sensitizing potential in a Buehler test that was not performed according to GLP. The study did not conform to OECD methodological guideline 406 (OECD, 1992) in that too few animals were used in the treatment

group. Three inducing doses of 1 ml of 1% cyhexatin in polyethylene glycol were given topically to the clipped dorsal skin of six male and six female Dunkin-Hartley guinea-pigs at a rate of one dose per week. A similar number of control animals were given the vehicle without any cyhexatin. Two weeks after the third induction, the test and control animals were challenged with a topical dose of 1 ml of 0.5% cyhexatin in polyethylene glycol. The application sites were examined for skin reactions at 14 h and 48 h after the challenge dose. Slightly patchy erythema was seen in both the test group and controls at both time-points, with the reaction of the test group being no greater than in the controls. The Meeting concluded that there was no evidence to suggest that cyhexatin could cause skin sensitization (Jones, 1984).

(c) Studies of toxicity with repeated doses

(i) Oral toxicity with repeated doses

Mice

In a 90-day study of toxicity that did not comply with GLP, groups of 10 male and 10 female B6C3F_1 mice were fed diets containing cyhexatin (purity, 98%) at concentrations that were adjusted to achieve doses of 0, 6 or 10 mg/kg bw per day. Blood samples were taken for haematology from the tails of all animals after 83 days of treatment. In addition, blood samples were taken from the orbital sinus at the end of the treatment period and were used for haematology and clinical chemistry. No urine analysis was performed. All animals were autopsied at the end of the treatment period, organs were weighed, and a microscopic examination was performed on a wide selection of organs and tissues from all animals. Apart from omitting urine analysis, the conduct of the study was broadly in line with OECD methodological guideline 408 (OECD, 1998a).

No treatment-related effects were seen on mortality, clinical signs, body-weight changes or food consumption. There were some sporadic changes in some haematological and clinical chemistry parameters, but there was a lack of dose–response relationship and these changes were not considered to be treatment-related. There were no treatment-related effects on gross pathology, organ weights or histopathology.

The NOAEL was the highest dose tested, 10 mg/kg bw per day (McCollister et al., 1980).

Rats

In a 28-day study of toxicity, groups of five male and five female Sprague-Dawley rats were given diets containing cyhexatin (purity, 98.3%) at dietary concentrations that were adjusted weekly to achieve doses of 0, 1, 3 and 6 mg/kg bw per day. The study was performed in accordance with GLP and with OECD methodological guideline 407 (OECD, 1995). All animals were killed and autopsied at the end of the study. Blood samples were taken and selected organs weighed at the time of autopsy. Histopathology examinations were performed on a wide range of organs and tissues from animals in the control group and in the group receiving the highest dose. Only the liver, lungs, kidneys and any gross lesions from the other treatment groups were examined microscopically. No mortality was observed and there were no treatment-related effects on clinical signs of toxicity, body-weight gain or food consumption. There were some statistically significant effects on haematological values, but all values remained within their respective historical control ranges. Prothrombin time was significantly increased ($p < 0.01$) in males at 6 mg/kg bw per day, but significantly decreased ($p < 0.05$) in females at 3 or 6 mg/kg bw per day. There was a small but significant decrease ($p < 0.01$) in mean corpuscular haemoglobin concentration and a significant increase ($p < 0.01$) in activated partial prothrombin time in males at the highest dose. There was a slight dose-related trend to increases in erythrocyte count, erythrocyte volume fraction and haemoglobin, and the levels were statistically significantly greater ($p < 0.01$) than concurrent controls in males at the highest dose (6 mg/kg bw per day). There were no treatment-related effects on any clinical chemistry parameters, gross pathology, organ weights or histopathology.

The NOAEL was 3 mg/kg bw per day on the basis of changes in haematological parameters at 6 mg/kg bw per day. The small decrease in prothrombin time seen in the females at 3 mg/kg bw per day was not considered to be treatment-related (Mertens, 2000a).

In a study of toxicity that did not comply with GLP, groups of 30 male and 30 female Wistar rats were fed diets containing cyhexatin (purity not reported) at a concentration of 0, 10, 50 or 100 ppm for up to 90 days. Ten animals of each sex from each group were killed at 50 days, 90 days and 120 days (30 days after treatment) of the study. All animals were autopsied, organs were weighed and histopathological examinations were made of a limited range of organs and tissues (liver, heart, kidneys, adrenals, lungs, brain, peripheral nerve, pituitary, thyroid, bladder, stomach, duodenum, colon, pancreas, testes, ovaries, uteri) from five control animals of each sex and 10 animals of each sex at the highest dose from the groups killed at 90 days. In addition, microscopic examinations were performed on the liver, testes and any organs that appeared abnormal at autopsy in animals of the other doses at 90 days or at the highest dose at the other two time-points. Terminal blood samples were taken for haematology and clinical chemistry measurements. Similarly, terminal urine samples were taken for urine analysis. The conduct of the study was not in accordance with OECD methodological guideline 408 (OECD, 1998a), as only a limited microscopic examination of organs and tissues had been performed and this examination had not been done on all groups of treated animals.

There were no treatment-related effects on mortality or clinical signs. Body weight was lower than in controls in both sexes at 50 or 100 ppm, with effects being first evident at on day 50 of treatment and still being present at day 90. There was no effect on body weight at the end of the 30-day recovery period. Food consumption of the animals at 50 or 100 ppm was low during the treatment period, but there was no difference between the food intakes of treated groups and controls during the recovery period. The mean erythrocyte counts of the group at 100 ppm were 13% (males) and 21% (females) less than the control values at 90 days, but not at the other times of sacrifice (days 50 of treatment and 30 days after the end of treatment). Serum alkaline phosphatase activity was 41–54% higher than control values for the males at the highest dose at all times of sacrifice and in the females at the highest dose it was increased by 35% at day 50 but not increased at the other time-points. Urine analysis revealed no treatment-related effects.

There were no treatment-related effects on organ weights. Autopsies showed no gross pathological lesions in the animals killed after 50 days of treatment or at the end of the recovery period, but there were some changes in the livers of some of the rats killed at 90 days. Three of the rats at 50 ppm and six of those at 100 ppm had thickened livers, which were described as having a patchy appearance. Microscopically, the livers of the animals that had been at 50 or 100 ppm and killed after 90 days showed pathological changes, including single-cell necrosis of hepatocytes, fatty change in hepatocytes, infiltration with lymphocytes and macrophages, an increased number of activated Kupffer cells and increased numbers of mitotic hepatocytes. Histopathological changes in the heart were also described in the animals at the highest dose that were killed at 90 days (the heart was not looked at in the animals killed at other times). The cardiac pathology consisted of generally low-grade increases in the number of monocytes in perivascular regions of the myocardium, and was seen in four males and four females of the treated group, compared with two males and no females in controls. The pathologist's report suggested that this might be related to a non-treatment-related increase in pulmonary infections that occurred in this group. Some changes were also seen in the testes of rats at the highest dose that were killed at either 50 or 90 days (see Table 6). The testicular changes observed included increased incidences (as compared with the controls killed at 90 days) of low-grade effects on degeneration of the developing sperm in single tubules, on interstitial oedema and on localized haemorrhages. No testicular lesions were seen in the animals killed after the recovery period.

The NOAEL was 10 ppm (equal to 0.68 and 0.75 mg/kg bw per day in males and females respectively) on the basis of liver changes at 50 ppm or more (Dickhaus & Heisler, 1981c).

Table 6. Incidences of histopathological lesions in the testes of rats given diets containing cyhexatin

Dietary concentration (ppm)	Time of sacrifice	No. of animals with low-grade degeneration of developing sperm in single tubules	No. of animals with low-grade interstitial oedema and localized haemorrhage
0 (control)	Day 90 of treatment	0	2
10	Day 90 of treatment	1	3
50	Day 90 of treatment	0	4
100	Day 90 of treatment	3	8
100	Day 50 of treatment	3	3
100	Day 30 after treatment	0	0

From Dickhaus & Heisler (1981c)

Dogs

In a study that did not comply with GLP, groups of four male and four female beagle dogs were given diets containing cyhexatin (purity not specified) for 90 days. The study was not performed to a modern protocol as laid out in OECD methodological guideline 409 (OECD, 1998b). A group of control animals received diet that did not contain cyhexatin. All the treatment groups were given a dietary concentration of cyhexatin that provided a dose of 1.5 mg/kg bw per day for the first week of the study. After the first week, the diets of the animals at the intermediate and highest doses were increased to 3 mg/kg bw per day and the animals at the lowest dose continued to receive cyhexatin at 1.5 mg/kg bw per day. After another week at this dose, the highest dose was increased to 6 mg/kg bw per day. For the rest of the study, the dogs received cyhexatin at dietary concentrations intended to provide doses of 0, 1.5, 3 or 6 mg/kg bw per day. Blood and urine samples were taken from each dog on days 0, 45 and 90 of the study. At the end of the treatment period, all of the dogs were killed by electric shock, autopsies were performed and a selection of organs and tissues were weighed and examined microscopically.

All animals survived until completion of the treatment period. No treatment-related effects were found on clinical signs, body-weight changes, food consumption, haematology, clinical chemistry, urine analysis, gross pathology, organ weights or histopathology.

The NOAEL was the highest dose tested, 6 mg/kg bw per day (Lindberg et al., 1977).

In a 1-year dietary study of toxicity that complied with GLP, groups of six male and six female beagle dogs were given diets containing cyhexatin (purity, 95.6%) at concentrations that were adjusted to provide doses of 0, 0.25, 0.50 and 0.75 mg/kg bw per day. The conduct of the study was broadly in line with OECD methodological guideline 409 (OECD, 1998b). Blood samples were taken from all dogs after 3, 6 and 12 months of treatment; and urine samples were taken 2 weeks before the end of the study. Ophthalmoscopy was performed on all dogs at the start of the study and at 2 weeks before the end of the treatment period. All dogs were killed and autopsied at the end of the treatment period. Organs were weighed. A microscopic examination was performed on a wide selection of tissues and organs from the control and high dose animals and on liver, kidneys and heart from the animals at the two lower doses.

All animals survived the treatment period. There were no treatment-related effects on clinical signs or food consumption. The body weights of females at the highest dose tended to be less than those of concurrent controls, but there was no statistical significant difference ($p > 0.05$) between the two groups. There were no treatment-related effects revealed by ophthalmoscopy, haematology, clinical chemistry or urine analysis. At the highest dose, heart weights were increased in both sexes, kidney weights were increased in females and liver weights were

increased in males. At the intermediate dose, kidney and liver weights were increased in females. However, there were no treatment-related adverse effects seen in any of these organs or other organs at autopsy or upon microscopic examination. Therefore, the effects on organ weights were not regarded as adverse effects.

The NOAEL was the highest dose given, 0.75 mg/kg bw per day (Bond et al., 1986).

In a feeding study that did not comply with GLP, groups of 12 male and 12 female beagle dogs were fed diets containing cyhexatin (purity, 96.4%) at concentrations that were adjusted to achieve doses of 0, 3, 6 or 12 mg/kg bw per day. An in-house protocol was used. The dogs used in the study varied greatly in age and body weight. Initially the dogs refused to eat the test diets, so lower doses were introduced, and gradually increased over 4 weeks until the full doses were achieved. The test diets were administered to the dogs for up to 2 years, except in the case of the highest dose. The dogs at the highest dose were switched to a control diet after 8 months of treatment and the surviving animals were killed after 2 months on the control diet (8 months after the start of the study). Apart from this, two dogs of each sex at each dose were killed after 3, 6 or 12 months of the study. Blood samples were taken for haematology and clinical chemistry after 3, 6, 12 and 24 months. Urine samples were collected at the end of the study for urine analysis. In addition to the usual parameters, the concentrations of various metals (potassium, sodium, calcium, magnesium and copper) were measured in the urine and serum. At the end of the study, all dogs were given a physical examination, including an unspecified neurological examination and ophthalmoscopy. Autopsies were performed on all animals that died prematurely and all of those killed at scheduled times. Selected organs were weighed and examined microscopically. It was reported that concentrations of tin were measured in serum, urine and a selection of tissues, but the results of the analysis were not presented in the report.

After an initial reluctance to eat with a subsequent decrease in body-weight gain at all doses, there was no treatment-related negative effect on food intake, indeed the intakes of treated diets became slightly higher than those of the controls. Body weights of the groups at 6 and 12 mg/kg bw per day were consistently lower than those of the concurrent controls. The number of animals dying prematurely at the lowest, intermediate and highest doses were, respectively: males: two, three and three; and females: one, one and three. In contrast, no animals died prematurely in the control group. Autopsies on the decedents did not show any dose-related pathology. There were no treatment-related effects on clinical appearance, ophthalmoscopy, haematology, clinical chemistry or urine analysis. The only treatment-related gross change seen at autopsy was a tan discolouration of the entire length of the small intestines and sometimes the pancreas, which was noted in all animals killed after 24 months of treatment. The animals at the lowest and intermediate doses that were killed after 24 months also had increased relative heart weights and increased absolute and relative liver weights compared with controls. No treatment-related histopathology was seen.

The NOAEL was 3 mg/kg bw per day on the basis of the body weights of the animals at the intermediate and highest doses becoming comparatively lower than in the control group (Eisenlord et al., 1970a).

(ii) Studies of toxicity after repeated doses administered by inhalation

In a study that did not comply with GLP, groups of five male and five female Wistar rats were exposed by nose only to a dust of cyhexatin (purity, 95%) at air concentrations of 0, 77, 207 or 596 mg/m^3 (0, 0.077, 0.207 or 0.596 mg/l) for 6 h per day, 5 days per week, for 2 weeks. Most (85–90%) of the dust was of particle size < 7 μm and the mass mean aerodynamic diameter (MMAD) of 3.95–5.25 μm for the different groups. Blood and urine samples were taken at the end of the treatment period and all animals were killed for necropsy. Organs were weighed and examined microscopically. None of the rats died during the treatment period and no clinical signs were reported. Body-weight gain was decreased in both sexes at the highest dose. Increased prothrombin time, glutamic pyruvate transaminase (also known as alanine aminotransferase),

alkaline phosphatase and blood urea nitrogen were seen in blood samples taken from both sexes at the highest dose and increased glutamic pyruvate transaminase and alkaline phosphatase activity was seen in the males at the intermediate dose. Traces of albumin and bilirubin were noted in urine samples from both sexes at the highest dose. There was a dose-related increase in lung weight in both sexes at doses of 207 mg/m^3 or more (0.207 mg/l or more). Gross examination at necropsy showed nasal exudates, inflamed tracheobronchial mucosa and pulmonary congestion at the intermediate and highest doses. Microscopy revealed treatment-related lesions in the lungs (interstitial pneumonitis), liver (hepatocellular necrosis), kidneys (tubular degeneration) and nasal muscosa (inflammation) of these animals.

The NOAEC was 77 mg/m^3 (0.077 mg/l) (Shiram Institute for Industrial Research, 1986).

(iii) Transdermal toxicity after repeated doses

In a study that complied with GLP, groups of five male and five female New Zealand White rabbits were cyhexatin (purity, 95.5%) at a dose of 0, 0.1, 0.3 or 1 mg/kg bw per day as a suspension in corn oil applied to clipped skin for 6 h per day, 5 days per week, for 3 weeks. Local inflammation of dose-related intensity was seen at the application site at all doses. Blood samples were taken at the end of the treatment period and all animals were killed for necropsy.

There were no treatment-related effects on mortality, body weight or haematology. Serum alkaline phosphatase activity was raised by 79% in females at the highest dose, but was not affected in other groups. No gross pathology was noted at necropsy, other than skin lesions at the site of application. Organ weights were unaffected by treatment. Histopathological examinations were confined to the liver, gall bladder, kidneys and skin. The only treatment-related histopathology seen was in the skin at the application site.

The NOAEL for effects other than local skin lesions was 0.3 mg/kg bw per day on the basis of the increased serum alkaline phosphatase activity in females at the highest dose (Corley & Johnson, 1986).

2.2 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year study of toxicity/carcinogenicity that did not comply with GLP, groups of 60 male and 60 female B6C3F$_1$ mice were fed diets containing cyhexatin (purity, 98%) at dietary concentrations that were adjusted to achieve doses of 1, 3 or 6 mg/kg bw per day. A control group of 96 males and 96 females was given basal diet on a comparable regimen. The study broadly conformed to OECD methodological guideline 453 (OECD 1981b). Ten animals of each sex from each treatment group or control group were killed after 12 months of treatment. Blood samples were taken from each of these interim-kill animals and from 10 mice of each sex per group at the terminal kill at 24 months. Haematology and clinical chemistry were performed on the blood samples. No urine samples were analysed. Necropsies were performed on all animals killed at 12 or 24 months and on any animals dying at unscheduled times. Organs were weighed and an extensive selection of organs and tissues were examined microscopically.

Mortality over the study period ranged from 13.4% to 38.1% in the various groups. From month 11 onwards, there were statistically significant increases ($p < 0.05$) in mortality in the males at 6 mg/kg bw per day. In contrast, females at 6 mg/kg bw per day showed a statistically significant decrease ($p < 0.05$) in mortality during months 3 to 7, but this decreased mortality was not regarded as an adverse treatment-related effect. Between days 95 and 533, the body weights of males at 6 mg/kg bw per day were significantly lower ($p < 0.05$) than those of concurrent controls. Food intakes of both sexes of mice at 6 mg/kg bw per day were sometimes significantly lower ($p < 0.05$) than in controls, especially during the first month of the study. No treatment-related effects were seen on clinical signs, haematology or clinical chemistry. No treatment-related effects were seen on gross pathology, organ weights or histopathology. There were no increased numbers of palpable masses or of any type of tumour found in the treated mice.

The Meeting concluded that cyhexatin was not tumourigenic in this study and that the NOAEL was 3 mg/kg bw per day on the basis of effects on mortality and body weight at the highest dose (Keyes et al., 1981).

Rats

In a non-GLP long-term study of toxicity/carcinogenicity, groups of 45 male and 45 female Long-Evans rats were given diets containing cyhexatin (purity, 96.4%) at concentrations that were adjusted to obtain doses of 0, 3, 6 or 12 mg/kg bw per day, which were maintained for up to 2 years. The conduct of this study did not conform to the modern standards set out in OECD methodological guideline 453 (OECD, 1981b). Groups of up to 10 rats of each sex at each dose were killed at 6 weeks, 3 months, 12 months, 14 months and 18 months, and all survivors from each group were killed at 24 months. Blood samples were collected at termination from five rats of each sex from each dose. A limited number of haematological and clinical chemistry tests were performed on the blood samples: erythrocyte count, total and different leukocyte counts, erythrocyte volume fraction, haemoglobin, blood urea nitrogen, glutamic-oxalacetic transaminase, alkaline phosphatase, cholesterol, glucose and bilirubin. No urine analysis was performed. All animals killed at scheduled times or that died prematurely were subjected to necropsies. A limited selection of organs were weighed: heart, liver, brain, kidneys, spleen, adrenals, pituitary and testes. A wider selection of organs and tissues were examined microscopically, along with any gross lesions.

There were no treatment-related effects seen on mortality, clinical signs, haematology or clinical chemistry. Body-weight gain and food consumption were decreased in both sexes at the highest dose. No treatment-related changes in gross pathology was seen. Relative spleen weight was increased in females at the highest dose (12 mg/kg bw per day) at the 12 months and 24 months. Relative liver weight was increased in females at 6 or 12 mg/kg bw per day, but was only statistically significant ($p < 0.01$) at 12 months. No treatment-related changes in histopathology were noted. The pattern of tumours seen was not related to dose. The Meeting concluded that cyhexatin was not carcinogenic in this study.

The NOAEL was conservatively estimated to be 3 mg/kg bw per day on the basis of an equivocal increase in relative weight of liver without any supporting evidence from histopathology (Eisenlord et al., 1970b).

In a long-term study of toxicity/carcinogenicity that did not comply with GLP, groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing cyhexatin (purity, 97.2%) at dietary concentrations that were adjusted to achieve doses of 0, 1, 3 or 6 mg/kg bw per day for 24 months. The study did not conform to the standards set out in OECD methodological guideline 453 (OECD, 1981b). No haematology, clinical chemistry or urine analysis measurements were made. Necropsies were performed on all animals that died spontaneously and all those that survived until the end of the 24-month treatment period. For all animals, major organs were weighed and a wide selection of tissues and organs were examined microscopically.

Mortality over the treatment period ranged from 26% to 60%, but there was no dose–response relationship. There were no treatment-related effects on clinical signs of toxicity. The mean body-weight gains of male and female rats in the groups at 3 or 6 mg/kg bw per day were statistically significantly decreased at most measurements throughout the study. The mean body weight of the females at 1 mg/kg bw per day was significantly higher than concurrent control values on day 420 and the mean body weights of males were significantly higher on days 84 and 126. No treatment-related gross pathology was seen at necropsy. The absolute weights of several organs (kidneys, liver, testes) were in the groups at 3 or 6 mg/kg bw per day than in controls, but these were associated with decreases in body weight in the affected animals. The absolute and relative (to body weight) mean weights were increased in the pituitary of males and females at 6 mg/kg bw per day and in the spleen of females at 6 mg/kg bw per day. Histopathological examinations revealed statistically significant ($p < 0.05$) and dose-related increases in the

incidence of bile-duct hyperplasia at all doses, as shown in Table 7. Areas of biliary fibrosis were frequently found in the cyhexatin-treated animals. No other treatment-related histopathology was seen. There was no treatment-related increase in the incidence of any type of tumour.

The Meeting concluded that cyhexatin was not carcinogenic in this study, but no NOAEL could be identified owing to the finding of increased incidences of bile-duct hyperplasia at all doses (NOAEL, < 1 mg.kg/bw per day) (Warner et al., 1977).

In a long-term study of toxicity/carcinogenicity, groups of 70 male and 70 female Sprague-Dawley rats were fed diets containing cyhexatin (purity, 98.3–99.2%) at a concentration of 0, 7.5, 30 or 180 ppm for 24 months. The dietary concentrations provided average doses of 0, 0.34, 1.39 and 8.71 mg/kg bw per day for males and 0, 0.43, 1.75 and 10.21 mg/kg bw per day for females. The study complied with GLP and conformed with OECD methodological guideline 453 (OECD, 1981b). Tests for locomotor activity and a functional observational battery (including home-cage, handling, open-field, sensory and neuromuscular observations) and were conducted on 10 rats of each sex from each group during week 50 of the study. Blood samples were collected from 20 animals of each sex per group at months 3, 6, 12 and 24 for haematological examinations. Blood samples for clinical chemistry and urine samples for urine analysis were collected from 10 animals of each sex per group at the same time intervals. Ophthalmoscopy was performed on all animals during weeks 1, 51 and 103. After 52 weeks, 10 rats of each sex were selected for neuropathological examination and were perfused with a fixative while under anaesthesia and then the central and peripheral nervous systems of five rats of each sex from the control group and the group at 180 ppm were dissected and examined. Another 10 rats of each sex per group were killed at 52 weeks for necropsy. Necropsies were also performed on the rats that were scheduled to be killed after 104 weeks and on animals that died prematurely. Major organs were weighed and histopathology was performed on a wide selection of organs and tissues.

Mortality, clinical signs, the number of palpable masses, ophthalmoscopy, locomotor activity tests and the functional observation battery of tests were not affected by treatment. Statistically significant ($p < 0.05$) and dose-related decreases in body-weight gain and decreases in food consumption were noted occasionally throughout the study in the males and females at 30 or 180 ppm. Some slight but non-significant decreases in body-weight gain were seen at 7.5 ppm. Haematology revealed significant reductions in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration at most sampling times in both sexes at 180 ppm. Activated partial prothrombin time was increased in males at 180 ppm at 52 and 104 weeks. In the clinical chemistry measurements, total protein and glucose were decreased and alkaline phosphatase activity was increased in the males and females at 180 ppm, at all sampling times during the first year. The pH of urine was also increased at the same sampling times.

At necropsy, no treatment-related gross pathology was seen and there were no effects on organ weights. Examination by microscopy showed increased incidences of bile-duct hyperplasia in all groups (see Table 8). The increased incidences of bile-duct hyperplasia were statistically

Table 7. Incidence of bile-duct hyperplasia (%) in rats given diets containing cyhexatin for 24 months

Dose (mg/kg bw per day)	Males	Females
0	8	8
1	39	50
3	62	72
6	72	72

From Warner et al. (1977)

significant in the males at 180 ppm and the females at 30 or 180 ppm when only the animals killed on schedule at 104 weeks were considered. However, when the data from all animals (week 52, week 104, and decedents) were considered, the effect was statistically significant in the males at 30 and 180 ppm and at all doses in females. The severity of the hyperplasia was minimal or mild in most animals, and there appeared to be no dose–response relationship to the degree of severity. It was considered likely that biliary hyperplasia was not caused by the treatment with cyhexatin and was possibly attributable to fortuitously low findings in controls. In addition to this, the incidence of retinal atrophy was statistically significantly ($p < 0.05$) higher in males at 180 ppm and in females at 30 or 180 ppm than in controls, and the atrophy was more severe at the higher doses (see Table 8). The incidence of hepatocellular adenomas was slightly greater in the animals at 180 ppm than in controls, with incidences in the groups at 0, 7.5, 30 and 180 ppm being, respectively, 1/60, 1/60, 2/60 and 3/60 in males and 0/60, 0/60, 3/60 and 4/60 in females. The increased incidence in hepatocellular adenomas was only statistically significant ($p > 0.05$) in females at the highest dose.

The Meeting concluded that there was no clear evidence that cyhexatin was tumourigenic in this study.

Table 8. Incidence of bile-duct hyperplasia and retinal atrophy in rats fed diets containing cyhexatin for 24 months

	Dietary concentration (ppm)			
	0	7.5	30	180
Males				
Retinal atrophy:				
No. of eyes examined	48	47	53	48
Total	2	4	6	17*
Minimal	2	3	2	7
Mild	0	0	2	8
Moderate	0	1	1	1
Severe	0	0	1	1
Bile-duct hyperplasia :				
No. of livers examined	60	60	60	60
Total	17	22	31*	38*
Minimal	13	16	18	24
Mild	3	4	12	12
Moderate	1	2	1	2
Females				
Retinal atrophy:				
No. of eyes examined	58	52	51	50
Total	2	7	13*	34*
Minimal	2	6	6	18
Mild	0	1	5	8
Moderate	0	0	0	7
Severe	0	0	2	1
Bile-duct hyperplasia:				
No. of livers examined	60	60	60	60
Total	16	30*	45*	33*
Minimal	12	17	31	26
Mild	4	13	13	6
Moderate	0	0	1	1

From Mertens (2004)

* Statistically significantly different from controls: $p < 0.05$.

The NOAEL was 7.5 ppm (equal to 0.34 mg/kg bw per day in males and 0.43 mg/kg bw per day in females) on the basis of retinal atrophy seen at dietary concentrations of 30 ppm or more in female rats (Mertens, 2004).

2.3 Genotoxicity

Cyhexatin was tested for genotoxicity in several assays, covering all relevant end-points (including mutation in bacteria, mutation in mammalian cells, clastogenicity in mammalian cells, DNA repair in mammalian cells and genotoxicity in vivo), as summarized in Table 9. Certificates of compliance with GLP were supplied with all reports of the studies, apart from Mendrala (1985).

Table 9. Results of studies of genotoxicity with cyhexatin

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100.	0.63–200 µg/plate in DMSO ±S9[a,b]	95.6	Negative	Mendrala (1985a)
Reverse mutation	*S.typhimurium* TA1535, TA1537, TA98, TA100, TA102 and *E.coli* WP2 *uvrA*	0.5–500 µg/plate in DMSO ±S9[c,d]	93	Negative	Stankowski (1996)
Forward mutation (XPRT locus)	Chinese hamster ovary (AS52-CHO) cells	50–5000 ng/ml in DMSO +S9[b] and 1.67–167 ng/ml –S9	93	Positive +S9 and equivocal –S9	Stankowski (1997a)
Forward mutation (HGPRT locus)	Chinese hamster ovary (CHO-K_1BH_4) cells	2.7–4.5 µmol/l in DMSO +S9[b] and 10–50 nmol/l –S9	95.6	Negative	Mendrala (1986)
Cytogenetics assay	Chinese hamster ovary (CHO) cells[e]	0.5–4.0 µg/ml in DMSO +S9[b]; and 0.05–0.4 µg/ml –S9	NS	Equivocal	Kennelly & Kirkland (1985)
Unscheduled DNA synthesis	Primary culture of hepatocytes from male Fisher 344 rats	1.6×10^{-8} to 5×10^{-6} mol/l in DMSO	95.6	Negative	Mendrala (1985b)
In vivo					
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male and female CD-1 mice	Single intraperitoneal injections of 0.6–6.0 mg/kg bw in corn oil	93	Negative	Stankowski (1997b)
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male and female CD-1 mice	Single oral doses of 18–180 mg/kg bw in corn oil	95.6	Negative	Bruce et al. (1985)

[a] S9 from an unspecified mammalian source.

[b] No independent repeat.

[c] S9 from the livers of Sprague-Dawley rats pretreated with an intraperitoneal injection of Araclor 1254.

[d] Two independent experiments.

[e] Independent experiments using two different clones of CHO cells.

NS, not stated

The studies were broadly in line with the relevant OECD methodological guidelines: 471 (OECD, 1997a), 476 (OECD, 1997b), 473 (OECD, 1997c), 482 (OECD, 1986) and 474 (OECD, 1997d).

Some of the tests for genotoxicity in vitro produced results that gave some cause for concern. The assay for cytogenetic changes (Kennelly & Kirkland, 1985) gave equivocal results. Positive results were obtained in the presence of metabolic activation in the assay for mutation of the gene for xanthine-guanine phosphoribosyl transferase (XPRT) (Stankowski, 1997a). However, the results of the assay for mutation of the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene (Mendrala, 1986) were not clear-cut, but appeared to be negative.

In the HGPRT assay in CHO cells in vitro (Mendrala, 1986), cyhexatin produced statistically significant increases ($p < 0.01$) in the number of mutant colonies produced at concentrations of 3.2, 4.1 and 4.5 μmol/l (the highest dose) in the presence of metabolic activation. The increases were dose-related, but were all within the range of historical controls for the laboratory. When an independent repeat of the experiment was performed, there was no increase in the number of mutations at any concentration in the presence or absence of metabolic activation. Therefore the result of this test was interpreted as negative.

The XPRT assay in CHO cells in vitro (Stankowski, 1997a) showed statistically significant increases ($p < 0.01$) in mutation frequency at the highest doses tested in the presence (5000 ng/ml) and absence (167 ng/ml) of metabolic activation, and also at two other concentrations (16.7 and 50 ng/ml) in the absence of metabolic activation. However, there was no dose–response relationship.

When the experiment was repeated, there were significant increases ($p < 0.01$) in mutation frequency at the two higher doses tested in the presence of metabolic activation (330 and 500 ng/ml) and at the penultimate dose tested in the absence of metabolic activation (200 ng/ml). The frequency of mutation was not significantly increased at the highest dose (250 ng/ml) that was applied in the absence of metabolic activation. There was a dose–response relationship to the frequency rates in the presence of metabolic activation, but not in its absence. The response observed in the presence of activation met the criteria for a positive result in this test for genotoxicity, but the results in its absence remained equivocal. Overall, the test showed that cyhexatin has the potential to cause gene mutations at the XPRT locus.

Cyhexatin gave an equivocal result for clastogenicity in an assay for cytogenetics in vitro (Kennelly & Kirkland, 1985). In two separate experiments using different clones of CHO cells, cyhexatin produced small increases in the number of cells with chromosomal aberrations. In one experiment, there were statistically significant increases ($p < 0.05$), when compared with negative controls, in the number of chromosomal aberrations (including or excluding gaps) at the highest doses tested in the presence (4.0 μg/ml) or absence of metabolic activation (0.4 μg/ml). However, the increases were seen in only one out of two replicate cultures and only in these did the numbers of aberrations excluding gaps exceed the 99% confidence limits of historical solvent controls. The aberrations observed in the presence of activation mainly comprised hyperdiploid or polyploid cells, while in the absence of activation they were structural aberrations (deletions and exchanges).

In the other experiment, a significant increase ($p < 0.05$) over the negative control value was seen only in the total number of chromosome aberrations, including gaps, in cultures tested at the highest dose (0.4 μg/ml) in the absence of metabolic activation. There was also a significant ($p < 0.05$) dose-related positive trend in the number of chromosome aberrations, excluding gaps, that were seen in cultures tested in the absence of metabolic activation. In the presence of metabolic activation, there was a significant ($p < 0.05$) positive dose–response relationship in the number of chromosome aberrations, excluding gaps, and the number was significantly greater ($p < 0.05$) than controls for the highest dose tested (2 μg/ml). The incidences of chromosomal aberrations were, however, less than the upper 95% confidence limit of historical control values.

No clear conclusion could be drawn from the results of this cytogenetics assay with regard to the potential of cyhexatin to produce changes to the structure or number of chromosomes in a cell.

The other tests for genotoxicity in vitro that were performed—the assays for bacterial reverse mutation (Mendrala, 1985a; Stankowski, 1996) and the assay for unscheduled DNA synthesis (Mendrala, 1985b)—gave clearly negative results. The two tests for genotoxicity in vivo for the formation of micronuclei in the bone marrow of mice also gave clearly negative results (Stankowski, 1997b; Bruce et al., 1985).

The weight of evidence provided by the studies of genotoxicity indicates that cyhexatin is unlikely to be a genotoxic hazard to animals or humans that might be exposed.

2.4 Reproductive toxicity

(a) One- and two-generation studies

Two GLP-compliant two-generation studies (Breslin et al., 1987; Barrow, 1994b) and one GLP-compliant one-generation study (Barrow, 1994a) have been performed using dietary administration of cyhexatin to Sprague-Dawley rats. Both two-generation studies conformed to OECD methodological guideline 416 (OECD, 2001a). The two generation study by Barrow (1994b) included an investigation of developmental effects.

A one-generation study was performed in accordance with an in-house method. Groups of 25 male and 25 female rats were given diets containing 0 (controls given access to food ad libitum) or 30 ppm of micronized cyhexatin (purity, 96%) for 4 weeks before mating and throughout gestation and lactation. An additional group (peer-fed controls) was fed the same amount of untreated diet as was eaten by rats in the cyhexatin-treated group. Weekly analyses of the diet showed that the levels of cyhexatin in the diet ranged between 80% and 94% of the intended amount (i.e. 24–29 ppm). The intake of cyhexatin by the treated group rats was 1.9–2.5 mg/kg bw per day until the end of gestation, rising to 5.9 mg/kg bw per day in dams during lactation. No mortality occurred and no signs of toxicity were observed. During gestation, body-weight gain of the dams given cyhexatin was lower than for either of the control groups, but at other times there was no effect on parental body weight. Feed intake of treated rats was reduced at various times: during the first week for males and during the first 2 weeks of gestation and last 2 weeks of lactation for females. There was no adverse treatment-related effect on mating performance, pregnancy rate, duration of gestation, pup viability or pup weight at birth. Pup weight at weaning was significantly lower for males and females in the cyhexatin-treated group than the controls fed ad libitum. In the peer-fed control group, pup weight at weaning was also significantly lower than the values for the controls fed ad libitum, but the values in all groups were within the range of results for historical controls, so the Meeting concluded that the effect was not caused by the cyhexatin (see Table 10).

Tests for physical and functional development of the pups showed no treatment-related effect on eye opening, incisor eruption, pinnae unfolding, auditory reflex or pupil reflex. No treatment-related abnormalities were found at necropsy examinations of parental males, parental females or pups. There was no effect on the weights of testes and epididymes of the males in the parental group. The Meeting concluded that the treatment with cyhexatin in the diet caused no adverse effects in this study. The observed slight effects on body weight were likely to be caused by reduced feed intake in the treated group and peer-fed control group compared with that in the control group fed ad libitum. The NOAEL was the only dose used, 30 ppm (equal to 1.9 mg/kg bw per day) (Barrow, 1994a).

Table 10. Pup weight at weaning in a one-generation study of reproductive toxicity in rats fed diets containing cyhexatin

Sex	Control group fed ad libitum	Peer-fed control group	Cyhexatin-treated group
Male	63	55*	53*
Female	59	54*	50*

From Barrow (1994a)

* Statistically significantly less than the control group given access to food ad libitum ($p < 0.01$)

Groups of 30 male and 30 female Sprague-Dawley rats were fed diets containing cyhexatin (purity, 95.5%) for two generations. The dietary concentrations of cyhexatin were adjusted to provide doses of 0, 0.1, 0.5 or 6.0 mg/kg bw per day throughout the study. Details of the frequency of adjustment and the achieved intakes of cyhexatin were not available, but it was claimed that the dietary concentrations ranged between 89% and 103% of the intended values. Treatment started 10 weeks before the mating of the F_0 generation rats and continued throughout gestation and lactation for this generation and the F_1 generation. The F_0 generation was mated once, and 30 male and 30 females of the F_1 generation were mated twice to produce offspring of the F_{2a} and F_{2b} generation. Gross pathological examinations were performed on all adult animals of the F_0 and F_1 generations and on 10 pups of each sex from each dose of the F_1, F_{2a} and F_{2b} generations. Histopathological examinations were performed on livers of all adult F_0 and F_1 rats and on kidneys and reproductive organs of adult F_0 and F_1 rats in the 0 and 6.0 mg/kg bw per day doses (i.e. control and highest dose).

There were no treatment-related effects on mortality or clinical signs. Throughout the study, adult body weight was frequently significantly lower ($p < 0.05$) than concurrent control values at both F_0 and F_1 generations in males and females at the highest dose (6.0 mg/kg bw per day) and also in the F_0 females of the group at 0.5 mg/kg bw per day. The reductions in body weight were roughly mirrored by reductions in food intake by the same groups. There were no treatment-related effects on mating, conception and gestation indices, duration of gestation, gestation survival index, number of pups born alive or birth weight. The mean body weight at weaning was significantly less ($p < 0.05$) than control values in male and female pups of the F_1, F_{2a} and F_{2b} generations at 6.0 mg/kg bw per day. Pup survival to weaning was decreased in the F_{2b} pups at 6.0 mg/kg bw per day. Histopathological examination of the adult rats of the F_0 and F_1 generations showed increased incidences of bile-duct hyperplasia, periductal inflammation and decreased glycogen in the livers of males and females of both generations at the highest dose (6.0 mg/kg bw per day). No treatment-related histopathology was seen in the kidneys or reproductive organs.

The NOAEL was 0.5 mg/kg bw per day on the basis of adverse effects that were seen at 6.0 mg/kg bw per day, including effects on the liver of treated adults and effects on the body weight at weaning and survival to weaning of the treated rats' pups. The reductions in body-weight gain that were seen at 0.5 mg/kg bw per day in the F_0 females seemed to be related to reduced feed intake (possibly caused by unpalatability of the test material) and did not appear to be due to any inherent toxicity of cyhexatin (Breslin et al., 1987).

Groups of 25 males and 25 female Sprague-Dawley rats were fed diets containing micronized cyhexatin (purity, 96%) at a concentration of 0, 10, 30 or 100 ppm for two generations. Weekly analyses of the diets showed that the levels of cyhexatin remained within 10% of the intended concentrations. The dietary concentrations gave mean doses of 0, 0.7, 2.1 and 7.0 mg/kg bw per day for males and 0, 0.7, 2.4 and 7.5 mg/kg bw per day for females. The females ate more during lactation and subsequently were exposed during this period to mean doses of 0, 1.7, 4.8 and 12.8 mg/kg bw per day in the F_0 generation and 0, 1.7, 4.8 and 11.8 mg/kg bw per day in the F_1 generation. The F_0 males and females were treated for 10 weeks before mating and treatment continued throughout gestation and lactation for this generation and the F_1

generation. The F_0 generation was mated once, and 25 male and 25 females of the F_1 generation were mated twice to produce offspring of the F_{2a} and F_{2b} generation. A developmental toxicity phase was performed following second mating of the F_1 generation adults, with the dams being killed and examined on day 20 of gestation. The mean doses received by the F_1 dams during the gestation of their second litter (the developmental toxicity phase of the study) were 0, 1.0, 2.0 and 6.3 mg/kg bw per day. Histopathological examination of the reproductive organs was performed on all F_0 and F_1 adults.

There was no mortality and no treatment-related signs of toxicity were seen. There was a dose-related statistically significant ($p < 0.01$) reduction in body-weight gain at all doses in females of the F_0 generation during the pre-mating period and the lactation period. During gestation, the decreased body-weight gain was seen in F_0 females only at 100 ppm. Body-weight gain was reduced during gestation and lactation in the littering phase of the F_1 generation dams at 100 ppm only, while maternal body-weight gain was significantly reduced during the second gestation of the F_1 generation (developmental toxicity phase). Body-weight gain was significantly decreased ($p < 0.01$) only at 100 ppm in the F_0 males, but not in males at other doses or in other generations. The reductions in body-weight gain were roughly mirrored by reductions in food intake. There were no treatment-related effects on pregnancy rate, sex ratio of pups, mean pup birth weight at any dose in any generation. At the highest dose of 100 ppm, there were significant reductions in the mean number of uterine implantation sites in the F_0 and F_1 generation animals, the mean number of corpora lutea at the F_1 generation, and litter size at the F_0 and F_1 (both litters) generations. There was also a significant reduction in mean litter size in the F_0 generation rats at 30 ppm. Mean pup weight at weaning was significantly reduced ($p < 0.01$) at 30 and 100 ppm in both generations. The mean time of eye opening was significantly delayed in the offspring of F_0 generation rats at 100 ppm and the first litter of the F_1 generation rats at 30 or 100 ppm. There was an association between delayed eye opening and several other parameters: pup weight at weaning, maternal body-weight gain and maternal food intake. A few pups at 100 ppm in the F_{2a} generation failed to show a pupil reflex at day 21 post-partum. No treatment-related macroscopic or microscopic lesions were found in the parental animals from any generation. No treatment related abnormalities were observed on gross examination of newborn F_1 and F_{2a} pups. More detailed examination of the F_{2b} pups (developmental toxicity phase) showed no treatment-related fetal abnormalities of the skeleton or soft tissues.

The NOAEL was 10 ppm (equal to 0.7 mg/kg bw per day) on the basis of the low weight at weaning and delayed eye opening that was seen at 30 ppm (2.1 mg/kg bw per day for males; 2.4 mg/kg bw per day for females). The reductions in body-weight gain that were seen at 10 ppm in the F_0 females seemed to be related to reduced feed intake (possibly caused by unpalatability of the test material) and did not appear to be due to any inherent toxicity of cyhexatin. The low fetal weight and the delayed eye opening appeared to be secondary to reduced maternal body-weight gain (Barrow, 1994b).

(b) Developmental toxicity

Rats

In a study of developmental toxicity that complied with GLP, groups of 10 pregnant Sprague-Dawley rats were given cyhexatin (purity, 95.5%) at a dose of 0, 1, 5 or 10 mg/kg bw per day suspended in corn oil by oral gavage on days 6–15 of gestation. The rats were killed on day 16 of gestation and necropsies were performed on all dams. The liver and kidneys of the dams were weighed. Litter parameters were determined, including number of corpora lutea, number of implantation sites, and number and position of live and resorbed fetuses. The study did not conform to OECD methodological guideline 414 (OECD, 2001b), as the group sizes were too small and as the fetuses were examined at too early a time in their 21-day gestation period.

Maternal body-weight gain was reduced at 5 and 10 mg/kg bw per day. Relative liver weight was increased at 10 mg/kg bw per day. There were no effects on any litter parameters. No

developmental effects were seen in the limited number of fetuses that were available for examination.

The NOAEL was 1 mg/kg bw per day on the basis of reduced maternal body-weight gain (Scortichini et al., 1986).

The two-generation study by Barrow (1994b), which is described more fully in section 2.5.1, included an investigation of developmental effects. No developmental toxicity was seen in Sprague-Dawley rats given cyhexatin (purity, 96%) at a dietary concentration of 0, 10, 30 or 100 ppm (equal to 0, 1.0, 2.0 and 6.3 mg/kg bw per day) administered throughout gestation.

Rabbits

In a GLP-compliant two-phase study of developmental toxicity, groups of seven artificially inseminated New Zealand White rabbits were given oral doses of cyhexatin (purity, 95.5%) on days 6–18 of gestation. In the first phase of the study, cyhexatin was given at a dose of 0, 5, 10 or 20 mg/kg bw per day by gavage as a suspension in corn oil. In the second phase, doses of 0, 1, 5 or 10 mg/kg bw per day were given as a suspension in 0.5% Methocel. All surviving does were killed at day 19 (phase 1) or 20 (phase 2) of gestation and necropsies were performed on all does. The study did not conform to OECD methodological guideline 414 (OECD, 2001b), as the group sizes were too small, as the fetuses were examined at too early a time in their 31-day gestation period and as only a limited range of parameters were investigated.

In the first phase, there was a decrease in mean body weight over the treatment period in the groups at 10 or 20 mg/kg bw per day and all of the animals given these doses died prematurely. Two of the animals in the group at 5 mg/kg bw per day in the first phase also died, and the survivors showed signs of severe toxicity, with postmortem examination showing severe inflammatory lesions of the trachea and lungs. Four of the surviving does suffered total litter resorption. In the second phase, 0, 2, 1 and 1 animals died in the groups given 0, 1, 5 and 10 mg/kg bw per day and 0, 0, 1 and 4 animals in these groups suffered total litter resorption. There was a loss of body weight and increased numbers of resorbed embryos at doses of 5 and 10 mg/kg bw per day. Multifocal erosions were found in the stomachs of three of the survivors at 10 mg/kg bw per day and in one animal at 5 mg/kg bw per day. No evidence of maternal toxicity (apart form the death of two animals) or embryotoxicity was seen at 1 mg/kg bw per day. However, it is not possible to reliably identify a NOAEL for this study as a result of the limited nature of the study and the choice of inappropriate doses (Berdasco et al., 1986).

In a GLP-compliant study of developmental toxicity, artificially inseminated Dutchland New Zealand White rabbits were given cyhexatin (batch AGR 213445; purity, 95.5%) suspended in a 0.5% aqueous solution of methylcellulose by oral gavage. Groups of 20 does were given doses of 0, 0.5, 1.0 or 3.0 mg/kg bw per day on days 7–19 of gestation. The does were killed on day 29 of gestation, their livers were weighed and litter data were recorded. All fetuses were examined by fresh dissection and then prepared for skeletal examination. There was no treatment-related effect on mortality, although a small number of animals from each group died prematurely. Necropsy findings of the decedents showed red fluid in the thoracic cavity, lung adhesions and clotted material in the respiratory tract, so it was suspected that the deaths were caused by either dosing errors and/or respiratory infection. Maternal body-weight gain was decreased at the highest dose compared with controls. Liver weights were slightly greater in all treated groups than in the controls (statistically significant only at the intermediate dose), but there was no dose–response relationship. Four does at the highest dose and one at the lowest dose aborted. One doe at the lowest dose and one at the highest dose had total litter resorption, and one doe at the intermediate and one at the highest dose gave birth before necropsy. Resorption incidence and postimplantation loss were increased and litter size was reduced at the highest dose. Hydrocephaly was seen in eight fetuses from four litters at the highest dose and one of the aborted fetuses from this group

was seen to have a domed head. Hydrocephaly was not seen in any of the other groups. There was no treatment-related effect on the incidences of any other type of fetal abnormality.

The results of this study indicated a NOAEL of 1.0 mg/kg bw per day for teratogenicity on the basis of hydrocephaly and for embryotoxicity as indicated by postimplantion loss (Schardein, 1986).

A GLP-compliant study of developmental toxicity was carried out in Dutchland New Zealand White rabbits. The protocol did not conform to OECD methodological guideline 414 (OECD, 2001b). Groups of 27 artificially inseminated does were given cyhexatin (batch AGR 213445; purity, 94.8%) at a dose of , 0.75 or 3.0 mg/kg bw per day suspended in 0.5% Methocel by oral gavage on days 7–19 of gestation. The does were killed on day 28 of gestation, the liver and gravid uterus were weighed and the fetuses were examined by fresh dissection under low-power magnification. Skeletal examination was not performed on the fetuses.

The number of does that died prematurely in the groups at 0, 0.74 and 3 mg/kg bw per day were 2, 7 and 4, respectively. The necropsy findings suggested that all the deaths were caused by either dosing error and/or respiratory infection. No clinical signs of toxicity were observed. Mean maternal body-weight gain was severely reduced at 3 mg/kg bw per day, with the majority of does losing weight over the treatment period. The number of does that aborted were 0, 2 and 12 in the control group, and at the lower dose and higher dose, respectively. Postimplantation loss was increased at the higher dose. The number of malformed fetuses and the number of litters containing malformed fetuses were increased in a dose-related manner, with the incidence of hydrocephaly being greater than values for concurrent and historical controls. The incidences of these malformations are shown in Table 11.

It was noticed that the incidence of hydrocephaly in the concurrent control group was high in comparison with that for historical controls. It was not possible to identify a NOAEL for this study as developmental toxicity was seen at all doses tested (0.74 and 3 mg/kg bw per day) (Kirk et al., 1987a).

A GLP-compliant study of developmental toxicity was performed in two phases. In the first phase, groups of 24 mated female hybrid Hy/Cr New Zealand White rabbits were given cyhexatin (batch 243; purity, 96%) at a dose of 0, 0.5, 0.75 or 1.0 mg/kg bw per day by oral gavage as a suspension in 0.5% aqueous carboxymethylcellulose. In the second phase, similar groups of mated females were given a dose of 0 or 3.0 mg/kg bw per day on the same days of gestation. The general study was conducted in accordance with OECD methodological guideline 414 (OECD, 2001b).

The does were killed on day 29 of gestation, gravid uteri were weighed and litter parameters were recorded. All fetuses were examined by fresh dissection. The heads of half of the fetuses were examined by serial section. The remaining fetal carcasses (with and without heads) were processed for skeletal examination.

Table 11. Incidences of malformations in fetuses (and in litters) in a study of developmental toxicity in rabbits given cyhexatin by gavage

End-point	Dose (mg/kg bw per day)		
	0 (control group)	0.75 (lower dose)	3.0 (higher dose)
No. of fetuses (or litters) examined	167 (21)	133 (16)	47 (7)
Total malformed	3 (3)	10 (7)	11 (5)
No. with hydrocephaly	2 (2)	7 (5)	9 (4)

From Kirk et al. (1987a)

There was no treatment-related effect on mortality, although a small number of animals from each group died prematurely. Necropsy findings on the decedents showed pulmonary lesions that were consistent with intubation errors or pulmonary infection. There were no treatment-related effects on clinical signs, body weights, feed intakes, number of abortions, resorption incidence, postimplantation loss, litter sizes, premature birth or fetal weight. There were three or four malformed fetuses in the control group and in the groups at 0.5, 0.75 or 1 mg/kg bw per day, but only one malformed fetus at the highest dose of 3 mg/kg bw per day. The malformed fetus at the highest dose was the only one with hydrocephaly.

The NOAEL was the highest dose, 3.0 mg/kg bw per day (Monnot, 1989a).

A GLP-compliant study was performed to compare three types of cyhexatin: a form of high purity (batch 2186-44; purity, 99.7%; median particle size = 27 μm), a non-micronized technical material from the USA (batch PCRCX-901K; purity, 97%; median particle size = 161 μm), and a micronized technical material from the Netherlands (batch 3527; purity, 98%; median particle size = 38 μm). The technical materials were given as suspensions in 0.5% carboxymethylcellulose by oral gavage to groups of 15–18 artificially inseminated Charles River New Zealand White rabbits on days 6–19 of gestation. The doses given of the two technical materials were 0, 0.75, 1.5 and 3.0 mg/kg bw per day. The high-purity cyhexatin was given to groups of 8 or 9 does at a dose of 3.0 mg/kg bw per day as either a suspension in 0.5% carboxymethylcellulose or a suspension in 1% Cremophor. The does were killed on day 29 of gestation and litter data were recorded. All fetuses were examined for visceral abnormalities by fresh dissection. The heads of half of the fetuses were examined by serial section. The remaining fetal carcasses (with and without heads) were processed for skeletal examination. There was no treatment-related effect on mortality with any of the treatments, although up to four does from each group did not survive to term. The results for the groups given the high-purity cyhexatin were difficult to interpret as there were only three litters available for examination. However, for both groups given the high purity material, maternal body-weight gain and feed intake were decreased, there was an increased number of aborted litters and there was no treatment related fetal abnormality. With the non-micronized technical cyhexatin, maternal body weight and feed intake were severely reduced at the highest dose. One doe aborted at each of the groups given 1.5 or 3.0 mg/kg bw per day. Litter parameters, including resorption incidence, were not affected by treatment. There was an equivocal non-dose-related increased incidence of slightly folded retinas in all doses. There was a very slight dilation of brain ventricles seen in two fetuses from different litters at the highest dose and in one fetus at the intermediate dose, but the incidence was within the range for historical controls. None of the fetuses in the control group or at the lowest dose had dilated ventricles. With the micronized technical cyhexatin, four does at the highest dose were killed in extremis after body-weight loss. Body-weight gain and feed intake were decreased at the highest dose and to a lesser extent at the lowest dose. One doe at the lowest dose and two at the highest dose aborted their litters. Postimplantation loss was increased at 3.0 mg/kg bw per day. There were no fetal abnormalities apart from an equivocal non-dose-related increased incidence of slightly folded retinas.

It was not possible to identify a NOAEL for the high-purity cyhexatin. The NOAEL for the non-micronized technical cyhexatin was 1.5 mg/kg bw per day on the basis of maternal toxicity. The NOAEL for the micronized technical cyhexatin was 1.5 mg/kg bw per day on the basis of maternal toxicity and embryotoxicity, as indicated by postimplantation loss (Ross, 1990).

In a study of developmental toxicity that complied with GLP and that was conducted in accordance with OECD methodological guideline 414 (OECD, 2001b), groups of 24 mated female hybrid Hy/Cr New Zealand White rabbits were given either “pure” cyhexatin (batch 243P; purity, 99.1%) or technical cyhexatin (batch 243; purity, 96%) at a dose of 3.0 mg/kg bw per day by oral gavage on days 16–18 of gestation. The test materials were administered as a suspension in 0.5% aqueous carboxymethylcellulose. A control group was dosed with the carboxymethylcellulose solution at the same times. The does were killed on day 29 of gestation. Litter data were recorded and gravid uteri were weighed. All fetuses were examined for visceral

abnormalities by fresh dissection. The heads of half of the fetuses were examined by serial section. The remaining fetal carcasses (with and without heads) were processed for skeletal examination.

There was no treatment-related effect on mortality, clinical signs or food consumption. Body-weight gain was lower in the animals treated with cyhexatin animals during the treatment period, being about one third of the control value on average, but the effect was statistically significant ($p < 0.05$) only for the group given "pure" cyhexatin. Abortions occurred in three of the does given technical cyhexatin, but none occurred in the other groups. Postimplantation loss was slightly increased in the group given technical cyhexatin and decreased in those given "pure" cyhexatin, but the effects were not statistically significant ($p > 0.05$). There were no treatment-related effects on resorption incidence, litter size or fetal weight. There was one malformed fetus in the control group (spina bifida), one malformed fetus in the technical cyhexatin group and three malformed fetuses in the group receiving "pure" cyhexatin. The malformed fetus from the group receiving technical cyhexatin and two from the same litter in the group receiving "pure" cyhexatin had either dilated brain ventricles or hydrocephaly. The other malformed fetus in the group receiving "pure" cyhexatin had multiple visceral and skeletal defects. The author of the study concluded that there was no indication of teratology and that the NOAEL was 3.0 mg/kg bw per day (Barrow, 1994c).

Embryotoxicity was seen at a dose of 3 mg/kg bw per day in several studies (see Table 12). The highest NOAEL for embryotoxicity in these studies was 1.5 mg/kg bw per day, which was taken as the overall NOAEL for this end-point.

In a GLP-compliant study, groups of 16 artificially inseminated Dutchland New Zealand White rabbits were given cyhexatin (batch AGR 213445; purity, 95.5%) at a dose of 0, 0.5, 1.0 or 3.0 mg/kg bw per day in 0.5% Methocel by application to clipped skin on days 7–19 of gestation. The protocol of the study was broadly in line with OECD methodological guideline 414 (OECD, 2001b). Caesarian examinations were performed on day 28 of gestation and the liver and gravid uterus were weighed. Fetuses were examined by fresh dissection. Skeletal examination was not performed.

No maternal mortality occurred. In all treated animals there were signs of irritation (including erythema, eschar, oedema, fissuring and scaling) at the application sites. There were no treatment-related effects on clinical signs, maternal body weight, numbers of abortions, litter resorptions and premature births or on liver weight. There was no effect seen on the total incidence of fetal abnormalities. However, hydrocephaly was seen in four fetuses from three litters in the group at 3 mg/kg bw per day. No hydrocephaly was seen in controls or at other doses.

The NOAEL for effects other than dermal irritancy was 1 mg/kg bw per day on the basis of the hydrocephaly seen at 3 mg/kg bw per day (Kirk et al., 1987b).

A similar GLP-compliant study was performed, using a protocol that conformed to OECD methodological guideline 414 (OECD, 2001b). Groups of 24 pregnant New Zealand White rabbits were given cyhexatin (purity, 96%) dermally at a dose of 0, 0.5, 1.0 or 3.0 mg/kg bw per day in 0.5% carboxymethylcellulose on days 6–18 of gestation. Does were killed and examined on day 29 of gestation. Fetuses were examined for external, soft tissue and skeletal abnormalities. Skin reactions (erythema, atonia and desquamation) were seen at the application site for all doses, but were more severe (skin cracking) at 1 and 3 mg/kg bw per day. No treatment-related effects were seen on mortality, clinical signs, body weight, food consumption, gravid uterine weight, gross appearance of ovaries and uteri, number of fetuses, numbers of early and late resorptions, total implantations, corpora lutea, fetal weight and sex ratio of fetuses. There were also no treatment-related effects on the incidences of externa, soft tissue and skeletal abnormalities.

The NOAEL for effects other than dermal irritancy was the highest dose tested, 3 mg/kg bw per day (Monnot, 1989b).

Table 12. Embryotoxicity reported in studies in rabbits given cyhexatin by oral gavage

Test substance	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)	Details	Reference
Micronized cyhexatin	1	3	7.9, 11.1, 16.9 and 25.2% postimplantation loss at 0, 0.5, 1 and 3 mg/kg bw per day	Schardein (1986)
Non-micronized technical cyhexatin	0.75	3	19.7, 17.9 and 48.4% resorption of implantations and 65, 59 and 80% of litters with resorptions at 0, 0.75 and 3 mg/kg bw per day	Kirk et al. (1987a)
Non-micronized technical cyhexatin	3	—	0.9, 0.7, 0.5, 0.9 and 1.1 resorptions per full-term gestating female at 0, 0.5, 0.75, 1 and 3 mg/kg bw per day	Monnot (1989a)
Non-micronized technical cyhexatin	3	—	8.5, 9.1, 16.5 and 3.8% postimplantation loss at 0, 0.75, 1.5 and 3 mg/kg bw per day	Ross (1990)
Micronized technical cyhexatin	1.5	3	8.5, 8.8, 3.7 and 19.5% postimplantation loss at 0, 0.75, 1.5 and 3 mg/kg bw per day	Ross (1990)
High-purity cyhexatin	3	—	8.5 and 9.1% postimplantation loss at 0 and 3 mg/kg bw per day	Ross (1990)
Technical cyhexatin	3	—	9.9 and 10.5% postimplantation loss at 0 and 3 mg/kg bw per day	Barrow (1994c)
High-purity cyhexatin	3	—	9.9 and 7.3% postimplantation loss at 0 and 3 mg/kg bw per day	Barrow (1994c)
Azocyclotin	1	—	10.6 and 7.1% postimplantation loss at 0 and 1 mg/kg bw per day	Tesh & Ross (1981)
Azocyclotin	1	—	11.4, 11.4, 9.8 and 3.0% postimplantation loss at 0, 0.1, 0.3 and 1 mg/kg bw per day	Tesh et al. (1981)

2.5 Special studies

(a) Neurotoxicity

In a study of neurotoxicity, which was an add-on experiment to a long-term study of toxicity/carcinogenicity (Mertens, 2004), groups of Sprague-Dawley rats were fed diets containing cyhexatin (purity, 98.3%) at a concentration of 0, 7.5, 30, 180 or 360 ppm for 90 days. The group sizes were 15, 10, 10, 10 and 15 animals of each sex, respectively. Five animals of each sex from the control group and at the highest dose were maintained on basal diet for a 28-day recovery period, but the rest of the animals were killed at the end of the treatment period. The study was conducted in accordance with OECD methodological guideline 424 (OECD, 1997e).

The animals at the highest dose suffered unexpected toxicity in the first week of treatment (deaths of four males and two females, body-weight loss, markedly reduced food consumption, and various clinical signs). The group at the "highest dose" were then fed basal diet for the second week and over the next 2 weeks the dose was raised stepwise to a dietary concentration of 240 ppm, which was maintained for the rest of the study. The average intakes of cyhexatin in the groups at 0, 7.5, 30, 180 and 360/240 ppm were, respectively, 0, 0.47, 1.99, 10.94 and 13.57 mg/kg bw per day for males and 0, 0.56, 2.16, 11.42 and 15.28 mg/kg bw per day for females. For neurological evaluation, a functional observation battery of tests and test for locomotor activity were performed before the start of treatment, in weeks 3, 7 and 12 of treatment and (on the recovery animals) in week 17 of the study. The tests for locomotor activity measured ambulatory activity and total motor activity. The functional test battery consisted of home cage

observations, handling observations, open-field observations, sensory observations, neuromuscular observations and physiological observations. Necropsies were performed on all animals and the weight and dimensions of the brain were recorded. During necropsy the animals were perfused in situ with a fixative, and subsequently five rats of each sex from the control group and at the highest dose underwent a detailed histopathological examination of the nervous system.

Apart from the early deaths at the highest dose, there was no treatment-related effect on mortality. Clinical signs, including emaciation, pale extremities, abnormal faeces and hypoactivity were seen in several animals in the groups at 180 or 360/240 ppm. Mean body-weight gains and food consumption were decreased in both sexes at 180 and 360/240 ppm. There were no treatment-related effects on the functional battery or on locomotor activity. No gross pathology was observed at necropsy. The only effects on brain measurements were statistically significant ($p < 0.05$) decreases in the mean weight and mean length of the brains of the females at the highest dose in the recovery group that was killed at week 17 of the study. However, as the effect was not seen in any group that was killed immediately after treatment, it was considered unlikely that the effect on brain measurements was caused by treatment with cyhexatin. There were no treatment-related effects on neurohistopathology. The Meeting concluded that cyhexatin was not neurotoxic at any of the doses tested in the study.

The NOAEL was 30 ppm (1.99 mg/kg bw per day) on the basis of clinical signs and decreases in body-weight gain and food consumption at 180 ppm (Mertens, 2000b).

(b) Toxicity of metabolites

The safety of dicyclohexyltin oxide was tested in a non-GLP 90-day feeding study in Long-Evans rats. Groups of 10 males and 10 females were given diets containing dicyclohexyltin oxide (purity not stated) at concentrations that were adjusted to achieve doses of 0, 1, 3 or 6 mg/kg bw per day. A range of observations were made including clinical signs, body-weight changes, food consumption, haematology, clinical chemistry, urine analysis, gross pathology, organ weights and histopathology. There were no treatment-related effects on any of the parameters measured.

The NOAEL for dicyclohexyltin oxide was the highest dose tested, 6 mg/kg bw per day (Wazeter et al., 1968).

(c) Effects on bile-duct hyperplasia

An experiment was performed to investigate the bile-duct hyperplasia that had been seen in some long-term studies of toxicity in rats. Groups of 10 male Fisher F344 rats were given cyhexatin (purity, 95.5%) at a dose of 0, 10 or 20 mg/kg bw per day by gavage in corn oil for 14 or 28 days. Animals were observed for mortality, clinical signs of toxicity, effects on body weight, serum enzyme activities (alkaline phosphatase, gamma-glutamyl transferase and transaminases), gross pathology, organ weights and histopathology. In the 14-day segment, one rat at the highest dose died; in the 28-day segment, three rats died in each of the groups given cyhexatin. Clinical signs of toxicity included perineal soiling, facial staining, roughened fur, weakness and dyspnoea. The last of these signs may have been caused by inadvertant inspiration of test material during oral intubation. Body-weight gains in both segments (14 and 28 days) were less than control values with animals at the higher dose being more severely affected than those at the lower dose. Serum activity of alanine aminotransferase was significantly increased ($p < 0.05$) at both doses at 14- and 28-days and alkaline phosphatase activity was increased at both doses at 28 days. Erosions and/or ulcers of the glandular gastric mucosa were seen in some animals from all treated groups. Relative liver weights were increased at both doses at 14 and 28 days. Histopathological examination of livers showed increased eosinophic staining of hepatocytes in rats at 20 mg/kg bw per day and killed at 14 days. There was an increase in cytoplasmic vacuolation of hepatocytes in rats at 20 mg/kg bw per day and killed at 48 days. No treatment-related effect on bile ducts was observed. It was decided that short-term oral exposure of rats to

cyhexatin was not a good model for studying the effects of cyhexatin on bile-duct hyperplasia (Corley et al., 1987).

3. Observations in humans

Health records and annual medical tests of workers at a plant manufacturing cyhexatin in Italy have revealed no adverse health effects of cyhexatin over a period of 10 years. The tests performed included audiometry, spirometry, urine analysis, haematology and clinical chemistry (transaminases, blood urea nitrogen, glucose and bilirubin), plus thoracic X-ray and/or electroencephalography in some individuals (Cerexagri SA, 2004).

Comments

Biochemical aspects

Oral doses of cyhexatin were absorbed to a limited extent in rats (about 1.6–10% from the gut lumen). In rabbits given oral doses of cyhexatin, less than 10% of the administered dose was absorbed from the gut.

Cyhexatin is metabolized by hydroxylation, which splits off cyclohexyl rings to produce dicyclohexyltin and monocyclohexylstannoic acid. The products of the initial reactions can undergo oxidation to produce unidentified polar metabolites. In addition, hydroxylated and destannylated derivatives have been identified in the faeces of animals treated with cyhexatin, but it is not clear whether these were the products of bacterial and chemical breakdown in the gut lumen or the products of metabolism of absorbed material that had been excreted in bile. There was extensive distribution of metabolites of azocyclotin and cyhexatin to various organs and tissues of the body, with the highest amounts being found in the liver and the kidneys. Elevated levels of tin and ^{14}C radiolabel were detected in fetuses, amniotic fluid and placenta in pregnant rabbits given oral doses of ^{14}C-labelled cyhexatin.

In all species investigated (rat, rabbit and guinea-pig), excretion of the metabolites of cyhexatin was mostly in the urine and to a lesser extent in the bile. As a result of poor absorption, large proportions of orally administered doses of cyhexatin were found in the faeces. Minimal amounts were exhaled as carbon dioxide.

Toxicological data

Cyhexatin has moderate acute toxicity by the oral route. The LD_{50} value for cyhexatin in rats was 265 mg/kg bw when administered by the oral route. Cyhexatin had very low acute systemic toxicity when applied dermally, with LD_{50} values for rats of > 2000 mg/kg bw, but high acute toxicity after exposure by inhalation, with LC_{50} values for rats of approximately 0.016 mg/l.

Cyhexatin is a severe irritant to skin and eyes of rabbits and does not cause skin sensitization in tests in guinea-pigs.

Inhalation exposure of rabbits to cyhexatin at 0.21 mg/l or more for 6 h per day, 5 days per week, for 2 weeks, caused inflammation of the respiratory tract, pulmonary congestion and toxicity to the liver and kidneys. The NOAEC was 0.077 mg/l.

Increased serum alkaline phosphatase activity was found when cyhexatin at a dose of 1 mg/kg bw per day was applied to the skin of rabbits for 6 h per day, 5 days per week, for 3 weeks. The NOAEL was 0.3 mg/kg bw per day.

In short-term studies with cyhexatin, the main toxicological effects seen in rats were local effects on the gastric mucosa, haematological changes and hepatotoxicity. However, no treatment-related adverse effects were seen in a 90-day repeat-dose dietary study of toxicity that delivered cyhexatin at doses of up to 10 mg/kg bw per day to mice. When cyhexatin was given at doses of 10 or 20 mg/kg bw per day by gavage for 14 or 28 days, erosions and/or ulcers of the glandular gastric mucosa were seen in some animals at both doses. A 28-day dietary study with cyhexatin in

rats showed haematological changes related to changes in erythrocyte and blood clotting parameters at 6 mg/kg bw per day. The NOAEL was 3 mg/kg bw per day. In a 90-day study with cyhexatin in rats, there was hepatotoxicity and liver regeneration at dietary concentrations of 50 ppm or more, with a NOAEL of 10 ppm (equal to 0.68 mg/kg bw per day).

The toxicity of dietary doses of cyhexatin in dogs was investigated in studies with durations of 90 days, 1 year and 2 years. In the 90-day and 1-year studies, no treatment-related adverse effects were seen at up to the maximum doses tested of 6 and 0.75 mg/kg bw per day, respectively. In the 2-year study, the body weights of the dogs given cyhexatin at a dose of 6 or 12 mg/kg bw per day were reduced compared with those of controls. The NOAEL was 3 mg/kg bw per day.

The NOAEL for cyhexatin in a long-term study in mice was 3 mg/kg bw per day on the basis of increased mortality and decreased body weight at 6 mg/kg bw per day. Three long-term studies of toxicity/carcinogenicity were performed with cyhexatin in Sprague-Dawley rats. Increased incidence of retinal atrophy was seen at dietary concentrations of 30 and 180 ppm in one of these studies, with a slight increase in severity at 180 ppm. In the same study there was an increased incidence of minimal to mild bile duct hyperplasia in treated rats. This effect was of equivocal toxicological significance because there was no progression in severity with increasing doses. The NOAEL was 7.5 ppm (equal to 0.34 mg/kg bw per day) on the basis of retinal atrophy.

In a long-term study of toxicity/carcinogenicity in mice, exposure to cyhexatin did not cause tumours. In one out of three long-term studies in rats, there were slightly increased incidences of hepatocellular adenomas in both sexes at 30 and 180 ppm. However, only the increased incidence in the females at 180 ppm was statistically significant. As the increased incidence of benign tumours was seen only in one sex at one dose in one of four studies with cyhexatin in rats, and as the effect was not seen in studies with azocyclotin in mice and rats, the Meeting concluded that cyhexatin and azocyclotin were unlikely to be carcinogenic in rodents.

Azocyclotin was not genotoxic in an extensive range of tests for genotoxicity in vitro and in vivo. Cyhexatin gave negative results in most tests for genotoxicity in vitro, but gave positive results in a test for mutation of the xanthine-guanine phosphoribosyl transferase (XPRT) gene in vitro in the presence of metabolic activation, and equivocal results in the absence of metabolic activation. It also gave equivocal results in a test for chromosomal effects in vitro. Cyhexatin gave negative results in a test for micronucleus formation in bone marrow of mice in vivo.

The Meeting concluded that cyhexatin and azocyclotin are unlikely to be genotoxic in vivo.

In the absence of genotoxicity in vivo and with the finding of an equivocal increase in the incidence of benign liver tumours at a high dose in female rats in only one out of four studies of carcinogenicity in rodents, the Meeting concluded that use of azocyclotin or cyhexatin as pesticides is unlikely to pose a carcinogenic risk to humans.

The lowest NOAEL identified in any of three studies of reproduction in rats given cyhexatin was 0.5 mg/kg bw per day for maternal hepatotoxicity (periductal inflammation, decreased glycogen content and bile-duct hyperplasia) and on the weaning weight and survival to weaning of the pups. In one of the two-generation studies of reproduction with cyhexatin there was delayed eye opening in male and female pups at a dietary concentration of 100 ppm (equal to 7.0 mg/kg bw per day), with an NOAEL of 30 ppm (equal to 2.1 mg/kg bw per day). There were associations between the delayed eye opening and low pup weight at weaning, decreased maternal body weight and decreased maternal feed intake. The Meeting concluded that the pup toxicity was secondary to maternal toxicity.

Two studies of developmental toxicity with azocyclotin in rats treated orally by gavage found no fetotoxicity or teratogenicity at any dose tested up to 30 mg/kg bw per day and no effects on embryotoxicity at doses that were not maternally toxic. Similarly with cyhexatin, a limited study of developmental toxicity in rats showed no developmental effects at doses of up to 10 mg/kg bw per day and a NOAEL for maternal toxicity of 1 mg/kg bw per day was identified. In addition, a developmental toxicity phase included in one of the two-generation studies of

reproduction in rats treated with cyhexatin gave no indication of developmental toxicity at dietary concentrations of up to 100 ppm (equal to 7 mg/kg bw per day).

Six studies of developmental toxicity in rabbits have been performed with cyhexatin and two with azocyclotin. There was no embryotoxicity, fetotoxicity or teratogenicity in rabbits given azocyclotin at doses of up to 1 mg/kg bw per day by gavage. The NOAEL for maternal toxicity was 0.3 mg/kg bw per day. Maternal toxicity caused by cyhexatin, as indicated by reduced body-weight gain, was seen with an overall NOAEL of 1.5 mg/kg bw per day (in rabbits treated by gavage). Embryotoxicity (postimplantation loss) was seen at a dose of 3 mg/kg bw per day in three of the studies in rabbits given cyhexatin by gavage. The highest NOAEL for embryotoxicity in these studies was 1.5 mg/kg bw per day (in rabbits treated by gavage). In two of the studies in rabbits (Dutchland New Zealand White rabbits from the same supplier) given cyhexatin by oral gavage, there were statistically significant increases in the incidence of hydrocephaly and/or dilated brain ventricles. Equivocal effects were recorded at 0.75 mg/kg bw per day and above in one study. Hydrocephaly was also seen in a study of dermal toxicity in Dutchland New Zealand White rabbits from the same supplier to test the same batch of cyhexatin. Other studies used other batches of cyhexatin either in Charles River New Zealand White rabbits or hybrid Hy/Cr New Zealand White rabbits. In these studies, hydrocephaly and/or dilated ventricles were either not seen at all or seen only at very low incidences at higher doses of cyhexatin. The Meeting concluded that the hydrocephaly observed in two studies was probably a consequence of the unique susceptibility of the substrain of New Zealand White rabbits and/or of a unique toxicity of the batch of cyhexatin used in the study. As a consequence, the finding of hydrocephaly was not relevant to the risk assessment. The Meeting concluded that neither azocyclotin nor cyhexatin were teratogenic or fetotoxic, and that cyhexatin was embryotoxic with a NOAEL of 1.5 mg/kg bw per day.

A 90-day study of neurotoxicity in rats showed that cyhexatin was not neurotoxic at dietary concentrations of up to 240 ppm (equal to 13.6 mg/kg bw per day). At the start of the study, the highest dietary concentration had been 360 ppm, but this was reduced to 240 ppm because of high mortality, feed refusal, body-weight loss and adverse clinical signs. There were adverse effects on body-weight gain, food consumption and clinical signs (emaciation, pale extremities, abnormal faeces and hypoactivity) at doses of 180 ppm (equal to 10.9 mg/kg bw per day) or more. The NOAEL was 30 ppm (equal to 1.99 mg/kg bw per day).

The toxicity of the metabolite, dicyclohexyltin oxide, was tested in a 90-day dietary study in rats. No treatment-related adverse effects were seen at any dose up to the highest used, 6 mg/kg bw per day. The results of this study showed that dicyclohexyltin oxide was less toxic than either cyhexatin or azocyclotin.

Monitoring of workers at a factory manufacturing cyhexatin over a period of 10 years showed no adverse health effects.

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

Toxicological evaluation

The Meeting recognized that some of the reported adverse effects of both azocyclotin and cyhexatin were a secondary consequence of an irritating effect on the gastrointestinal mucosa and therefore were not relevant for establishing reference values.

The Meeting established a group ADI for azocyclotin and cyhexatin of 0–0.003 mg/kg bw based on the NOAEL of 0.34 mg/kg bw per day for retinal atrophy in a long-term study of toxicity/carcinogenicity with cyhexatin in rats and using a safety factor of 100.

The Meeting established a group ARfD for azocyclotin and cyhexatin of 0.02 mg/kg bw based on the NOAEL of 1.5 mg/kg bw per day for embryotoxicity in studies of developmental toxicity with cyhexatin in rabbits, and using a safety factor of 100. The ARfD is applicable to

women of childbearing age. No ARfD is necessary for the rest of the population, as the only other acute responses were related to dietary refusal and/or local irritation of the gut.

The Meeting recognized that the ARfD might be conservative, but it was not possible to determine whether the embryotoxicity was the result of systemic toxicity to the conceptus or the result of reduced nutrition caused by reduced maternal food intake and local adverse effects to the maternal gastrointestinal mucosa as a result of the irritant nature of the cyhexatin.

Levels relevant to risk assessment

(ii) Studies with cyhexatin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Long-term study of toxicity/carcinogenicity[d]	Toxicity	3 mg/kg bw per day[b]	6 mg/kg bw per day[b]
		Carcinogenicity	6 mg/kg bw per day[a, b]	—
Rat	Long-term study of toxicity/carcinogenicity[d]	Toxicity (retinal atrophy)	7.5 ppm (equal to 0.34 mg/kg bw per day)	30 ppm (equal to 1.39 mg/kg bw per day)
	Multigeneration study[d]	Toxicity	0.5 mg/kg bw per day[b]	6.0 mg/kg bw per day[b]
		Toxicity	0.5 mg/kg bw per day[b]	6.0 mg/kg bw per day[b]
		Developmental toxicity	7.0 mg/kg bw per day[a, b]	—
	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	5 mg/kg bw per day
		Developmental toxicity	10 mg/kg bw per day[a]	—
	Neurotoxicity[d]	Toxicity	30 ppm (equal to 1.99 mg/kg bw per day)	180 ppm (equal to 10.94 mg/kg bw per day)
Dog	2-year study[d]	Toxicity	3 mg/kg bw per day	6 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Developmental toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day

[a] Highest dose tested

[b] Dietary concentrations were regularly adjusted to achieve set doses

[c] Gavage administration

[d] Dietary administration

Estimate of acceptable daily intake for humans

0–0.003 mg/kg bw

Estimate of acute reference dose

0.02 mg/kg bw for women of childbearing age

Unnecessary for the rest of the population

Studies that would provide information useful to the continued evaluation of the compound

The metabolic fate of the 1,2,4-triazole that splits off from azocyclotin when it breaks down to form cyhexatin is unknown.

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Limited absorption in rats (1.6–10%) and rabbits (10%)
Distribution	Extensive, with the largest amounts being found in the liver and kidneys
Potential for accumulation	Accumulation is unlikely
Rate and extent of excretion	Excretion was mainly in the urine and to a lesser extent in bile
Metabolism in mammals	Splitting off of cyclohexyl rings and oxidation to produce a variety of substances (most of which were unidentified)
Toxicologically significant compounds (animals, plants and environment)	Cyhexatin
Acute toxicity	
Rat LD_{50} oral	407 mg/kg bw for males; 265 mg/kg bw for females
Rat LD_{50} dermal	7600 mg/kg bw for males; 3600 mg/kg bw for females
Rabbit LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	0.016 mg/l
Rabbit, skin irritation	Irritant
Rabbit, eye irritation	Severely irritant
Skin sensitization (test method used)	No skin sensitization potential in guinea-pigs (Buehler test)
Short-term studies of toxicity	
Target/critical effect	Hepatotoxicity (rats); low body weight (dogs)
Lowest relevant oral NOAEL	0.68 mg/kg bw per day (rats)
Genotoxicity	
	Not genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effects	Mortality and body weight (mice); retinal atrophy (rats)
Lowest relevant oral NOAEL	0.34 mg/kg bw per day for retinal atrophy (rats)
Carcinogenicity	Unlikely pose a carcinogenic. risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Decreased pup weight at weaning and decreased survival to weaning at parentally toxic doses
Lowest relevant reproductive NOAEL	Parents and offspring: 0.5 mg/kg bw per day Reproductive toxicity: 7.5 mg/kg bw per day, highest dose tested (rats)
Developmental target/critical effect	Embryotoxicity (postimplantation loss) in rabbits
NOAEL for maternal toxicity	1.5 mg/kg bw per day in studies of developmental toxicity in rabbits (low body-weight gain). 0.5 mg/kg bw per day in a two-generation study in rats (hepatotoxicity)
Lowest relevant developmental NOAEL	1.5 mg/kg bw per day for embryotoxicity in rabbits.
Medical data	
Health monitoring of workers	No adverse effects seen

Summary for azocyclotin and cyhexatin

	Value	Study	Safety factor
Group ADI	0–0.003 mg/kg bw	Rat, 2-year study, NOAEL	100
Group ARfD*	0.02 mg/kg bw	Rabbit, developmental toxicity, NOAEL	100

*For women of childbearing age, unnecessary for the rest of the population

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