5.15 HEXYTHIAZOX (176)

TOXICOLOGY

Hexythiazox is the ISO approved name for (*trans*-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide (CAS No. 78587-05-0). Hexythiazox is an acaricide that acts against egg, larval and nymph stages. The precise mechanism of acaricidal action is unknown.

Hexythiazox was evaluated previously by the JMPR in 1991 when an ADI of 0–0.03 mg/kg bw was established based on a NOAEL of 3.2 mg/kg bw per day identified in a 2-year study in rats and with a safety factor of 100. Hexythiazox was reviewed by the present Meeting as part of the CCPR periodic review programme. Two additional studies of genotoxicity and some revised study reports were available to the present Meeting.

Most of the pivotal studies met the basic requirements of the relevant OECD or national test guidelines. Only a small number of study reports contained certificates of compliance with good laboratory practice (GLP).

Biochemical aspects

The absorption, distribution and excretion of [^{14}C]hexythiazox was rapid in rats at 10 mg/kg bw, but much slower at 880 mg/kg bw. The extent of absorption at 10 mg/kg bw was approximately 30% on the basis of the level of urinary excretion, but significantly lower at 880 mg/kg bw. The maximum plasma concentrations of radiolabel were observed about 3–4 h after administration of the lower dose. The elimination half-life was about 10 h at 10 mg/kg bw, and was prolonged to about 20 h at 880 mg/kg bw, presumably reflecting saturation. This was confirmed by data on excretion. Most (about 60–90%, depending on the administered dose) of the radiolabel was excreted in the faeces within 3 days. About 10–20% of the administered dose was excreted as intact hexythiazox at the lower dose and 65–70% at the higher dose. The highest concentrations of tissue residues were found in fat, adrenals, liver and ovaries; the main component in fat was hexythiazox. Metabolism was extensive, but most of the radioactive material was not attributed to specific metabolites. The main metabolic reactions identified were hydroxylation of the cyclohexane ring and cleavage of the amide bond to the cyclohexane ring.

Toxicological data

Hexythiazox was of low acute toxicity when administered orally (LD_{50} > 5000 mg/kg bw), dermally (LD_{50} > 5000 mg/kg bw) or by inhalation (LC_{50} >2.0 mg/L) routes. No deaths were reported in any of the submitted studies. Hexythiazox was not irritating to the skin of rabbits; was a slight, transient eye irritant and produced no evidence for skin sensitizing potential in a Magnussen and Kligman maximization study in guinea-pigs.

The toxicity of hexythiazox given as repeated doses has been investigated in mice, rats and dogs. Effects on body weight and the liver (which showed hypertrophy and, in some studies, necrosis) were seen relatively consistently. However, a number of other effects were seen at lower doses in dogs.

In a 28-day study in mice, body-weight gain was reduced, total cholesterol concentration in plasma was decreased, liver weights were increased and there were alterations in liver pathology at 1800 ppm and above. The NOAEL was 300 ppm, equal to 55 mg/kg bw per day.

In a 90-day study in rats, there were reductions in body-weight gain, alterations in erythrocyte parameters and increases in liver, kidney and adrenal weights and fatty degeneration of the adrenals at 500 ppm, equal to 36 mg/kg bw per day, and above. At 3500 ppm, the incidence of hepatocellular

hypertrophy was increased in males and females and the incidence of glomerulonephrosis was increased in males. The NOAEL was 70 ppm, equal to 4.9 mg/kg bw per day.

In a preliminary 4-week study in groups of two male and two female dogs, the NOAEL was 125 ppm, equal to 5.5 mg/kg bw per day, on the basis of increased adrenal weights at 500 ppm and above. In a 1-year study in dogs, adrenal weights were increased and there was an increased incidence of adrenocortical hypertrophy at 500 ppm, equal to 13 mg/kg bw per day, and above. Also at 500 ppm, erythrocyte parameters and serum concentrations of inorganic phosphorus were reduced. At 5000 ppm, increased liver weights were associated with hepatocellular hypertrophy. The NOAEL in the 1-year study in dogs was 100 ppm, equal to 2.9 mg/kg bw per day.

The toxicity and carcinogenicity of hexythiazox has been investigated in long-term dietary studies in $B6C3F_1$ mice and F344 rats. In both studies, survival was > 70% in all groups at 2 years. Hexythiazox had no effect on survival in either study.

In mice, non-neoplastic findings included reduced body-weight gain, decreases in erythrocyte parameters, and increases in liver weight, hepatic necrosis, hepatic nodules and adrenal weights at 1500 ppm. At 250 ppm and above, there were reductions in leukocyte counts throughout the study and increases in the frequency of proteinaceous casts in the kidneys. Reductions in body-weight gain at 40 and 250 ppm were not considered to be biologically significant as the absolute body-weight values were similar to those of the historical controls. The incidences of hepatocellular adenoma and carcinoma were increased in males at 1500 ppm, but not statistically significantly ($p > 0.05$). In females the incidence of hepatocellular adenoma was increased significantly ($p = 0.033$) at 1500 ppm, but there was no change in the incidence of hepatocellular carcinoma in females. Low incidences of hepatoblastoma were seen in 3 out of 70 males at 1500 ppm**,** compared with a mean incidence in historical controls of 0.2% (range, 0 out of 50 to 1 out of 50). Two of the three mice with hepatoblastoma also had hepatocellular adenoma and carcinoma, and the hepatoblastomas were considered to be part of the general pattern of liver tumours in these aged mice. The incidences of adenomas and carcinomas were related to age or duration of treatment as they were not increased in mice terminated or dying before week 78, the normal duration of a study of carcinogenicity in mice. The NOAEL for non-neoplastic effects was 40 ppm, equal to 6.7 mg/kg bw per day, and the NOAEL for carcinogenicity was 250 ppm, equal to 42 mg/kg bw per day.

In rats, non-neoplastic findings included increased adrenal weights and severity of vacuolation; withdrawn/swollen testes and the severity of chronic nephritis at 430 ppm and above, although the latter was statistically significant only in males at the highest dose at 12 months. At 3000 ppm, there were increases in liver weight and the incidence of hepatocellular cytoplasmic alterations, and increased ovary and spleen weights. The incidence of mammary-gland fibroadenoma was increased in males at 3000 ppm compared with values for historical controls, but not statistically significantly according to a pair-wise comparison with concurrent controls. The incidence of testicular interstitial-cell adenoma was increased at 3000 ppm at the interim 12-month kill relative to values for historical controls; the incidence in rats in the control group at study termination was > 90%, as is typical for the F344 strain. The size of interstitial-cell tumours and the occurrence of withdrawn/swollen testes might be related, but no specific measurements of tumour size were reported. The NOAEL for non-neoplastic effects was 60 ppm, equal to 3.2 mg/kg bw per day, and the NOAEL for carcinogenicity was 430 ppm, equal to 23 mg/kg bw per day.

Hexythiazox produced negative results in an adequate and extensive range of assays for genotoxicity in vitro and in vivo. An equivocal result in a non-standard study in yeast was not considered to be of significance when compared with the overall database.

The Meeting concluded that hexythiazox was unlikely to be genotoxic.

No investigations had been performed into potential mechanisms behind the increases in incidence of tumours.

On the basis of the negative results of tests for genotoxicity, the relatively high doses producing tumours and the NOAELs for non-neoplastic effects, the Meeting concluded that the

increased incidences of tumours in rodents exposed to hexythiazox were likely to be threshold phenomena and that hexythiazox was unlikely to present a carcinogenic risk to humans at exposure levels associated with residues in food.

In a two-generation study of reproductive toxicity, parental rats showed toxicity consistent with other studies in rats; effects included: reduced body-weight gain, increased liver, kidney and adrenal weights. The NOAEL for adults was 400 ppm, equal to 24 mg/kg per day. The NOAEL for offspring was also 400 ppm, equal to 24 mg/kg bw per day, on the basis of reduced pup weights and associated changes in developmental landmarks at 2400 ppm. There were no effects on mating performance, gestation, litter size or pup survival at the highest dose tested, 2400 ppm, equal to 136 mg/kg bw per day.

In studies of developmental toxicity, hexythiazox did not induce specific malformations in either rats or rabbits. In the study of developmental toxicity in rats, the NOAEL for maternal toxicity (reduced body-weight gain) was 240 mg/kg bw per day; the NOAEL for developmental effects (reduced metatarsal ossification) was also 240 mg/kg bw per day. In the study of developmental toxicity in rabbits, there was no evidence of maternal toxicity at the highest dose tested, 1080 mg/kg bw per day, a dose that produced a slight increase in the number of fetuses with overlapping of the vertebral arches. The NOAEL for foetotoxicity in rabbits was 360 mg/kg bw per day.

The Meeting concluded that hexythiazox was not teratogenic and was not selectively toxic to the developing fetus.

No specific studies on neurotoxicity were submitted. Hexythiazox did not produce any neurotoxic effects in routine studies.

The effects of hexythiazox on the central nervous system, cardiovascular and respiratory system, skeletal muscle, isolated smooth muscle, intestinal motility, gastric secretion and on blood coagulation were studied in a general pharmacology study in a range of species. In these assays, hexythiazox did not demonstrate any pharmacological activity that would be of concern at dietary exposure levels.

A number of rat metabolites were investigated, all of which gave negative results in Ames tests. PT-1-2 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine-carboxamide) and PT-1-3 (5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine) were of moderate acute oral toxicity (LD_{50}s of about 1500 and 420 mg/kg bw, respectively). Other metabolites were of low acute oral toxicity (LD_{50}s of > 5000 mg/kg bw) in rats.

No reports of adverse effects or any unusual patterns in the data were evident in medical assessments of personnel from manufacturing plants spanning 20 years. Hexythiazox had not been linked to any epidemiological reports of adverse effects. A single poisoning incident was been reported in the Philippines, but no details were available.

The Meeting concluded that the existing database on hexythiazox was adequate to characteriz e the potential hazards to fetuses, infants and children

Toxicological evaluation

An ADI of 0–0.03 mg/kg bw was established for hexythiazox based on the NOAEL of 3.2 mg/kg bw per day, identified in the 2-year study in rats on the basis of increases in fatty vacuolation of the adrenals in both sexes, the severity of chronic nephritis and the incidence of swollen/withdrawn testes in males at 23 mg/kg bw per day and with a safety factor of 100. This was supported by the NOAEL of 2.9 mg/kg bw per day in the 1-year study in dogs.

The Meeting concluded that the establishment of an ARfD for hexythiazox was unnecessary on the basis of its low acute toxicity, the absence of developmental toxicity in rats and rabbits, the lack of evidence for any acute neurobehavioral effects, and the absence of any other toxicologically relevant effect that would be elicited by a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	40 ppm, equal to 6.7 mg/kg bw per day	250 ppm, equal to 42 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 42 mg/kg bw per day	1500 ppm, equal to 267 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity [a]	Toxicity	60 ppm, equal to 3.2 mg/kg bw per day	430 ppm, equal to 23 mg/kg bw per day
		Carcinogenicity	430 ppm, equal to 23 mg/kg bw per day	3000 ppm, equal to 163 mg/kg bw per day
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	2400 ppm, equal to 136 mg/kg bw per day[c]	—
		Parental toxicity	400 ppm, equal to 24 mg/kg bw per day	2400 ppm, equal to 136 mg/kg bw per day
		Offspring toxicity	400 ppm, equal to 24 mg/kg bw per day	2400 ppm, equal to 136 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	240 mg/kg bw per day	2160 mg/kg bw per day
		Embryo and fetal toxicity	240 mg/kg bw per day	2160 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	1080 mg/kg bw per day[c]	—
		Embryo and fetal toxicity	360 mg/kg bw per day	1080 mg/kg bw per day
Dog	One-year study of toxicity[a]	Increased adrenal weight and adrenal hypertrophy	100 ppm, equal to 2.9 mg/kg bw per day	500 ppm, equal to 13 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

Unnecessary

Information that would be useful for continued evaluation of the compound

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

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid: $Cm_{ax,}$ 3–4 h; limited, about 30%, based on concentrations in urine.
Distribution	Extensive; highest concentrations in fat, liver, adrenals and ovaries.
Potential for accumulation	Slight, some persistence of low concentrations of hexythiazox in fat
Rate and extent of excretion	Rapid, > 70% in 24 h and relatively extensive, > 90% in 3 days
Metabolism in animals	Extensive but not fully identified. Major reactions are hydroxylation and cleavage of the amide bond to the cyclohexane ring
Toxicologically significant compounds (animals, plants and environment)	Hexythiazox and metabolites PT-1-2 and PT-1-3
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 2 mg/L (highest technically achievable), 4 h, whole body
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slight transient irritation
Guinea-pig, skin sensitization	Not a sensitizer (Magnussen & Kligman)
Short-term studies of toxicity	
Target/critical effect	Reduced body-weight gain; increased liver and adrenal weight and associated pathology changes.
Lowest relevant oral NOAEL	2.9 mg/kg bw per day (1-year study in dogs; 100 ppm)
Lowest relevant dermal NOAEL	No data

Lowest relevant inhalation NOAEL	No data
Genotoxicity	
	No genotoxic potential in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body-weight gain, hepatotoxicity, adrenal fatty vacuolation, nephritis; testes (rat); haematology (mice).
Lowest relevant NOAEL	3.2 mg/kg bw per day (2-year study in rats, 60 ppm)
Carcinogenicity	None relevant at levels of human dietary exposure
Reproductive toxicity	
Reproduction target/critical effect	No adverse effect on reproduction
Lowest relevant reproductive NOAEL	136 mg/kg bw per day (rats, 2400 ppm, highest dose tested)
Developmental target/critical effect	Reduced ossification of metatarsals (rats)
	Increase in overlapping of the vertebral arches (rabbits)
Lowest relevant developmental NOAEL	240 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No specific studies; no indications from routine studies
Other toxicological studies	
	Screen for pharmacological activity did not identify any potent activity.
	All rat metabolites tested were negative in Ames tests. Two, PT-1-2 & PT-1-3, were of moderate acute oral toxicity; other metabolites were of low acute oral toxicity in rats
Medical data	
	No adverse reports from health surveillance of production plant workers. No reports of adverse findings in the published literature. One report of a poisoning case in the Philippines.

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg bw	Rat, 2-year study	100
ARfD	Unnecessary	—	—