HEXYTHIAZOX

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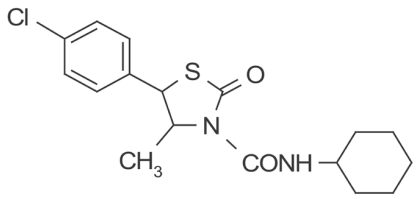
Explanation

Hexythiazox is the International Organization for Standardization (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 acceptable daily intake (ADI) of 0–0.03 mg/kg bw was established based on a no-observed-adverse-effect level (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 Codex Committee on Pesticide Residues (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 Organization for Economic Co-operation and Development (OECD) or national test guidelines. Only a small number of study reports contained certificates of compliance with good laboratory practice (GLP).

Figure 1. Chemical structure of hexythiazox



Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

Three groups of five male and five female Fischer F344 rats (age 10 weeks) rats were given a single oral dose of [thiazolidine-5-^{14}C]hexythiazox (specific activity, 6.6 mCi/mmol; purity, > 99%). The three different treatment protocols included a single oral dose at a lower dose (10 mg/kg bw), a single oral dose at the lower dose after fourteen oral doses of unlabeled hexythiazox and a single oral dose at a higher dose (880 mg/kg bw). The lower dose equated to approximately 50 µCi/kg bw and the higher dose, approximately 90 µCi/kg bw. The vehicle chosen for administration of the lower dosedimethyl sulfoxide (DMSO), while the vehicle chosen for the higher dose was olive oil, because of the solubility limitations of hexythiazox in DMSO. Blood and excreta were collected regularly over the 3 or 4 days after dosing. Concentrations of radioactivity were determined in fifteen tissues, in addition to plasma, and digestive organs and their contents. Expired gases were not collected because no volatile radioactivity (less than 0.01%) was recovered in a preliminary study. Analyses of the metabolites in urine and faeces were carried out on days 0–1 and 1–2 only, because subsequent samples contained only low levels of radioactivity. The metabolites of hexythiazox in excreta were determined by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). In a preliminary experiment, faecal metabolite identification was performed using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The levels of bound ^{14}C-residues in tissues were evaluated by polar solvent extraction. The study protocols are outlined in Table 1.

The maximum concentrations of radioactivity in plasma were observed about 3–4 h after administration in the groups at 10 mg/kg bw groups (groups B & C) and 12 h after dosing at at 880 mg/kg bw (group D). At those times the mean plasma concentrations of radioactivity in groups B and C were 1.8–2.2 ppm for males and 2.3–2.6 ppm for females, and in group D, 37 ppm for males and 27 ppm for females; indicating saturation of absorption at the higher dose. At 72 or 96 h after administration approximately 0.1 ppm of the radioactivity remained in the plasma in groups B and C; the concentrations of radioactivity in plasma in group D had decreased to approximately 2 ppm. The plasma absorption and elimination followed first-order kinetics, with rate constants of 0.53 h^{-1} and approximately 0.075 h^{-1} respectively, at 10 mg/kg bw in males. The elimination rate constant

Table 1. Design of a study of the absorption, distribution, metabolism and excretion of radiolabelled hexythiazox in rats treated orally

Parameter	Preliminary group	Group B	Group C	Group D
Nominal dose (mg/kg bw)	10	10	10 (after 14 days of pre-treatment with non-labelled hexythiazoz at 10 mg/kg bw per day)	880
No. of rats (male/female)	3/0	5/5	5/5	5/5
Duration (h)	48	72	96	96
Samples	Exhaled air, urine, faeces	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues	Urine, faeces, blood, tissues
Methods of analysis	Total ^{14}C	Total ^{14}C TLC, HPLC, NMR, MS	Total ^{14}C TLC, HPLC	Total ^{14}C- TLC, HPLC

From Soeda (1983); Soeda (1985a, 1985b, 1985c, 1985d)

HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectrometry; TLC, thin-layer chromatography;

corresponded to a half-life of approximately 9 h. In females, the half-life was slightly longer at 11.4 h and the half-life was also prolonged in males and females at the higher dose, at 17.3 and 21.7 h, respectively.

In groups B and C approximately 30% of the administered radioactivity was excreted in the urine and approximately 60–70% of the administered radioactivity was recovered in the faeces. In group D, 9.5% of the radioactivity was found in the urine and 89.1% in the faeces. Of the administered dose, 1.1–10.1% was associated with tissues at 96 h. The highest concentrations of radioactivity were found in fat, adrenal, liver, ovary and digestive organs and their contents. The highest concentrations of radioactivity in tissues were observed in fat 96 h after dosing, reaching approximately 2.3, 1.2 and 76 ppm in males and 5.4, 3.3 and 129 ppm in females, in groups B, C and D, respectively. Approximately 36–71% of the radioactivity in the liver and less than 2% of the radioactivity in fat remained as bound ^{14}C after extraction. Residue concentrations in fat were generally twice as high in females as in males. Concentrations of radioactivity in fat at the end of the studies were low, but more than 20fold those in plasma, indicating some potential for bioaccumulation. There were no remarkable differences in patterns of absorption and excretory patterns between males and females or after repeated doses (Soeda, 1983; Soeda, 1985a, 1985b, 1985c, 1985d).

The dermal penetration of hexythiazox has been investigated in rats in vivo. Groups of eight male Crl:CD(SD)BR rats were exposed to ^{14}C-hexythiazox in aqueous suspensions of a wettable powder formulation at three different dilutions. The application volume was 200 µl/25 cm^2. Four rats per group were fitted with jugular-vein cannulae and were exposed for 10 h, during which time samples of blood and excreta were collected and analysed by liquid scintillation counting (LSC). The remaining four rats per group were exposed for 1 h and samples of excreta were collected. At termination (at 10 h or 1 h in cannulated and non-cannulated rats, respectively) the concentration of radioactivity in a range of samples was determined by LSC. After an initial rapid absorption phase between 0 h and 0.5 h, absorption was linear, showing no diminution at the end of the 10 h exposure period. Only a small proportion of the applied radioactivity was absorbed (< 2%) in cannulated

rats. The shortcomings of this study, in terms of exposure time for the non-cannulated rats, no measurements taken more than 10 h after the start of exposure and no information on the residue at the application site, do not permit a definitive determination of the dermal penetration of hexythiazox to be made from this study (Grube, 1986).

1.2 Biotransformation

Rats

Samples from the studies of Soeda (1983) and (1985a, 1985b, 1985c, 1985d) (see above) were extracted and analysed for metabolites. In the groups at the lower dose, the primary identified compound was hexythiazox, present in the urine at approximately 2% of the administered radioactivity and in faeces at approximately 40% of the administered radioactivity. Unidentified compounds extracting into methanol represented approximately 80% of the radioactivity in urine and 30% of the faecal radioactivity. The primary, identified metabolic reactions were hydroxylation of the cyclohexane ring and cleavage of the amide-cyclohexane bond. The major identified radiolabelled metabolite in excreta was PT-1-8 (*cis*), which comprised 8–12% of the administered radioactivity in excreta in the groups at the lower dose. The remaining identified metabolites were present at low concentrations (each < 2% of the administered radioactivity): PT-1-2, PT-1-3, PT-1-4, PT-1-8 (*trans*), PT-1-9, PT-1-10 and PC-1-1 (see Figure 1 for metabolite names and structures). The major identified radiolabelled component in fat was the parent compound; the predominant metabolite in liver and kidney was PT-1-4. With such a high proportion of unidentified material, the metabolism of hexythiazox in rats cannot be considered to have been fully characterized (Gomyo, 1991).

The proposed metabolic pathway of hexythiazoz is given in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

Hexythiazox was of low acute toxicity when administered orally, dermally or by inhalation. No deaths were seen in any of the submitted studies (Table 2).

Table 2. Acute toxicity of hexythiazox

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Reference
Rat	SLC:SD	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983a)
Mouse	SLC:ICR	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983b)
Dog	Beagle	Males & females	Oral	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1984)
Rat	SLC:SD	Males & females	Dermal	> 5000 (water + Tween 80)	—	98.3	Saika et al. (1983c)
Rat	SLC:SD	Males & females	Inhalation (4 h, whole body)	—	> 2.0 [a] (dust aerosol; MMAD, 4.2 µm)	92.3	Saika et al. (1983d)

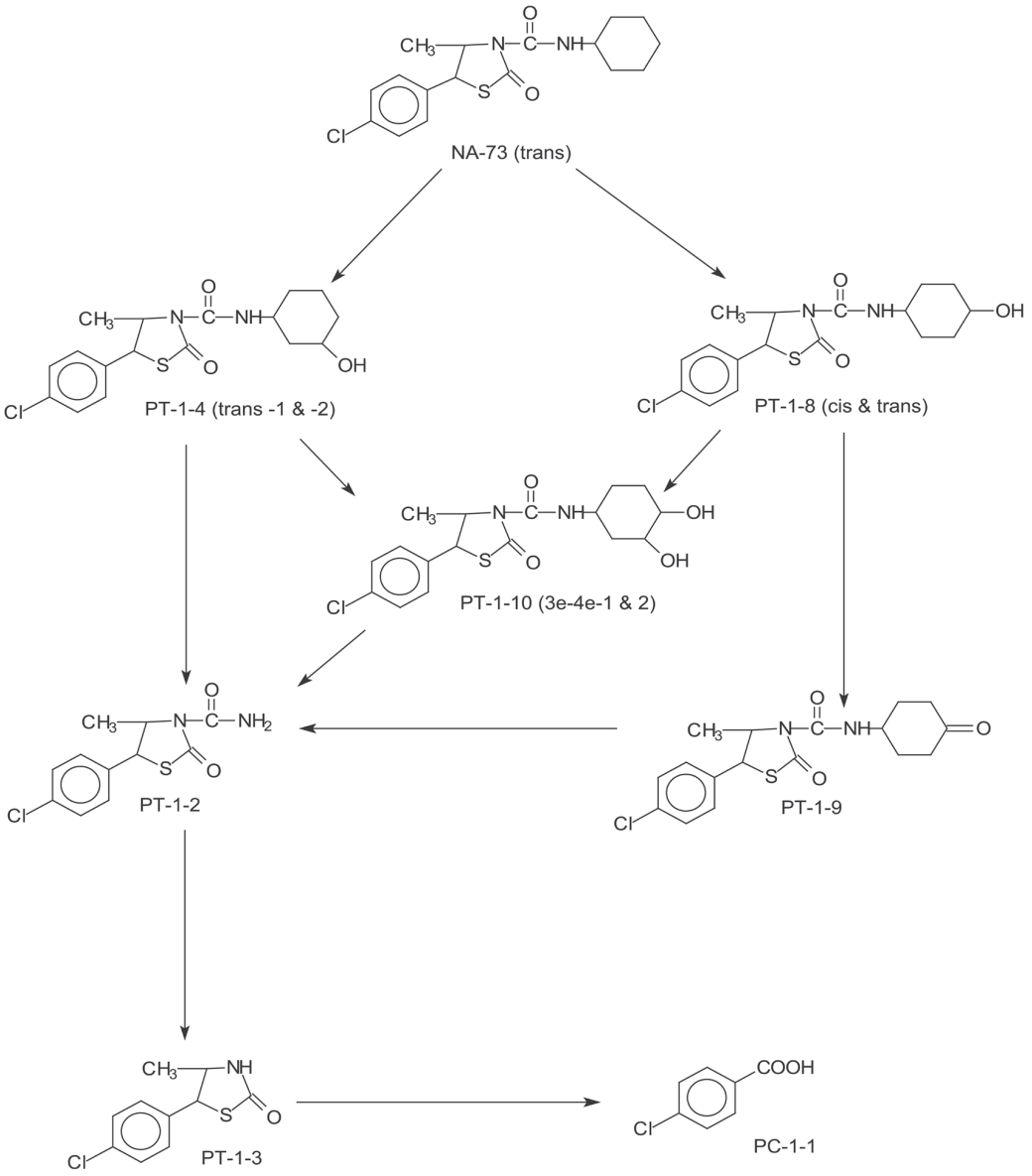
MMAD, mass median aerodynamic diameter.

[a] Maximal technically achievable concentration.

(b) Dermal and ocular irritation and dermal sensitization

Hexythiazox was not irritating to the skin of rabbits (Souma et al., 1983a); was a slight, transient eye irritant (Souma et al., 1983b) and produced no evidence of skin sensitizing potential in maximization tests (Souma et al., 1983c; Takaori, 2006).

Figure 2. Proposed metabolic pathway of hexythiazox in the rat



NA-73: hexythiazox
PC-1-1: 4-chlorobenzoic acid
PT-1-2: 5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine-carboximide
PT-1-3: 5-(4-chlorophenyl)-4-methyl-2-oxo-3-thiazolidine
PT-1-4: 3-hydroxy hexythiazox
PT-1-8: 4-hydroxy hexythiazox
PT-1-9: 4-oxo hexythiazox
PT-1-10: 3,4-dihydroxy hexythiazox

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female B6C3F$_1$ mice (age 6 weeks) were given diets containing hexythiazox (purity, 98.3%) at a concentration of 0, 50, 300, 1800 or 10 800 ppm for 28 days. Diets were supplied biweekly and stored in a freezer (–20 °C) until used. Haematological tests and clinical chemistry analyses were carried out at termination of the study. Urine samples were collected during week 3. Gross post-mortem examinations were performed, organ weights were determined and histopathological examination of liver, kidneys, heart, lung and spleen was performed on all mice. Histopathological evaluation of a wide range of tissues was conducted for mice in the control group and mice at the highest dose.

The homogeneity and content of the diet were not confirmed analytically. Mean daily intake of hexythiazox was reported as 10/13; 55/63; 319/388 and 1908/2045 mg/kg bw per day for males and females in the four treatment groups, respectively.

A male mouse in the group at 50 ppm died from a bite wound at 22 days. Adverse clinical signs were not observed in any of the mice treated with hexythiazox. Body-weight gain was decreased significantly (approximately 15%) at 3 and/or 4 weeks in males at 50, 1800 and 10 800 ppm, but not at 300 ppm. There was no notable change in food consumption. Total cholesterol was decreased significantly in males and females at 10 800 ppm (30–40%) and in males at 1800 ppm (approximately 20%). Specific gravity of urine was increased in males at 10 800 ppm. Enzyme markers of liver toxicity were similar in rats receiving hexythiazoz and rats in the control groups. A dose-dependent increase in liver weight and liver/body weight ratio was observed at 1800 ppm (approximately 10%) and 10 800 ppm (approximately 30%), in males and females. Swollen centrilobular liver cells were observed in the majority of males and females at 10 800 and in males at 1800 ppm. These cells were characterized by slightly enlarged nuclei and eosinophilic cytoplasm, not stainable by periodic acid-Schiff (PAS) stain. Fat droplets, which were usually visible in the liver of rats in the control group, were not apparent in these swollen cells.

The NOAEL was 300 ppm, equal to 55 mg/kg bw per day, on the basis of decreased body-weight gain and decreased total cholesterol in males at 1800 ppm (Takaori et al., 1983a).

Rats

Groups of 20 male and 20 female Fischer F344 rats were given diets containing technical hexythiazox (purity, 98.3%) at a concentration 0, 10, 70, 500 or 3500 ppm for 90 days. Satellite groups of 20 males and 20 females were used for interim measurements. An additional group of 20 males and 20 females was used for determination of baseline values for haematology, blood chemistry and cholinesterase activity. Haematological tests were performed pre-test and at 1.5 and 3 months. Blood chemistry determinations were performed pre-test and at 2 and 3 months. Cholinesterase activities (brain, plasma and erythrocyte) were determined after overnight fasting at 0, 1, 2 and 3 months, in 10 males and 10 females. Urine analysis was performed after 1.5 and 3 months. Gross necropsy and histopathology were performed on a wide range of tissues from all rats.

The homogeneity and content of the diet were confirmed analytically. Mean daily intake of hexythiazox was 0.7/0.8, 4.9/5, 36/38 and 266/258 mg/kg bw per day for males and females, respectively.

All rats survived until study termination without any clinical signs of toxicity. Body weight and body-weight gain were reduced in males and females at 3500 ppm and females at 500 ppm. Body-weight gain was reduced in males and females at 3500 ppm for almost the entire study period, starting from around week 4, and slightly in females at 500 ppm from week 11 (Table 3). There was a slight reduction (< 10%) in food consumption in males and females at 3500 ppm; on occasional weeks, the

reduction was statistically significant compared with values for the control group, but there was no obvious trend with duration of treatment. Food efficiency in females at 3500 ppm and 500 ppm was decreased at 1 week of feeding.

A number of haematological changes were seen in males at 3500 ppm (Table 3). A reduction in leukocyte count and effects on urine analysis results at 1.5 months, but not at 3 months, were not considered to be adverse findings given the absence of consistency over time. Significant changes in clinical chemistry results were seen in males and females at 3500 ppm; minor changes observed in rats at 500 ppm were not considered to be adverse (Table 3). Plasma cholinesterase activity was statistically significantly decreased at 500 (12 and 13% at 2 and 3 months, respectively) and 3500 ppm (10%, 25% and 24% at 1, 2 and 3 months, respectively) in females, but erythrocyte and brain acetylcholinesterase activities were not inhibited.

Absolute liver (approximately 8% and approximately 40%, respectively) and relative liver/body weights (approximately 10% and approximately 47%, respectively) were increased in males and females at 3500 and 500 ppm (Table 4). At 3500 ppm, absolute spleen weight and spleen/body weight ratio were decreased in males and females, kidney/body weight, adrenal/body weight and gonad/body-weight ratios were increased in males and females, absolute adrenal weight was increased in males, absolute thymus weight was decreased in females and brain/body-weight ratio was increased in females (Table 4).

All males at 3500 ppm suffered from glomerulonephrosis and 60% of males (12 out of 20) in all other groups, including controls. Hepatocellular hypertrophy was observed in all males and females at 3500 ppm. Fatty degeneration of adrenal cortex (zona fasciculate) was found in all males and 65% (13 out of 20) of females at 3500 ppm and in all males and 20% (4 out of 20) of females at 500 ppm versus zero in the control group and the groups at the lower doses.

The NOAEL was 70 ppm, equal to 4.9 mg/kg bw per day, on the basis of reduced body-weight gain, and increased fatty degeneration of the adrenal cortex (Takaori et al., 1983b).

Dogs

Groups of two male and two female beagle dogs were given diets containing hexythiazox (purity, 97.7%) at a concentration of 125, 500, 2000 or 8000 ppm for 4 weeks. Dogs in the control group received basal diet. The weights of brain, liver, kidney, heart, spleen, testis, ovary, pituitary, thyroid/parathyroid and adrenal were recorded. However, only liver and adrenal samples were prepared and examined microscopically.

The homogeneity and content of the diet were not confirmed analytically. Mean daily intakes were reported to be 5.6/5.5; 23/22; 89/79 and 324/346 mg/kg bw per day for males and females in the four treatment groups, respectively.

No deaths occurred during the course of the study. Food consumption was reduced (approximately 25%) in males at the highest dose, although when expressed per kg bw, food consumption was only marginally less than in the controls (–8.4%). Body-weight gain was decreased in females at 8000 ppm (30%), although this might have been linked to a higher mean starting weight. Relative liver weights were increased at 8000 ppm in males (30%) and females (25%) and at 2000 ppm in females (25%). Absolute and relative adrenal weights were increased in a dose related manner (30–60%) in males at 500 ppm and above and in females at 8000 ppm (50%). A slightly irregular heart rhythm was present in one female at 8000 ppm. There were no test substance related changes in gross necropsy and histopathology. The increased liver weights were not considered to be adverse in the absence of any histopathological correlates.

The NOAEL was 125 ppm, equal to 5.5 mg/kg bw per day, on the basis of increased adrenal weights in males (Spicer, 1984a).

Table 3. Selected findings in rats given diets containing hexythiazox for 90 days

Parameter	Dietary concentration (ppm)				
	0	10	70	500	3500
Body weight (g):					
Males	327	324	323	323	315*
Females	187	185	184	181*	172***
Body-weight gain (g):					
Males	190	187	186	186	178*
Females	82	81	79	76**	68***
Cholesterol (mg %):					
Males	22	20	21	24	30***
Females	33	32	31	32	41***
Total protein (g %):					
Males	5.9	5.8	5.9	6.0	6.6***
Females	6.0	5.9	5.9	6.1	6.6***
Albumin (g %):					
Males	3.8	3.8	3.8	3.9*	4.2***
Females	3.9	3.8	3.8	3.9	4.2***
Calcium (mg %):					
Males	9.8	9.8	9.9	9.9	10.1**
Females	9.5	9.4	9.5	9.6	9.8**
Alkaline phosphatase (mU/ml):					
Females	88	86	85	85	71***
> 300 mg protein/dl urine:					
Males	0	0	0	0	4
Erythrocytes (10^6/mm^3):					
Males	8.27	8.28	8.19	8.17	7.93**
Packed cell volume[a] (%):					
Males	51.0	51.1	50.7	50.4	48.4**
Haemoglobin (g/dl):					
Males	16.8	16.8	16.8	16.6	16.0**
MCV:					
Males	61.6	61.7	61.9	61.7	61.0*
Platelets (10^6/mm^3):					
Males	0.618	0.641	0.641	0.630	0.650*
Leukocytes (10^3/mm^3)					
Males, month 1.5	8.0	8.0	7.8	7.2*	7.0*
Males, month 3	7.5	7.2	6.9	6.9	7.0

From Takaori et al. (1983b)

MCV, mean corpuscular volume.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student t-test)

[a] Packed cell volume is equivalent to erythrocyte volume fraction × 100.

Table 4. Organ weights in rats given diets containing hexythiazox for 90 days

Parameter	Dietary concentration (ppm)				
	0	10	70	500	3500
Body weight (g):					
Males	327	324	323	323	315*
Females	187	185	184	181*	172***
Absolute liver weight (g):					
Males	8.21	8.22	8.13	8.77**	11.53***
Females	4.40	4.46	4.45	4.79***	6.11***
Relative liver weight:					
Males	2.64	2.66	2.69	2.85***	3.82***
Females	2.52	2.59	2.60*	2.85***	3.82***
Absolute spleen weight (g):					
Males	0.631	0.643	0.626	0.609	0.580***
Females	0.437	0.435	0.430	0.419	0.363***
Relative spleen weight:					
Males	0.203	0.209	0.207	0.199	0.192**
Females	0.250	0.252	0.251	0.250	0.228***
Absolute adrenal weight (g)					
Males (right/left)	0.025/0.026	0.026/0.029	0.026/0.028	0.026/0.028	0.029***/0.032*
Females (right/left)	0.029/.032	0.030/0.031	0.028/0.030	0.028/0.031	0.028/0.031
Absolute kidney weight (g)					
Males (right/left)	1.06/1.07	1.07/1.07	1.05/1.05	1.07/1.06	1.08/1.10
Females (right/left)	0.67/0.66	0.68/0.68	0.67/0.67	0.67/0.68	0.66/0.67
Relative kidney weight					
Males (right/left)	0.34/0.34	0.35/0.35	0.35/0.35	0.35/0.35	0.36**/0.36**
Females (right/left)	0.39/0.38	0.39/0.39	0.39/0.39	0.40/0.40*	0.41***/0.42***
Relative testis weight (right/left)	0.46/0.48	0.47/0.48	0.48/0.46	0.47/0.49	0.48/0.51**
Relative ovary weight (right/left)	0.018/0.020	0.022/0.020	0.20/0.21	0.021*/0.022*	0.023***/0.022*

From Takaori et al. (1983b)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Student t-test)

Groups of four male and four female beagle dogs were given diets containing hexythiazox (purity, 97.7%) at a concentration of 100, 500 or 5000 ppm (corresponding to mean daily intakes of 2.9/3.2; 13.1/13.9; 153/148 mg/kg bw per day in males and females, respectively) for 1 year. Body weights and food consumption were recorded weekly. Dogs were observed for moribundity and mortality and overt toxicity twice per day throughout the study; detailed observations were conducted at least once a week. Ophthalmoscopic examinations were conducted pre-test and at weeks 27 and 51. Blood biochemical, haematological and urine-analysis parameters were determined before study initiation and at 3, 6 and 12 months. Cholinesterase activities of erythrocytes and serum were determined. Gross necropsy and histopathology of an extensive range of organs and tissues were performed on all dogs.

The homogeneity and content of the diet were confirmed analytically.

All dogs survived to study termination. An increase in salivation (ptyalism) was reported in some dogs during the later stages of the study, although there was no clear dose–response relationship. There were no statistically significant differences in mean body weight values in any treated group when compared with the control group. However, body-weight gain was decreased in males at the highest dose and all treated females when compared with controls. Food consumption was decreased in all treated groups generally throughout the study (Table 7), but without any clear dose–response relationship.

Values for erythrocytes, haemoglobin and haematocrit (erythrocyte volume fraction) were reduced statistically significantly in males at 500 and 5000 ppm males at 3 months (Table 5); reticulocyte numbers were similar in test and control groups. Serum alkaline phosphatase (significant in males and females) and alanine aminotransferase (significant only in females) activities were increased in males and females (Table 5). There were significant reductions in total protein at 12 months at 5000 ppm in males and females and at 500 ppm in males. Phosphorus was significantly decreased in females at 500 and 5000 ppm and non-significantly in males at 5000 ppm at study termination (Table 5). There were no changes in serum or erythrocyte cholinesterase activities.

Absolute adrenal and adrenal/body weights were significantly ($p < 0.01$) increased at 5000 ppm in males and females (Table 7). This finding was attributed to adrenocortical hypertrophy seen in all three zones of the cortex (Table 6). Increases in adrenal weights were apparent at 500 ppm, the values were not statistically significant but there were trace levels of adrenal hypertrophy (Table 6). Liver/body weight was significantly ($p < 0.05$) increased in males and non-significantly in females at 5000 ppm (Table 7). Liver-weight increase was related to hepatocellular hypertrophy of generally mild severity (Table 6) and serum-enzyme changes (Table 5). Thyroid and parathyroid weights were increased in males at 5000 ppm and in females at 500 and 5000 ppm, but the differences from control values were not statistically significant and there were no consistent histopathological findings. There were no test substance-related changes in urine analysis or ophthalmoscopy.

The NOAEL was 100 ppm, equal to 2.9 mg/kg bw per day, on the basis of increased adrenal weights and adrenal hypertrophy in males and females and indications of altered erythrocyte parameters in males at 500 ppm (Spicer, 1984b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 80 male and 80 female B6C3F$_1$ mice were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 40, 250 or 1500 ppm for up to 2 years (104 weeks). Fifty males and 50 females per group were exposed for 104 weeks, while satellite groups of 10 males and 10 females were scheduled for interim termination at weeks 26, 52 and 78. Body weight and food consumption were determined regularly. Haematological, clinical chemistry and urine analysis examinations were performed on 8–10 males and 8–10 females of each group at weeks 26, 52, 78 and 104. A gross examination was performed on all rats at termination, when the weights of a range of organs were determined. Histopathology was performed on an extensive range of tissues from 10 males and 10 females per group killed at week 52 weeks and at week 104 on all mice that survived until study termination, as well as on all mice that died or were killed in a moribund condition during the study. Livers from the mice killed at week 78 were investigated microscopically and the results presented in a separate report (Anonymous, 1985)

Table 5. Selected haematological and biochemical parameters in a 1-year study in dogs given diets containing hexythiazox

Parameter	Month	Dietary concentration (ppm)			
		0	100	500	5000
Males (n = 4)					
Erythrocytes (×°10^6/mm^3)	0	6.05	6.05	5.71	6.18
	3	6.81	6.55	6.27*	6.16**
	12	6.97	6.99	6.40	6.25
Haemoglobin (g/dl)	0	15.6	15.8	14.8	15.9
	3	18.4	17.5	16.9*	16.7*
	12	18.4	18.4	17.4	17.1
Haematocrit[a] (%)	0	43.9	43.7	41.3	44.2
	3	49.8	48.1	46.1*	45.4**
	12	50.7	51.1	47.3	46.6
Alkaline phosphatase (IU/l)	0	76	72	70	71
	3	46	47	48	81
	12	40	39	41	108**
Alanine aminotransferase (IU/l)	0	31	33	32	35
	3	45	43	41	60
	12	37	41	29	47
Total protein (g/dl)	0	5.6	5.7	5.6	5.8
	3	6.3	5.9	5.9	5.8
	12	6.9	6.4	5.9*	5.9*
Phosphorus (mg/dl)	0	7.2	7.6	7.7	7.4
	3	4.9	5.3	5.2	4.6
	12	4.0	4.1	4.1	3.4
Females (n = 4)					
Alkaline phosphatase (IU/l)	0	81	54*	68	64
	3	57	31*	43	104**
	12	58	25**	32*	106**
Alanine aminotransferase (IU/l)	0	37	35	34	36
	3	43	41	43	57
	12	27	28	31	40**
Total protein (g/dl)	0	5.3	5.3	5.3	5.5
	3	6.0	5.6	5.9	5.7
	12	6.6	6.0	6.0	5.6*
Phosphorus (mg/dl)	0	6.9	7.1	7.0	7.4
	3	4.6	4.9	4.6	4.8
	12	4.2	4.1	3.7*	3.6*

From Spicer (1984b)

* $p < 0.05$, ** $p < 0.01$ (t-test; Dunnet test)

[a] Haematocrit is equivalent to erythrocyte volume fraction × 100.

Table 6. Histopathology findings in a 1-year study in dogs given diets containing hexythiazox

Observation [a]	Dietary concentration (ppm)			
	0	0	0	0
Adrenal cortex (males / females):				
Trace hypertrophy	0/0	0/0	4/4	0/0
Mild hypertrophy	0/0	0/0	0/0	4/4
Liver (males/females):				
Trace hypertrophy	0/0	0/0	0/0	0/1
Mild hypertrophy	0/0	0/0	0/0	4/3
Thyroid (males/females):				
Parafollicular cell hyperplasia, mild	1/3	3/1	1/0	3/2

From Spicer (1984b)

[a] Four males and four females at each dose.

Diet content and homogeneity were confirmed analytically. Mean consumption of the test substance was 6.7/8.4, 42/51 and 267/318 mg/kg bw per day for males and females in the three treatment groups, respectively.

Survival in all groups was > 90% at week 78 and > 70% at week 104. There was no treatment-related alteration in survival rates or in the incidence of clinical findings in the control and treated groups. An apparent decrease in body weight in all groups of males receiving hexythiazoz could have been due to the unusually high body weight of the controls, particularly in the second half of the study (Table 8); this argument would not apply to the group at the highest dose as there were still notable (> 10%) deficits compared with the values for historical controls. The reductions in body weights in the groups at 40 and 250 ppm relative to those of the concurrent controls were most marked towards the end of the study, when the mice had started to lose body weight; the body weight values for the groups at 40 and 250 ppm were considered to be consistent with those of the historical controls. Body weights of females receiving hexythiazoz were comparable to those of females in the control groups. There were sporadic fluctuations in food consumption and food conversion efficiency, but these were not considered to be an effect of hexythiazox.

A range of changes in erythrocyte parameters were seen at 1500 ppm (Table 9); some were also seen at lower doses, but were not consistently related to duration of dosing (Table 9). Platelet counts were increased in males and females at 1500 ppm at week 52 and in males at 1500 ppm at week 104. Reticulocyte counts were increased at week 26 in all males receiving hexythiazoz and in females at 1500 ppm, and at week 52 in males at 1500 ppm (Table 9), but subsequently were similar to control values. There was a consistent, statistically significant reduction in leukocyte counts in males at 250 and 1500 ppm.

A number of clinical chemistry parameters were altered at 1500 ppm. Minor changes in clinical chemistry findings at 250 ppm (increased phosphate concentration and reduced uric acid) were not considered to be adverse as they were small in magnitude and/or not consistent over time (Table 9). There were no significant findings in the results of urine analysis.

Absolute liver and/or liver/body weights were increased in males and females at 1500 ppm throughout the study (Table 10). Increases in relative testes, brain and kidney weights were considered to be secondary to reduced body weights because there was no increase in absolute organ weights. Absolute adrenal and adrenal/body weights of males at 1500 ppm were increased at week 104, as were adrenal/body weights at week 52 (Table 10).

Table 7. Body, adrenal, liver and thyroid weights and food consumption data a 1-year study in dogs given diets containing hexythiazox

Parameter[a]	Dietary concentration (ppm)			
	0	0	0	0
Males (n = 4)				
Body weight (kg):				
Month 1	10.3	10.3	11.0	10.6
Month 3	12.3	12.2	13.5	11.1
Month 12	12.7	13.0	13.6	11.6
Food consumption (g/day):				
Month 1	309	349	337	315
Month 3	405	366	357	346
Month 12	414	322	309	264*
Females (n = 4)				
Body weight (kg):				
Month 1	8.5	8.1	8.6	8.9
Month 3	10.0	9.3	10.0	10.6
Month 12	11.3	10.1	10.4	10.9
Food consumption (g/day):				
Month 1	288	255	281	384
Month 3	410	328	286	331
Month 12	334	260	270	300
Organ weights				
Absolute adrenal weight (g):				
Males	1.10	1.16	1.41	1.76**
Females	1.28	1.19	1.59	2.26**
Absolute liver weight (g):				
Males	333	304	360	386
Females	308	249	271	353
Relative liver weight (to body weight):				
Males	2.71	2.44	2.82	3.51*
Females	3.03	2.60	2.72	3.35
Absolute thyroid/parathyroid weight (g):				
Males	1.21	0.96	1.38	1.58
Females	0.97	0.85	1.29	1.46
Relative thyroid/parathyroid weight (to body weight):				
Males	10.09	7.83	10.39	14.30
Females	9.36	8.67	12.80	14.00

From Spicer (1984b)

Table 8. Mean body weights (g) in male mice given diets containing hexythiazox for up to 2 years and in male mice in the database of historical controls

Time-point	Mean body weight (g)				
	Historical control groups[a]	Dietary concentration (ppm)			
		0 (concurrent control group)	40	250	1500
Week 0	21.3	22.1	22.2	22.1	22.0
Week 52	45.2	47.1	45.4	43.9**	42.7**
Week 78	45.4	48.2	45.7	44.0**	42.9**
Week 86	NA	47.9	44.9	44.5**	42.3**
Week 104	42.7	46.0	41.0**	42.3**	37.8**

From Inoue (1985)

NA, not available.

** $p < 0.01$ versus concurrent controls (group 0).

[a] Nine studies with 650 mice at initiation.

Table 9. Haematological and clinical chemistry parameters in mice given diets containing hexythiazox for up to 2 years

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
Males					
Erythrocytes (10^6/mm^3	26	8.94	8.99	8.82	8.66**
	52	8.87	8.45**	8.43**	8.24***
	78	8.80	8.66	8.33	8.01**
	104	10.35	9.07	9.03	9.47
Haematocrit [a] (%)	26	42.3	42.1	41.3	41.1*
	52	40.8	39.1*	39.1*	38.6**
	78	39.3	38.5	37.9	37.2*
Reticulocytes (%)	26	15	19*	21**	21*
	52	11	15	14	16*
Leukocytes (10^3/mm^3)	26	3.0	2.6	1.8*	1.2**
	78	3.0	2.5	1.7**	1.6**
	104	4.2	2.6	2.1*	1.6**
Uric acid (mg/dl)	52	3.7	4.2	3.7	2.4***
	78	4.3	4.2	3.9	3.7
Females					
Erythrocytes (10^6/mm^3)	26	9.31	9.34	9.15	8.91***
	52	8.82	8.86	8.65	8.74
	78	8.77	8.80	8.72	8.41*

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
	104	8.84	8.77	8.54	8.23
Haematocrit (%)[a]	26	43.4	43.3	43.0	42.0**
Reticulocytes (%)	26	17	19	17	24*
Uric acid (mg/dl)	52	3.2	2.6	2.1**	2.2**
	78	3.9	4.5	4.4	3.0***
Phosphate (mg/dl)	52	6.0	5.6	6.1	6.1
	78	4.4	5.0	5.2**	5.9***
	104	6.0	6.1	6.3	6.4

From Inoue (1985)

[a] Haematocrit is equivalent to erythrocyte volume fraction × 100.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 10. Mean organ weights in mice given diets containing hexythiazox for up to 2 years

Parameter	Week	Dietary concentration (ppm)			
		0	40	250	1500
Absolute liver weight (g), males/females:	26	1.25 / 1.10	1.21 / 1.09	1.31 / 1.12	1.43** / 1.26*
	104	1.95 / 1.46	2.02 / 1.61	1.87 / 1.51	2.59* / 1.69*
Absolute adrenal weight (mg), males	52	4	4	4	6
	104	6	6	7	7*

From Inoue (1985)

* $p < 0.05$ relative to controls; ** $p < 0.01$ relative to controls.

An increased incidence of liver nodules in males and females at 1500 ppm was observed on gross pathological examination. Cytological alterations in liver were increased in treated males at interim kill at week 52. Liver necrosis was increased in males at 1500 ppm at study termination (Table 11) but not in mice dying or killed earlier in the study. Changes in ovarian atrophy and hyaline droplet formation in the central nervous system were not considered to be adverse effects as there was no clear dose–response relationship and these effects are known to be age-related; it is also of note that the mean ovarian weights in mice in the control group were very high and showed a large standard deviation. Proteinaceous casts in the kidney were increased in frequency in males and females at 250 and 1500 ppm at study termination, but with no clear dose–response relationship (Table 11). Adrenal findings were similar in groups receiving hexythiazoz and in the control groups. 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; National Toxicology Program, 1999). 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 hepatocellular adenomas and carcinomas were related to age and/or extended duration of treatment as they were not increased in mice killed or dying before week 78, the normal duration of a study of carcinogenicity in mice. The histopathology findings, particularly the liver, were re-evaluated and although absolute numbers of mice with a particular lesion varied between the reports the overall picture was consistent (Enomoto, 1986; Inoue & Enomoto, 1987a & 1987b).

Table 11. Re-evaluation of incidence of non-neoplastic and neoplastic histopathology findings in mice given diets containing hexythiazox for up to 2 years

Finding	Dietary concentration (ppm)				No. of mice examined
	0	40	250	1500	
Males					
Liver:					
Hepatic nodules:					
Week 104	15	9	16	35***	70
Hepatocellular adenoma:					
Week 78	9	3	3	2	70
Week 104	20	23	17	27	
Hepatocellular carcinoma:					
Week 78	0	1	1	0	70
Week 104	11	9	10	14	
Hepatoblastoma: week 104	0	0	0	3	70
Hepatic necrosis:					
Dying during study	4	4	3	1	70
Terminal sacrifice	0	1	3	7*	
Kidney:					
Proteinaceous casts: week 104	12	9	20*	19	60
Females					
Liver:					
Hepatic nodules	6	6	3	19**	70
Hepatocellular adenoma:					
Week 78	2	0	0	0	70
Week 104	7	1*	5	16*	
Hepatocellular:					
Week 78	0	0	0	0	70
Week 104	0	3	3	3	
Hepatoblastoma: week 104	0	0	0	1	70
Kidney:					
Proteinaceous: week 104	24	29	37*	32	60
Ovarian atrophy: week 104	31	43	47	43	60

From Inoue (1985); re-evaluated by Enemoto (1986); Inoue & Enomoto (1987a & 1987b)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Fisher exact test).

The NOAEL for toxicity was 40 ppm, equal to 6.7 mg/kg bw per day, on the basis of consistently reduced leukocyte counts in males and increased proteinaceous casts in the kidney of males and females at 250 ppm. The NOAEL for carcinogenicity was 250 ppm, equal to 42 mg/kg bw per day, on the basis of the increased incidence of hepatocellular adenoma in females at 1500 ppm (Inoue, 1985).

Rats

Groups of 80 male and 80 female Fischer F344 rats were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 60, 430 or 3000 ppm for up to 2 years. Groups of 10 males and 10 females per dose were selected from a satellite group of 30 males and 30 females designated for water consumption measurements, clinical pathology and interim necropsy. Ophthalmoscopic examinations were conducted on all rats pre-test and on all rats in the control group and the group at the highest dose (excluding those in the satellite groups) at weeks 6, 13, 25, 52, 78 and 104. Haematological and clinical chemistry measurements and urine-analysis determinations were performed on 10 males and females per group at weeks 26, 52, 78 and 104. Necropsy was performed on all rats that died spontaneously or were killed in extremis, on rats killed for interim necropsy and on all survivors at week 104. Organ weights for adrenals, brain (with stem), heart, kidneys, liver, lung, ovaries, spleen and testes were determined. Samples of a range of tissues were collected from all rats for microscopic examination. In addition, three transverse sections through the head, including tongue, nasal cavity, turbinates, paranasal sinuses, nasopharynx, portions of oral cavity and middle ear, were examined for 10 males and 10 females per group. If tumours occurred at one of these sites, sections from that site were examined microscopically for all rats.

The homogeneity and content of the diet were confirmed analytically. Mean intakes of test substance were 3.2/4.0, 23/29 and 163/207 mg/kg bw per day for males and females in the three treatment groups, respectively.

There were no major differences in mortality among the groups; overall survival was good at > 70% in all groups at week 104. Mean body weights were statistically significantly lower throughout the study at 3000 ppm when compared with those of the controls. At 430 and 60 ppm, body weights were slightly decreased in males and females, but the magnitude was < 5% and although the difference compared with controls was occasionally statistically significant, it was not considered to be adverse. Food consumption was increased approximately 10% in males and females at 3000 ppm on a number of occasions. Food efficiency showed great variability throughout the study. Water consumption was generally comparable in controls and treatment groups. The only finding of note on clinical examination was an increase in swollen/withdrawn testes from month 15 months at 430 and 3000 ppm (Table 12). There were no treatment-related adverse findings in haematological, clinical chemistry or urine-analysis examinations. No test substance -elated ophthalmoscopic findings were observed.

Absolute and relative liver weights were increased in males and females at 3000 ppm at study termination. Absolute and relative adrenal weights were increased in males at 60 and 3000 ppm at study termination, but not at 430 ppm; with no dose–response relationship, the finding at 60 ppm was considered to be incidental. Relative kidney weights were increased significantly at 3000 ppm at study termination; absolute kidney weights were increased significantly in females, and increased but not statistically significantly in males (Table 13). Absolute and relative spleen weights were decreased (10–15%) in males at 3000 ppm at 12 months, but increased at 24 months (Table 13). Increased absolute and relative testis weights were observed at 3000 ppm at interim kill and increased testis/body weight at study termination. Relative ovary weights were increased significantly with a non-significant increase in absolute ovary weight at 3000 ppm (Table 13). Relative weights of the brain, heart and lung were increased at 3000 ppm, but as there was no associated increase in absolute weights these were considered to be secondary to the lower body weight.

Table 12. Clinical findings in the testes of rats (n = 70) given diets containing hexythiazox for up to 2 years

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Testes withdrawn or swollen				
Week 66–78	5	2	6	26*
Week 79–91	23	19	43*	51*
Week 92–106	31	29	45*	45*

From Spicer (1984c)

* $p < 0.05$

Table 13. Body and organ weights in rats given diets containing hexythiazoz for up to 2 years

Body or organ	Dietary concentration (ppm)			
	0	60	430	3000
Males				
Body weight (g)	378	374	362*	343*
Absolute adrenal weight (g)	64	70*	64	68*
Relative adrenal weight [a] (% × 10^3)	17	19*	18	20*
Absolute kidney weight (g)	3.6	3.5	3.5	3.6
Relative kidney weight [a] (% × 10)	1.0	0.9	1.0	1.1**
Absolute spleen weight (g)	1.3	1.3	1.4	1.7*
Relative spleen weight [a] (%)	0.33	0.35	0.40*	0.50*
Absolute liver weight (g)	15.7	15.6	15.8	18.1**
Relative liver weight [a] (%)	4.2	4.2	4.4	5.3**
Absolute testis weight (g)	5.9	5.5	5.9	6.6
Relative testis weight [a] (%)	1.6	1.5	1.6	1.9**
Females				
Body weight (g)	279	279	272	247**
Absolute adrenal weight (mg)	75	68**	70*	73
Relative adrenal weight [a] (% × 10^3)	27	25**	26	30**
Absolute kidney weight (g)	2.6	2.6	2.7	2.8**
Relative kidney weight [a] (%)	0.9	0.9	1.0	1.1**
Absolute spleen weight (g)	0.8	0.9	0.9	0.8
Relative spleen weight (%)	0.30	0.33	0.35	0.32
Absolute liver weight (g)	11.9	11.9	11.8	13.1**
Relative liver weight [a] (%)	4.3	4.3	4.4	5.4**
Absolute ovary weight (mg)	128	122	130	134
Relative ovary weight (% × 10^2)	4.6	4.4	4.8	5.4**

From Spicer (1984c)

[a] Compared with body weight.

* $p < 0.05$; ** $p < 0.01$

The incidences and severity of vacuolar fatty changes in the adrenal were increased in females at 430 and 3000 ppm at termination and in males at 3000 ppm at interim and terminal kill (Tables 14 and 15). Chronic nephritis was a very common finding both in the control group and in rats receiving hexythiazoz, but the severity was increased in rats treated with hexythiazoz, most notably at interim kill, at dietary concentrations of 430 ppm and greater, although statistical significance was apparent only in males at the highest dose (Table 14). Cytoplasmic alteration in males at 3000 ppm was the only histopathological finding in the liver that was increased when compared with incidence in rats in the control group. Seminal tubule vesiculitis was increased in males at 3000 ppm. Changes in thyroid histopathology were not consistent in males and females and did not attain statistical significance. Reduced incidences of eye mineralization and of heart muscle degeneration were seen in males and females at 3000 ppm. Other findings were typical of aged F344 rats and were similar in control and treated groups. The incidence of mammary-gland fibroadenoma was increased in males at 3000 ppm compared with values for historical controls (0–6%; Anonymous, 2007). The incidence of testicular interstitial-cell adenoma was increased at 3000 ppm at the interim 12-month kill (Table 14) relative to values for historical controls (0–15%); the incidence in rats in the control group at study termination was > 90%, as is typical for the F344 strain (Table 15). 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. Overall rates of tumour incidence (benign and malignant) were similar in rats receiving hexythiazoz and in the control group.

The NOAEL for general toxicity was 60 ppm, equal to 3.2 mg/kg bw per day, on the basis of increases in fatty vacuolation of the adrenals in males and females and the severity of chronic nephritis and incidences of swollen/withdrawn testes in males. The NOAEL for carcinogenicity was 430 ppm, equal to 23 mg/kg bw per day, on the basis of increases in mammary-gland fibroadenomas in males at 24 months (2 years) and in testicular interstitial-cell adenomas at 12 months relative to historical control incidences (Spicer 1984c).

Table 14. Histopathology findings at 12-month interim kill in male rats given diets containing hexythiazox

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Adrenal gland:				
Fatty vacuolation	2/10	1/10	5/10	9/11*
Kidney:				
Chronic nephritis (total)	7/10	6/10	9/10	8/11
Chronic nephritis, > trace	1/10	1/10	4/10	6/11*
Testes:				
Interstitial-cell adenoma	0/10	0/10	2/10	3/11 [a]
Interstitial-cell hyperplasia [b]	4/10	3/10	3/10	6/10

From Spicer (1984c)

* $p < 0.05$, Fisher exact test.

[a] $p = 0.0245$ (chi-squared test for linear trend).

[b] No hypertrophy reported in any rat.

Table 15. Histopathology findings in rats (n = 70) given diets containing hexythiazox for 2 years

Finding	Dietary concentration (ppm)			
	0	60	430	3000
Males				
Adrenal, cortex:				
Fatty vacuolation, total	13	12	17	24*
Fatty vacuolation, > mild	10	11	15	15
Adrenal, medulla:				
Pheochromocytoma	5	9	9	7
Pheochromocytoma, malignant	0	0	0	1
Kidney:				
Chronic nephritis, total	68	66	70	69
Chronic nephritis, mild	35	18	28	18
Chronic nephritis, moderate	28	46	34	50*
Chronic nephritis, severe	2	0	0	0
Liver:				
Cytoplasmic alteration, clear	3	4	4	7
Cytoplasmic alteration, > trace	1	4	4	6
Mammary gland:				
Fibroadenoma	0	1	2	6
Adenocarcinoma	0	0	0	1
Seminal vesicle:				
Vesiculitis, total	5	2	6	12
Testis:				
Interstitial-cell adenoma	67	66	66	68
Thyroid:				
Parafollicular cell adenoma	3	3	2	7
Follicular carcinoma	0	0	1	1
Females				
Adrenal, cortex				
Vacuolar change, total	18	20	30*	28
Vacuolar change , ≤ mild	17	18	26	28*
Adrenal, medulla				
Pheochromocytoma	0	3	0	2
Pheochromocytoma, malignant	0	0	0	1
Kidney:				
Chronic nephritis, total	61	60	67	66
Chronic nephritis, mild	28	36	48	44
Chronic nephritis, moderate	11	10	5	14
Chronic nephritis, severe	0	1	0	0
Thyroid:				
Parafollicular cell hyperplasia	4	8	7	7
Parafollicular cell adenoma	3	3	3	3

From Spicer (1984c)

* $p < 0.05$

2.4 Genotoxicity

Hexythiazox has been tested for genotoxicity in a wide range of assays. Many of the studies were performed in the 1980s and did not comply with GLP or current test guidelines. However, the Meeting considered that the overall database was adequate to conclude that hexythiazox does not possess significant genotoxic potential. The results of Ames tests with *Salmonella typhimurium* and *Escherichia coli* were negative. The result of a test for mutation in *Saccharomyces cerevisiae* was considered equivocal in the presence of metabolic activation. A test for mutation at the *Hprt* gene locus in Chinese hamster V79 cells gave negative results. The results of an assay for bacterial recombination (supplemental study) and a test for unscheduled DNA synthesis in rat primary hepatocytes in vitro were negative. The result of an adequate test for chromosomal aberration in vitro in Chinese hamster ovary (CHO) cells was negative. An inadequate test for chromosomal aberration in vivo gave negative results, as did two assays for micronucleus formation in vivo. The weight of evidence is that hexythiazox has no significant genotoxic potential.

The results of studies of genotoxicity with hexythiazox are summarized in Table 16.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Wistar rats (age 5 weeks) were given diets containing hexythiazox (purity, 98.2%) at a concentration of 0, 60, 400 or 2400 ppm. There were two litters per generation. F_0 parents received treated diets for 13 weeks before the F_1a mating; the F_1b mating occurred 2 weeks after weaning. F_1b parents were first mated at age 17 weeks. All pups were observed until day 21 of lactation. Before matings, the estrus status of females was confirmed. Litters were culled by random selection to a total of eight pups (four males and four females) on postnatal day 4. Mortality, behaviour, clinical observations, body weights, food consumption, mating performance, duration of gestation, reproduction and litter data were examined. F_1a and F_2a pups were observed for mortality only. F_1b and F_2b offspring were examined for general condition and physical development (pinna-detachment, eruption of incisors, hair growth and eye-opening). Brain, heart, lungs, liver, kidneys, spleen, adrenals, testes and ovaries weights were recorded from 10 F_2 adult males and 10 adult females per dose; for F_0 and F_1 rats, tissues were taken from five males and five females per group. A wide range of tissues was collected for histopathological examination from these rats, but epididymides, seminal vesicles and coagulating glands were not preserved for histopathology. The study also included a satellite group subjected to limited investigation of developmental effects in five F_0 dams per dose.

The homogeneity and content of the diets were confirmed analytically. Achieved intakes were 3.6–9.2, 24–62 and 136–360 mg/kg bw per day at 60, 400 and 2400 ppm respectively.

One death was observed at 60 ppm in the F_0 generation. Pup mortality did not show dose-related effects. There were no remarkable clinical signs of toxicity. Mean body weight was significantly reduced in F_0, F_1 and F_2 males and females at 2400 ppm. Food consumption was reduced in the F_0 and F_2 generations, and in F_1 females at 2400 ppm and also at 400 ppm in the F_2 females. The food efficiency of F_0 and F_1 males at 2400 ppm tended to be lower than that in the control group. There were significant increases in organ weights at 2400 ppm, but no dose-related histopathological findings. Absolute and relative liver weights were increased in males and females at the highest dose in all generations.

There were no adverse effects on mating, pregnancy or litter parameters at any mating (Table 17). There were no adverse findings in the satellite group for investigation of developmental effects.

Table 16. Results of studies of genotoxicity with hexythiazox

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	100, 200, 400, 800, 1600, 3200 and 6400 µg/plate, ± S9, in DMSO	97.7	Negative ± S9	Inoue (1983a)
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537; *E, coli* (WP2 *uvr*A)	313, 625, 1250, 2500 and 5000 µg/plate, ± S9, in DMSO	99.9	Negative ± S9	Kanaguchi (2005)
Gene mutation	*Saccharomyces cerevisiae* S138, S211 and D_4	S138 and S211: 312, 625, 1250, 2500, 5000 and 10 000 µg/ml	98.4	Negative ± S9	Jagannath (1984)
		D_4: 1, 10, 100, 500, 1000, 2500, 5000 and 10 000 µg/plate		Equivocal + S9; negative – S9	
		Both tests ± S9, in DMSO			
Gene mutation (*Hprt)*	Chinese hamster ovary cells (V79)	9.38, 18.8, 37.5, 75.0 and 150 µg/ml[a] ± S9; in DMSO	98.6	Negative ± S9	Seeberg (1986)
Chromosomal aberration	Chinese hamster ovary (CHO) cells	20, 30, 40 and 50 µg/ml[a] – S9, in DMSO;	98.4	Negative ± S9	Galloway (1984)
		40, 100, 150, 200, 300 and 400 µg/ml[a] + S9, in DMSO			
Chromosomal aberration	Chinese hamster ovary (CHO) cells	5, 20, 35 and 50[b] µg/ml – S9 in DMSO;	Not reported	Negative ± S9	Ivett (1986)
		35, 50, 200, 350 and 500[b] µg/ml + S9, in DMSO			
Recombination	*Bacillus subtilis* H17 and M45	400, 800, 1600 and 3200 µg/disk, in DMSO	97.7	Negative	Inoue (1983b)
Unscheduled DNA synthesis	Rat primary hepatocytes	2.5, 5, 10, 25, 50,[b] 100 and 250 µg/ml	98.4	Negative	Cifone (1985)
In vivo					
Chromosomal aberration	Chinese hamster bone marrow	1000, 2000 and 4000 mg/kg bw, in peanut oil and DMSO	98.6	Negative, but several shortcomings	Mosesso (1986)
Micronucleus formation	CD-1 mouse bone marrow	100, 500 and 1000 mg/kg bw, in DMSO	98.4	Negative	Ivett (1984)
Micronucleus formation	ICR mouse bone marrow	500, 1000 and 2000 mg/kg bw, in corn oil	99.0	Negative	Gudi & Krsmanovic (2001)

S9, 9000 × *g* supernatant from rat livers.

[a] Precipitation and cytotoxicity at the highest dose.

[b] Cytotoxicity.

The mean body weights of male and female F_1b and F_2b pups decreased at 2400 ppm and slightly at 400 ppm in F_2b females. The eruption of incisors was promoted in F_1b pups and the hair growth of the abdomen was delayed in all treated F_1b pups and in F_2b pups at 2400 ppm. In addition, eye opening was delayed at 2400 ppm in the F_2b pups. The delays in developmental markers were probably secondary to the lower body-weight gain, in addition, the alterations in developmental markers were not consistent in the two generations.

Table 17. Litter data and body weights of pups during lactation in a multigeneration study in rats given diets containing hexythiazox

Parameter	Dietary concentration (ppm)			
	0	60	400	2400
Mean live litter size at birth:				
F_1a	10.3	9.5	10.1	9.7
F_1b	7.9	8.5	9.9	10.1
F_2a	8.9	7.8	9.7	7.9
F_2b	8.7	9.1	9.2	9.0
Mean litter size at day 21:				
F_1a	7.7	7.1	7.7	7.4
F_1b	6.7	6.9	7.3	7.5
F_2a	7.0	6.4	7.4	6.6
F_2b	6.8	6.3	7.4	7.1
Mean pup weight (g) on day 0:				
F_1b (male/female)	5.3 / 5.0	5.3 / 5.0	5.2 / 4.8	5.2 / 4.9
F_2b (male/female)	5.3 / 4.9	5.1* / 4.8	5.1 / 4.8	5.3 / 4.9
Mean pup weight (g) on day 4:				
F_1b (male/female)	8.7 / 8.4	8.9 / 8.5	8.5 / 8.1	7.8***/7.5***
F_2b (male/female)	8.0 / 7.6	7.8 / 7.6	7.5 / 7.3	7.2 / 6.8
Mean pup weight (g) on day 7:				
F_1b (male/female)	12.9 / 12.4	13.2 / 12.6	12.7 / 12.0	11.3***/11.1***
F_2b (male/female)	11.7 / 11.2	11.5 / 11.1	11.2 / 10.7	9.9***/9.4***
Mean pup weight (g) on day 28:[a]				
F_1b (male/female)	61.0 -/ 55.2	60.5 / 55.0	60.0 / 54.8	54.8**/50.5**
F_2b (male/female)	55.4 / 50.7	51.0 / 48.4	50.1 / 46.0	45.5*/43.6*

From Okugi & Enomoto (1984)

[a] No. of dams = 5

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

The NOAEL for effects on reproduction was 2400 ppm, equal to 136 mg/kg bw per day, the highest dose tested. The NOAEL for parental toxicity was 400 ppm, equal to 24 mg/kg bw per day, on the basis of decreased body weights and food consumption. The NOAEL for offspring toxicity was 400 ppm, equal to 24 mg/kg bw per day, on the basis of reduced body-weight gain during lactation (Okugi & Enomoto, 1984).

(b) Developmental toxicity

Rats

Groups of 24 mated female Sprague-Dawley (Crj:CD) rats received daily oral doses of hexythiazox (purity, 98.3%) at 0, 240, 720 or 2160 mg/kg bw per day by gavage in 5% aqueous gum arabic from day 7 to day 17 of gestation. The day of copulation was classed as day 0, therefore dosing should have started on day 6. Dams were killed on day 21, all viable fetuses were weighed, sexed and examined for external malformations and variations including the palate and eyes. Approximately one third of each litter was preserved in Bouin fixative and examined for visceral malformations and variations. The remaining two thirds of each litter was fixed in ethanol and cartilage and bone were differentially stained with alcian blue and alizarin red S and examined for skeletal malformations and variations.

Significant reductions in mean maternal body-weight gain were observed at 720 and 2160 mg/kg bw per day during dosing; these were probably secondary to reduced food consumption (Table 18). The changes in mean body weight over the first 2 day of dosing appeared to show a treatment-related effect, but there were no biologically significant differences between the groups when the ranges were taken into account. There was a significant decrease in absolute heart and spleen weights in dams at 720 mg/kg bw per day compared with controls. The relative spleen weight was also reduced at 720 mg/kg bw per day. Absolute and relative ovary weights were increased at 2160 mg/kg bw per day and the relative value was also increased at 720 mg/kg bw per day. There was no significant change in fetal viability, fetal weights, placental weights or any other reported parameters. The number of fetuses with subcutaneous haemorrhages, dilation of the renal pelvis and non-ossified metatarsi were slightly increased, especially at 2160 mg/kg bw per day. Only the increase in non-ossified metatarsi was statistically significant. The incidence of non-ossified metatarsi was also statistically significantly increased at 720 mg/kg bw per day. The observed changes did not indicate any teratogenic potential for hexythiazox.

Table 18. Findings of a study of developmental toxicity in rats given hexythiazox by gavage

Finding	Dose (mg/kg bw per day)			
	0	240	720	2160
Maternal body weight (g), range:				
Day 7 of gestation (min/max)	270 / 324	282 / 334	280 / 321	258 / 334
Day 9 of gestation (min/max)	262 / 331	263 / 337	265 / 321	236 / 338
Body-weight gain (g), range:				
Day 7–9 (min/max)	– 11.1 / + 12.3	– 22.1 / + 12.4	– 27.1 / + 5.2	– 33.4 / + 9.2
Maternal body-weight gain (g):				
Days 0–7 of gestation	30	30	28	30
Days 7–9 of gestation	2.9	– 0.4	– 3.3	– 6.0
Days 7–17 of gestation	55	54	44**	42**
Days 17–21 of gestation	52	57	57	57
Days 0–21 of gestation	136	141	129	129
Food consumption (g/rat per day):				
Days 7–9 of gestation	21.8	20.1	21.8	19.3*
Day 21 of gestation	19	20	19	20
Days 7–17 of gestation	255	250	232	231

Finding	Dose (mg/kg bw per day)			
	0	240	720	2160
Fetal body weights (g) :				
Males / females	5.29 / 5.04	5.26 / 4.98	5.20 / 4.93	5.17 / 4.87
Number of fetuses (litters) with:				
Subcutaneous haemorrhage	1	3	0	6
External malformations	1	1	0	1
Skeletal malformations	0	0	0	1
Visceral variations (dilation of renal pelvis)	7/110	9/118	9/112	11/111
Skeletal variations	24/222	29/240	20/226	24/225
No. of ossified metatarsi	9.9	9.9	9.7**	9.5**
Number of resorptions	15	14	19	15
Mean viable litter size	14.4	15.6	15.4	14.6

From Gotoh et al., (1983)

* $p < 0.05$

The NOAEL for maternal toxicity was 240 mg/kg bw per day on the basis of decreased maternal body-weight gain. The NOAEL for teratogenicity was 2160 mg/kg bw per day, the highest dose tested. The NOAEL for effects on fetal development was 240 mg/kg bw per day on the basis of reduced metatarsal ossification (Gotoh et al., 1983).

Rabbits

Groups of 15 mated female New Zealand White rabbits were given daily oral doses of hexythiazoz (purity, 97.7%) at 120, 360 or 1080 mg/kg bw per day by gavage in 5% arabic gum aqueous solution from day 6 to day 28 of gestation (copulation, day – 1). On day 28 of gestation, the rabbits were killed, the uterus was weighed and the fetuses were removed from the dams by caesarean section. Routine examinations of the uterine contents were performed and all viable fetuses were weighed and examined for external malformations and variations, including the palate and eyes, and fixed in alcohol. Each fetus was dissected, internally sexed and examined for visceral malformations and variations. Skeletal malformations and variations were examined after staining the skeleton with alizarin red S.

Two females died, one in the group at 120 mg/kg bw per day (hind-limb paralysis) and the other at 360 mg/kg bw per day (pulmonary congestion). There were no treatment-related clinical signs or adverse body weight changes during the study (Table 19). Weights of maternal organs and mean fetal body weights were not significantly different between the groups. Incidences of resorptions and malformations were similar in all groups. The number of fetuses with skeletal variations, primarily overlapping of the vertebral arch, was increased at 1080 mg/kg bw per day (Table 19). A later re-analysis (Gotoh & Nishibe, 1988) did not confirm the vertebral arch anomalies and the authors proposed that the these anomalies were an artefact; however, a true artefact would be expected to have a random distribution and not affect only seven animals from the same group.

The NOAEL for teratogenicity and maternal toxicity was 1080 mg/kg bw per day. The NOAEL for fetotoxicity is 360 mg/kg bw per day on the basis of an increased incidence of overlapping of the vertebral arch at the highest dose level, against a zero background rate (Gotoh et al., 1984).

Table 19. Findings of a study of developmental toxicity in rabbits given hexythiazox by gavage

Finding	Dose (mg/kg bw per day)			
	0	120	360	1080
Maternal mean body-weight gains (g):				
Days 0–6 of gestation	0.12	0.16	0.12	0.14
Days 6–18 of gestation	0.10	0.16	0.16	0.13
Days 18–28 of gestation	0.17	0.20	0.28*	0.22
Days 0–28 of gestation	0.39	0.52	0.56*	0.48
Number of resorptions	17	16	12	15
Mean viable litter size	7.0	8.3	8.3	6.8
Mean fetal body weights (g):				
Males / females	35.4 / 35.4	34.2 / 33.7	38.0 / 36.5	34.4 / 34.9
Fetuses/litters with malformations:	3/2	0	4/3	3/3
Cleft palate	0	0	1	0
Absence of tail	0	0	0	1
Cardia bifida	2	0	0	0
Ventricular septal defect	1	0	0	0
Fusion of sternebrae	0	0	1	2
Fusion of ribs	0	0	2	0
Fusion of the thoracic vertebral arches	0	0	1	0
Fetuses with skeletal variations (total):	3 / 98 (3.1%)	2 / 100(2.0%)	2 / 100(2.0%)	12 / 88 (13.6%)
Bilobed shape of the sternebrae	1	0	0	1
Splitting of the sternebrae	1	0	1	2
Bilobed shape of the thoracic vertebral body	1	1	1	1
Asymmetry of the sternebrae	0	1	0	2
Overlapping of the vertebral arch	0	0	0	7
Partial absence of the thoracic vertebral arch	0	0	0	1
Fetuses with visceral variations:	1/98	0	0	2/88

From Gotoh et al. (1984)

2.6 Special studies

(a) Studies on metabolites

Studies of acute toxicity and genotoxicity have been performed on a number of rat metabolites of hexythiazox. The results of these studies are summarized in Tables 20 and 21.

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 more acutely toxic orally than hexythiazox. All other metabolites are of relatively low acute oral toxicity and none was genotoxic in Ames tests.

Table 20. Acute toxicity with metabolites of hexythiazox

Test substance[a]	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	Reference
PT-1-2	Rat	SLC:SD	Males	Oral	2321	Approx. 99%	Saika et al. (1985)
			Females		1079		
PT-1-3	Rat	SLC:SD	Males	Oral	494	Approx. 99%	Saika et al. (1985)
			Females		341		
PT-1-4	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-5	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-6	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-8 (*cis*)	Rat	SLC:SD	Males	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-8 (*trans*)	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)
PT-1-9	Rat	SLC:SD	Males & females	Oral	> 5000	Approx. 99%	Saika et al. (1985)

[a] The vehicle for all test substances was water + Tween 80.

[b] No studies of exposure by inhalation were available.

(b) Screening for pharmacological activity

Hexythiazox was extensively investigated, mainly by acute parenteral administration, for pharmacological activity in mice, rats, rabbits and guinea-pigs. In none of the studies did hexythiazox show any indication of potent pharmacological activity.

(i) Effects on the central nervous system

Mice

Mice given hexythiazoz at a dose of 200, 1000 or 5000 mg/kg bw (intraperitoneal, i.p.) showed decreased reactivity/passivity, writhing, piloerection and a decreased heart rate. At 200 mg/kg bw, these signs were very slight and were no longer apparent after 120 min; recovery time was longer at higher doses. Pentobarbital sleeping time was prolonged at 1000 and 5000 mg/kg bw (i.p.) but not at 200 mg/kg bw. There was an anti-convulsive effect (delayed start of convulsions, reduced mortality) against pentetrazole at 45, 60 and 120 mg/kg bw (i.p.), but not at 30 mg/kg bw. There was no effect on strychnine-induced convulsions or mortality at doses of up to 114 mg/kg bw (i.p.), with a weak protective effect at 228 mg/kg bw (mortality, four out of six compared with six out of six in the control group). Locomotor activity in mice was increased at 30 mg/kg bw (intravenous, i.v.); there was no effect at 60 mg/kg bw, and at a dose of 120 mg/kg bw, locomotor activity was reduced.

Rabbits

Hexythiazox did not affect the body temperature of rabbits dosed intravenously at 30 or 60 mg/kg bw. Spontaneous electroencephalogram (EEG) in conscious rabbits was unaffected by dosing with hexythiazox at 50 mg/kg bw.

(ii) Effects on cardiovascular and respiratory system of rabbits

Slight hypotension and hypoventilation lasting for 15 min were observed in rabbits given hexythiazoz intravenously at a dose of 25 mg/kg bw, becoming more pronounced at 50 mg/kg bw. Both doses caused a slight decrease in heart rate.

Table 21. Results of studies of genotoxicity with metabolites of hexythiazox

Test substance (vehicle)	End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro						
PT-1-2	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	0.5–1000 µg/plate + S9 1–5000 µg/plate –S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-3	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	1–5000 µg/plate + S9 5–10 000 µg/plate – S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-4	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-5	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	10–50 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-6	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-8 (*cis*)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	5–10 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-8 (*trans*)	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	0.5–5000 µg/plate + S9 5–10 000 µg/plate – S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)
PT-1-9	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538; *E. coli* (WP2 *uvr*A)	10–50 000 µg/plate ± S9	Approx. 99%	Negative ± S9	Sasaki et al. (1985)

S9, 9000 × *g* supernatant from rat livers.

[a] The vehicle for all test substances was dimethyl sulfoxide (DMSO).

(iii) Effects on skeletal and smooth muscle

Rats

Intravenous administration of hexythiazox at doses up to 100 mg/kg bw did not affect rat skeletal muscle tension induced by indirect stimulation. Hexythiazox did not affect the body temperature of rats given intraperitoneal doses of 1250 or 2500 mg/kg bw.

Guinea-pigs

Hexythiazox at concentrations of 0.1 or 1 mg/ml inhibited the contraction of isolated ileum sections induced by acetylcholine; a concentration of 0.01 mg/ml had no effect. The contraction of isolated ileum induced by histamine was inhibited at by hexythiazoz at 1 mg/ml, but not at 0.1 mg/ml. The contraction of the ileum induced by barium chloride was inhibited by hexythiazoz at concentrations of 0.01 mg/ml and above. Hexythiazox at 1 mg/ml slightly inhibited contraction of the vas deferens induced by acetylcholine, but had no effects on contraction induced by adrenaline. Tracheal contractions induced by acetylcholine and relaxation caused by noradrenaline were unaffected by hexathiazox at concentrations of of 0.01 or 0.1 mg/ml. Contractions induced by histamine were slightly inhibited at both of these concentrations.

(iv) Effects on the gastrointestinal tract

Hexythiazox had no effect on intestinal motility in mice when given intravenously at doses of up to 150 mg/kg bw and did not alter gastric secretion in rats given doses of 3000 mg/kg bw by gavage.

(v) Effects on blood

Intraperitoneal administration of hexythiazox at a dose of 3000 mg/kg bw reduced blood coagulation time in rats by approximately 30%; a dose of 1000 mg/kg bw had no significant effects. Hexythiazox at concentrations of 0.1, 1 or 10% w/v induced mild haemolysis of rabbit erythrocytes in vitro, which did not increase in severity with hexythiazoz concentration. A similar degree of haemolysis was observed with the positive control, 0.4% Tween 20 (Souma et al., 1985).

3. Observations in humans

Personnel in the plant manufacturing hexythiazoz receive a medical examination at least once per year. The examinations include a physical assessment and haematology, urine analysis, and clinical-chemistry investigations. No reports of adverse effects or any unusual patterns in the data were evident in summaries covering 1985 to 1987 (Motogi, 1987) and 1998 to 2007 (Kojima, 2008).

Hexythiazox has not been linked to adverse effects in any epidemiological reports. A single poisoning incident has been reported in the Philippines, but no details were available (Panganiban, 2001).

Comments

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 air) 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 characterize 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 males and females, 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.

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 air (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	—	—

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