FENHEXAMID

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Explanation

Fenhexamid is the International Organization for Standardization (ISO) approved name for 2',3'-dichloro-4'-hydroxy-1-methylcyclohexanecarboxanilide, a hydroxyanilide fungicide that inhibits the growth of fungal spore germ tubes and mycelia. Fenhexamid is intended for the control of grey mould (*Botrytis cinerea*) on grapevines, strawberry, stone fruit, other berry fruit, kiwi fruit, tomatoes and ornamentals. It has no systemic activity in plants.

The Meeting has not previously evaluated fenhexamid.

All pivotal studies with fenhexamid were certified as complying with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, and excretion

Rats

In a series of experiments, groups of five male and five female Wistar rats (Bor:WISW[SPF Cpb]) were given [phenyl-UL-^{14}C]-labelled fenhexamid (purity, > 98%) as single oral doses at 1 or 100 mg/kg bw by gavage as a suspension in 0.5% aqueous tragacanth. One group received unlabelled fenhexamid (purity, 98.5–99.0%) at a dose of 1 mg/kg bw per day for 14 days followed by radiolabelled fenhexamid as a single oral dose at 1 mg/kg bw. One group of six male rats was bile-duct cannulated and given fenhexamid at a dose of 1 mg/kg bw administered intraduodenally. The administration volume was 10 ml/kg bw for oral dosing and 2 ml/kg bw for intraduodenal dosing.

Treated animals were kept in metabolism cages for the collection of excreta and carbon dioxide. Radioactivity in the exhaled air, urine, and faeces was analysed at various time intervals. Urine was collected on dry ice. Carbon dioxide was collected by trapping in a mixture of ethanolamine/ethanol (1:1 v/v). The distribution of radioactivity into various organs and tissues was determined at sacrifice 48 or 72 h after dosing. Whole-body autoradiographs of rat sections were prepared and evaluated qualitatively and quantitatively from one animal at each time-point (1, 4, 8, 24, 48 and 72 h after dosing) from males at the lower dose only. The terminal biotransformation products of fenhexamid in the rat were characterized, identified and quantified in the excreta by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) analysis, gas chromatography-mass spectroscopy (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy.

[^{14}C]Fenhexamid was rapidly absorbed from the gastrointestinal tract of the rats in all dose groups. Selected pharmacokinetic parameters are presented in Table 1. The curves for plasma showed that the absorption of radioactivity started immediately after dosing. The plasma concentration peaked within 5–10 min after single or repeated administration at the lower dose (1 mg/kg bw) and between 40 and 90 min after administration at the higher dose (100 mg/kg bw).

A second maximum plasma peak appeared 2–3 h after dosing at the lower dose, but was not evident at the higher dose. The maximum plasma concentration reached was 0.1 μg/g and 2.4–3 μg/g at the lower and higher doses, respectively, i.e. there was a subproportional enhancement with dose. There was no significant difference in maximum plasma concentration achieved after single or repeated dosing. The plasma maximum was independent of sex. At all doses, the plasma curves showed a rapid and continuous decrease of radioactivity after the maximum as a result of fast distribution and excretion.

In bile-duct cannulated rats, approximately 60% of the lower dose was recovered in the bile within 1 h, 85% within 5 h and > 97% within 48 h (94.7% identified), demonstrating rapid and

nearly complete absorption from the gastrointestinal tract. In bile-duct cannulated rats, < 5% of the administered dose was recovered in the urine, suggesting a high degree of enterohepatic recirculation in intact rats.

The total clearance was very high at all doses, ranging from 3.7 to 9.5 ml/min per animal without correction for the bioavailability. The renal clearance was also very high, ranging from 0.9 to 1.9 ml/min per animal. Apparent volume of distribution under steady-state conditions ranged between 1700% and 4900% of body volume, indicating widespread distribution of the radioactivity into organs and tissues. The mean residence time of total radioactivity in the central compartment was low at all doses (12.2–16.4 h), indicating rapid redistribution of radioactivity back into plasma before rapid elimination.

The distribution of radioactivity into various organs and tissues was determined by whole-body autoradiography of males receiving the lower dose at various time-points. At 1 h after oral dosing, the highest concentration of radioactivity was in the stomach. Concentrations of radioactivity in the liver and kidney were lower than those in the stomach, but higher than in the blood. Concentrations of radioactivity in all other organs and tissues were lower than or similar to those in the blood. A similar trend of distribution was evident 4, 8 and 24 h after exposure, i.e. stomach (gastrointestinal tract), liver and kidneys showing the highest concentrations and other organs and tissues showing detectable radioactivity at concentrations similar to or less than those in the blood.

With the exception of the gastrointestinal tract, concentrations of radioactivity in all organs and tissues including liver and kidneys were low and barely detectable at 48 h and 72 h, indicating no significant trend towards bioaccumulation. Total radioactive residue in the body excluding the gastrointestinal tract was ≤ 0.3% of the administered dose at 72 h at all doses. Apart from the gastrointestinal tract, the liver and kidney were the organs with the highest concentrations of radioactivity at all sacrifice times.

Biotransformation to volatile metabolites and carbon dioxide was negligible (0.02% of the administered dose in 72 h), indicating that the selected radiolabelling position in the molecule was stable. Distribution of radioactivity in selected organs and excreta is shown in Table 2. The rate of excretion was relatively rapid; > 70% of the administered dose was excreted within the first 24 h after dosing. Approximately 96% of the administered dose was excreted into the urine (15–36%) and faeces (62–81%) within 48 h, and the average ratio in faeces : urine was > 3 : 1.

Table 1. Selected pharmacokinetic parameters in rats given fenhexamid

Parameter	Single low dose (1 mg/kg bw)		Single high dose (100 mg/kg bw)		Repeated low dose (15 × 1 mg/kg bw)	
	Male	Female	Male	Female	Male	Female
Cmax (µg/g)	0.067	0.059	2.96	2.37	0.069	0.096
Tmax (h)	0.167	0.167	1.5	0.667	0.167	0.167
T1/2 (h)	10.4	10.2	10.1	11.9	10.1	9.5
AUC (µg/h)	0.903	0.569	57.9	34.98	0.58	0.74
Total clearance (ml/min/rat)	3.7	5.9	5.8	9.5	5.7	4.5
Renal clearance (ml/min/rat)	0.9	1.9	0.9	1.8	0.9	1.7
Vd (ml/kg)	16.6	25.8	25.2	49.1	24.9	18.4
Vd (% of the body volume)	1660	2580	2520	4910	2490	1840
Mean residence time (h)	13.6	12.2	14.3	16.4	13.2	12.6

From Anderson & Bornatsch (1996)

AUC, area under the curve for blood concentration–time; C_{max}, concentration achieved at peak blood concentration; T_{max}, time until peak blood concentrations achieved; $T_{1/2}$, apparent half-life of elimination; Vd, apparent volume of distribution.

Total radioactive residues (except in the gastrointestinal tract) were very low, being slightly lower in females than males after the single dose of 100 mg/kg bw. These sex differences in tissue concentrations were not apparent after repeated dosing at the lower dose; however, renal excretion of radioactivity was significantly higher for females than for males. The percentage renal excretion was higher in both sexes at the single lower dose than at a single higher dose. Percentage faecal excretion was higher after a single higher dose than after a single lower dose for females. There were no significant differences in excretion residues between repeated dosing at the lower dose and at a single lower dose.

1.2 Biotransformation

The quantitative distribution of metabolites in the urine, faeces and bile after oral or intraduodenal administration is shown in Table 3. At all doses, extracts from faeces almost exclusively contained unchanged parent compound. Bile contained one major metabolite, the glucuronide conjugate of the parent compound (M17; 72.69%) in addition to parent (20.75%) and traces of polar metabolites (1.34%). The major component in rat urine was the same major metabolite found in the bile, unchanged parent and with a slightly higher percentage of polar metabolites than in bile. Neither bile nor faeces contained any metabolite not detected in urine. Four main fractions were isolated from urine collected from male rats receiving the higher dose.

The parent compound accounted for 62–75% of the administered dose recovered in the urine and faeces, independent of sex and dose. The glucuronic acid conjugate of the parent ranged from 4–23% of the dose (the high value of 23% was observed in females at the lower dose, but the experiment was considered invalid and was repeated). Metabolite fractions 2 and 3 accounted for up to 3% and 7% of the dose, respectively. Metabolite fraction 2 was a mixture of isomeric compounds hydroxylated at the cyclohexyl ring in position 3 (M16) and 4 (M06). Metabolite fraction 3 was a mixture of sulfate and glucuronic acid conjugates (M19 and M18). Traces of the compound hydroxylated in position 2 (M03) were also detected.

The proposed metabolic pathway of fenhexamid in rats is shown in Figure 1. The main pathway of biotransformation proceeded via conjugation of the aromatic hydroxyl group with glucuronic acid. Before excretion in faeces, hydrolysis in the intestine converted the glucuronic

Table 2. Recovery of radioactivity in tissues and excreta of rats given fenhexamid and sacrificed 48 h after dosing

Fraction	Recovery of radioactive dose (%)						
	Single low dose		Repeated low dose		Single high dose		Bile-duct cannulated
	Male[a]	Female[a]	Male	Female	Male	Female	Male
Urine	24.42	31.89	16.49	36.44	15.22	19.20	1.659
Bile	—	—	—	—	—	—	90.54
Faeces	75.16	66.56	81.33	62.08	80.88	79.65	7.736
Skin		0.009	0.018	0.012	0.026	0.009	0.011
All organs	0.065	0.141	0.111	0.094	0.299	0.102	0.031
Body (not gastrointestinal tract)	0.065	0.150	0.129	0.106	0.325	0.110	0.042
Gastrointestinal tract	0.359	1.400	2.043	1.375	3.577	1.047	0.020
Total body	0.424	1.550	2.172	1.481	3.902	1.157	0.062

From Anderson & Bornatsch (1996). Data extracted from Table V on Page 182 of the study report.

[a] Sacrificed at 72 h after dosing. For comparison of the excreta (urine and faeces), 48 h values are presented.

acid conjugate to parent compound, giving rise to a pronounced enterohepatic circulation. Hydroxylation of the cyclohexyl ring on positions 2, 3 and 4 (the predominant positions for hydroxylation) also occurred. Both glucuronic acid and sulfate conjugates of these hydroxylated compounds were found in the excreta (Anderson & Bornatsch, 1996).

Table 3. Quantitative distribution of metabolites (percentage of the administered radioactivity) after oral or intraduodenal administration of [phenyl-UL-^{14}C]fenhexamid

Metabolite	Low dose		High dose		Repeated doses		Bile-duct cannulated
	Male	Female	Male	Female	Male	Female	Male
Urine							
KBR 2738	4.44	23.06	2.38	2.41	5.08	20.45	0.37
M17	10.03	3.82	3.74	13.05	6.26	8.19	0.96
M06 + M16	1.77	1.87	1.33	0.17	0.88	1.83	0.08
M18 + M19	6.04	1.46	6.72	2.09	3.65	2.17	0.47
Faeces							
KBR 2738	57.53	52.00	66.13	65.31	69.15	49.35	7.43
M17	0.26	ND	ND	ND	0.35	0.17	ND
M06 + M16	1.14	1.34	1.61	0.74	1.69	0.80	ND
M18 + M19	ND	ND	ND	ND	ND	ND	ND
M03	ND	ND	ND	ND	ND	Traces	ND
Total identified of administered dose	81.21	83.56	81.91	83.77	87.06	82.95	9.31
Total of the recovered products	88.52	88.07	89.77	91.14	89.58	91.99	8.85
Bile							
KBR 2738	—	—	—	—	—	—	20.75
M17	—	—	—	—	—	—	72.69
M06 + M16	—	—	—	—	—	—	ND
M18 + M19	—	—	—	—	—	—	1.34
Total identified	—	—	—	—	—	—	94.78

From Anderson & Bornatsch (1996) and Diesing & Brauner (2004).

ND, not detected

KBR 2738, fenhexamid

M03, 2-hydroxy-KBR 2738

M06, 4-hydroxy-KBR 2738 (metabolic fraction 2)

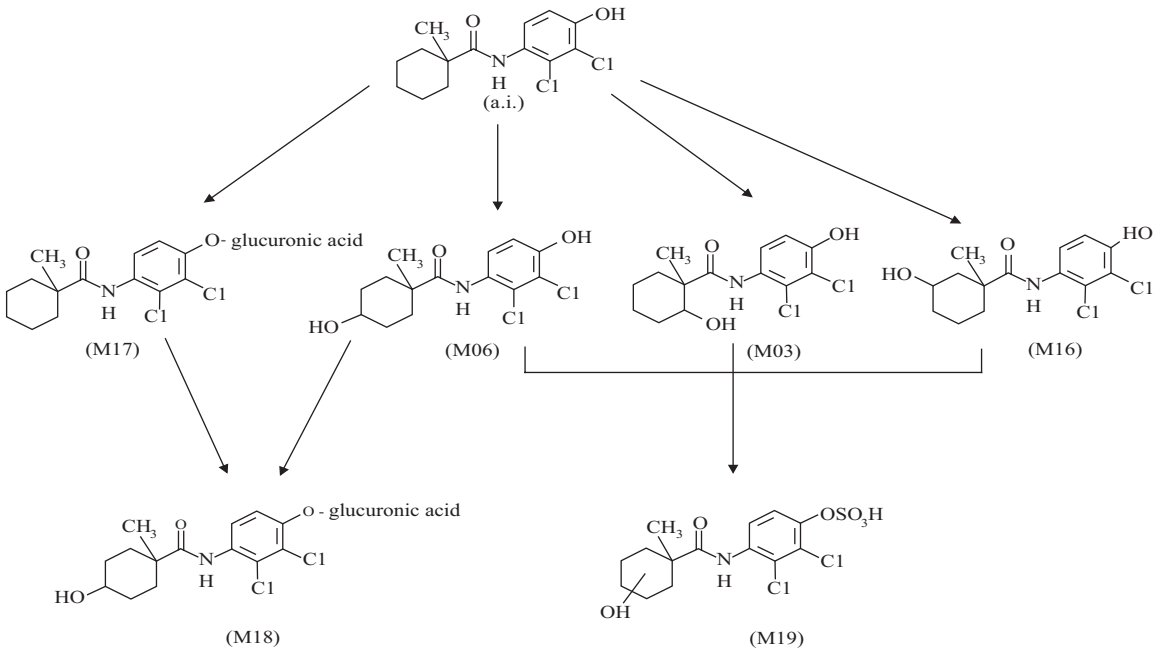
M16, 3-hydroxy-KBR2738 (metabolic fraction 2)

M17, glucuronide of KBR 2738 (metabolic fraction 1)

M18, glucuronide of 4-hydroxy-KBR 2738 (metabolic fraction 3)

M19, sulfate of isomeric hydroxy-KBR 2738 (metabolic fraction 3)

Figure 1. Proposed metabolic pathway for fenhexamid in rats



a.i., active ingredient, fenhexamid
M03, 2-hydroxy-fenhexamid
M06, 4-hydroxy-fenhexamid
M16, 3-hydroxy-fenhexamid
M17, glucuronide of fenhexamid
M18, glucuronide of 4-hydroxy-fenhexamid
M19, sulfate of isomeric hydroxyl-fenhexamid

1.3 Effects on enzymes and other biochemical parameters

No data were available.

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with fenhexamid are summarized in Table 4.

(a) Oral administration

Groups of five male and five female Wistar rats (Bor:WISW[SPF-Cpb]) were given fenhexamid (batch 17002/90; purity, 95.5%) at a single dose of 5000 mg/kg bw by gavage in 2% Cremophor EL (in demineralized water). Treated rats were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP regulations. There were no mortalities, no treatment-related clinical signs of toxicity, and no findings on necropsy, or changes in body weight observed during the 14-day observation period. The median lethal dose (LD_{50}) was > 5000 mg/kg bw (Bomann, 1991a).

Groups of five male and five female mice (Bor:NMRI[SPF-Cpb]) were given fenhexamid (batch 17002/90; purity, 95.5%) at a dose of 2500 or 5000 mg/kg bw by gavage in 2% Cremophor EL (in demineralized water) and observed for 14 days. Necropsy was performed at termination. This study was conducted in accordance with GLP regulations. No deaths occurred during the observation period. No treatment-related changes in the findings on necropsy or in body weights were observed at either dose. No treatment-related clinical signs of toxicity were observed in males or females at 2500 mg/kg bw. Clinical signs such as piloerection, apathy, and spastic gait were observed in males and females at 5000 mg/kg bw; these were resolved after day 1 of treatment. The LD_{50} was > 5000 mg/kg bw (Bomann, 1991b).

Table 4. Acute toxicity with fenhexamid[a]

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/m^3)	Other result	Reference
Rat	Bor:WISW (SPF-Cpb)	M & F	Oral	> 5000	—	—	Bomann (1991a)
Mouse	Bor: NMRI (SPF-Cpb)	M & F	Oral	> 5000	—	—	Bomann (1991a)
Rat	Bor: WISW (SPF-Cpb)	M & F	Dermal	> 5000	—	—	Bomann (1991a)
Rat	Bor: WISW (SPF-Cpb)	M & F	Intraperitoneal	> 1000	—	—	Bomann (1991a)
Rat	Bor: WISW (SPF-Cpb)	M & F	Inhalation	—	> 322 (aerosol) > 5057 (dust)	—	Pauluhn (1991a, 1996b)
Rabbit	NZW		Dermal irritation	—	—	Not irritating	Martins (1991, 1996)
Rabbit	NZW		Eye irritation	—	—	Not irritating	Martins (1991, 1996)
Guinea-pig	Bor: DHPW		Skin sensitization (Buehler)	—	—	Not sensitizing	Dreist (1992, 1996)
Guinea-pig	Hsd:Win:DH, previously Bor:DHPW		Skin sensitization (maximization)	—	—	Challenge with 12% formulation gave positive response Challenge with 3% formulation gave negative response	Stropp (1996, 1997)
Guinea-pig	Hsd:Win:DH Previously Bor:DHPW		Skin sensitization (maximization)	—	—	Not sensitizing (challenge with 6% formulation gave negative response)	Stropp (1996, 1997)
Guinea-pig	Dunkin-Hartley		Skin sensitization (maximization)	—	—	Not sensitizing (challenge with 10% formulation gave negative response)	Leuschner (2000)
Mice	Hsd:Win:NM-RI		Sensitization (local lymph node assay)	—	—	Not sensitizing	Vohr (2000)

[a] Purity, 94.6%–98.8% a.i

M, males; F, females; NZW, New Zealand White

(b) Dermal application

Five male and five female Wistar rats (Bor:WISW[SPF-Cpb]) were dermally exposed to fenhexamid (batch 17002/90, purity, 95.5%) at a dose of 5000 mg/kg bw, moistened in physiological saline for 24 h under an occlusive dressing. After 24 h, the occlusive dressing was removed and the treated site was rinsed with soap and water. Animals were observed for clinical signs and mortality for up to 14 days after dosing. This study was conducted in accordance with GLP. No deaths occurred. There were no treatment-related clinical signs or necropsy findings. Localized redness (slight or weak) was observed around the application site in three out of five females on days 2–3 after exposure. On day 4 after exposure, slight body-weight losses observed among three out of five females were considered by the study authors to be caused by the occlusive dressings. The body-weight losses were recovered by day 8. The dermal LD_{50} was > 5000 mg/kg bw (Bomann, 1991c).

(c) Exposure by inhalation

Young adult Wistar rats (Bor:WISW[SPF-Cpb]) were exposed by head–nose-only inhalation to fenhexamid (batch No. 17002/90, purity, 95.5%) as either dust or aerosol. Fenhexamid was sprayed in a mixture of polyethylene glycol 400 and ethanol as vehicle for the aerosol formation. Five males and five females were exposed for 4 h at 322, 492, and 5057 mg/m^3. Groups of five rats of each sex were also exposed to either air controls or vehicle controls for 4 h. Treated animals and controls were observed for 14 days and necropsied. This study was conducted in accordance with GLP regulations.

No mortality occurred. No treatment-related effects were observed on body weights, clinical signs of toxicity or necropsy findings. The median lethal concentration (LC_{50}) was > 322 and 5057 mg/m^3 for the aerosol (maximum achievable concentration) and dust, respectively (Pauluhn, 1991a, 1996b).

(d) Intraperitoneal administration

Groups of five male and five female Bor:WISW(SPF-CPB) Wistar rats were given fenhexamid (batch No. 17002/90, purity, 95.5%) as a single dose at 0, 50, 200, or 1000 mg/kg bw by intraperitoneal injection in demineralized water. Treated rats were subjected to gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP.

No mortality occurred except one female at the highest dose died on day 1. Clinical signs of toxicity such as apathy, laboured breathing, spastic gait, piloerection, reduced motility, vocalization after contact and temporary convulsions were observed at 200 and 1000 mg/kg bw. These clinical signs had mostly subsided by day 3 of treatment. Transient body-weight loss was observed on day 4 of treatment in the higher two doses; this recovered by day 8 or at termination. Gross pathology findings indicated black discolouration of the abdominal organs. The study author suggested that the black discolouration was most likely to be caused by deposition of the dark brown test powder in abdominal organs. In addition, "Massive ulcer-like foci were observed in the glandular abdomen". No abnormalities were observed at 50 and 200 mg/kg bw. One female had no abnormalities at 1000 mg/kg bw, but the remaining animals showed liver lobe adhesions and white skin covering the spleen, peritoneal adhesion, and dark discolouration. One male in the group at the highest dose had a very small right testicle, soft, dark blue and adjacent vessels with black discolouration. The intraperitoneal LD_{50} for both sexes was > 1000 mg/kg bw (Bomann, 1991d).

(e) Skin irritation

In a study of primary dermal irritation, three young adult female New Zealand White rabbits were dermally exposed to 0.5 g of fenhexamid (batch No. 17002/90; purity, 95.5%), moistened with water for 4 h on a body surface area of 6 cm^2 . Animals were then observed for

7 days. Irritation was scored by the Draize method at 1, 24, 48, and 72 h, and 7 days after exposure. This study was conducted in accordance with GLP.

No mortalities were observed. No evidence of irritation was observed. Based on the study results, it was concluded that fenhexamid is not a skin irritant (Martins, 1991, 1996).

(f) Eye irritation

In a study of primary eye irritation, 0.1 ml of pulverized fenhexamid (equivalent to approximately 70 mg; batch No. 17002/90, purity, 95.5%) was instilled into the conjunctival sac of one eye of each of three young adult female New Zealand White rabbits. After 24 h, the treated eye was rinsed with saline. Animals then were observed for 7 days. Irritation was scored by the Draize method at 1, 24, 48, 72 h and 7 days after exposure. This study was conducted in accordance with GLP.

No irritation of any kind was observed at any time, except for one out of three rabbits that had a slight discharge at 1 h after instillation only. Based on the study results, the Meeting concluded that fenhexamid is not an eye irritant (Martins, 1991, 1996).

(g) Skin sensitization

In a study of dermal sensitization with fenhexamid (batch No. 17002/90; purity, 95.5%), 12 young male guinea-pigs (Bor:DHPW) were tested using the Buehler method. An additional group of 12 males served as vehicle control animals. For the inductions and challenge phase, 500 mg of the test article was made into a paste with 0.45 ml Cremophor EL (2% v/v) and sterile physiological saline solution, and applied to the skin of each animal.

For the induction phase, animals were treated dermally with the test article three times over 7 days. The challenge took place 4 weeks after the first, and 2 weeks after the third epidermal induction. The skin reactions were scored 24 h after the induction exposures and 24, 48, and 72 h after start of the challenge. This study was conducted in accordance with GLP.

No dermal irritation was observed at any time during the induction or the challenge phases of the study in any of the treated or vehicle control animals. Fenhexamid was not a skin sensitizer in guinea-pigs as determined by the Buehler method (Dreist, 1992, 1996).

Three studies of skin sensitization were conducted using the maximization test in guinea-pigs. In the first dermal sensitization study with fenhexamid (batch No. 4258/76; purity, 94.60%), young guinea-pigs (Hsd:Win:DH, previously called Bor:DHPW) were tested using the method of Magnusson & Kligmann. This study was conducted in accordance with GLP. The test substance was formulated in physiological saline solution containing 2% Cremophor EL for intradermal induction and the challenge treatment. The test concentrations chosen for the main study were 5% for intradermal induction, 50% for topical induction, 12% for the first challenge and 3% for the second challenge. The skin reactions were assessed 48 and 72 h after the start of the application to induce the challenge.

After the first challenge with 12%, the test substance formulation produced skin reactions (grade 1) in six out of 10 (60%) animals in the test group compared with none of the animals in the control groups, under the study conditions of the maximization test. The second challenge dose with 3% test concentration did not produce any evidence of skin reactions. Fenhexamid exhibited a skin sensitization potential in this study (Stropp, 1996, 1997).

In another study of dermal sensitization with fenhexamid (batch 17003/94; purity, 96.3%), young female guinea-pigs (HsdPoc:DH) were tested using the maximization method of Magnusson & Kligmann. The test substance was formulated in physiological saline solution containing 2% Cremophor EL for intradermal induction and the challenge treatment. In this study,

the test concentrations chosen were 5% for intradermal induction, 50% for topical induction, and 6% for the challenge. This study was conducted in accordance with GLP.

The challenge treatment with 6% test formulation did not produce any evidence of skin reactions in the treated and control groups. Under the study conditions, fenhexamid did not indicate the potential for skin sensitization The study author suggested that the 12% test formulation in the first study was irritating to the skin and results may have been confounded because of this irritation (Stropp, 1996, 1997).

In a third study of dermal sensitization with fenhexamid (batch H0003; purity, 98.8%), Dunkin-Hartley guinea-pigs were tested using the method of Magnusson & Kligmann. In this study, 10 male guinea-pigs were used for the treatment group, 5 males for the vehicle control group and 20 males for the group treated with the positive control, potassium dichromate. The test substance was formulated in sesame oil for the induction and the challenge phase. Test substance concentrations of 5% for intracutaneous, 80% for topical induction and 10% for the challenge phase were used.

The first intracutaneous induction stage with 0.1 ml fenhexamid produced a discrete or patchy erythema to a moderate and confluent erythema in all animals. Topical induction with 2 ml of an 80% concentration of fenhexamid was not irritating to the skin. The challenge with 2 ml of 10% fenhexamid showed no skin reactions. The vehicle control group did not show any evidence of skin reactions. The positive control group with potassium dichromate exhibited skin sensitizing reactions as expected. On the basis of the results of this study, the Meeting concluded that fenhexamid was not a skin sensitizer in guinea-pigs under the study conditions used (Leuschner, 2000).

(h) Local lymph node assay in mice

The sensitizing potential of fenhexamid (batch No.H0003; purity, 98.8%) was evaluated in NMRI mice (Hsd Win:NMRI) using the local lymph node assay. Fenhexamid was formulated in DAE [mixture of dimethylacetamide (40%), acetone (30%), and ethanol (30%)]. The test concentrations for epicutaneous application were 0, 3, 10 or 30% fenhexamid in DAE. Six females per dose were utilized. The test concentrations were applied epicutaneously on the dorsal part of both ears in a volume of 25 µl per ear. This treatment was repeated on three consecutive days. The mice were anaesthetized with carbon dioxide and the auricular lymph nodes removed and were transferred into sterile physiological saline.

There was no evidence of significant dose-dependent treatment-related increases in the simulation indices for the weight index, cell count index, ear swelling or ear weights. All analyses of T-cell surface markers and macrophage activation markers did not reveal any treatment-related changes. Under the study conditions used in the local lymph node assay, fenhexamid did not show a significant specific immunostimulating response (Vohr, 2000).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a range-finding short-term study of oral toxicity, groups of 10 male and 10 female B6C3F_1/Bom mice, were given diets containing fenhexamid (batch No. 17002/90; purity, 97.8%) at a concentration of 0, 100, 1000 or 10 000 ppm (equal to 0, 26.5, 266.5, or 3283.5 mg/kg bw per day for males and 0, 51.6, 453.9, or 5151.1 mg/kg bw per day for females) mixed with 1% peanut oil (excipient) for 14 weeks. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and on public holidays. Body weight was determined before the

initiation of the study, once per week during the study and at termination on week 14. Water and food consumption for individual mice was determined once a week for 14 weeks. Ophthalmoscopic examinations and urine analyses were not performed. Blood was collected from all animals at termination (weeks 11–13) for measurement of haematological and clinical parameters. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs (brain, heart, liver, spleen, kidneys and testes) were weighed. Histopathological examinations were performed on liver, adrenals, lungs, testes and spleen of all animals in the control group and at 10 000 ppm only. The kidneys of all animals at all doses were examined microscopically. This study was conducted in accordance with GLP.

Stability and homogeneity data indicated that the test article was homogenously distributed in the diet and was stable in the diet. The analysis of test substance concentration indicated that the measured concentration ranged between 94% and 99% of the nominal concentrations.

A total of seven mice (three males and four females) died during the study, but there was no dose–response relationship. The study authors concluded that no treatment-related change in the mortality was observed since no group-specific findings were determined at necropsy. Clinical signs, body weight and body-weight gain were unaffected by treatment. Male mice in the group receiving the highest dose consumed approximately 21% more food than did the controls, and therefore food efficiency was decreased. Cumulative water intake at the highest dose was increased by 59% in males and 55% in females. There were no treatment-related changes in the erythrocyte data, haemoglobin concentration, erythrocyte volume fraction or thrombocyte counts. The mean reticulocyte count was statistically significantly lower than that of the controls for females at 1000 ppm and above; however, the decreases was not considered to be treatment-related as the individual values were within the range for historical controls. Slightly higher leukocyte and lower polymorphonuclear granulocyte (PMN) counts were observed in female mice at 1000 ppm and above. These changes were not considered to be treatment-related because there was no dose–response relationship and the values were within the range for historical controls. Slightly elevated cholesterol, creatinine and bilirubin concentrations were found in both sexes at the highest dose only. Activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were decreased significantly in females receiving the highest dose only, but these values were within the reference range for these parameters. Moreover, the decreased liver enzyme activity observed in females does not correlate with the marginally increased liver weight. Therefore, these findings are not considered to be toxicologically relevant.

Kidney weights (both absolute and relative) were decreased in males at the highest dose (16.6%/14.0%) and females (18.7%/16.8%). The relative kidney weights were decreased in females (4.2%) at 1000 ppm. The reduced relative kidney weight in females at 1000 ppm is not considered to be adverse because there were no corroborating histological findings. Absolute liver weights were higher in males at the highest dose (6%) and females (8%) than in the controls. These changes were not considered to be toxicologically relevant because there were no significant changes in liver enzymes, gross examination or histopathology. Histologically, an increased incidence of basophilic tubules in the renal cortex (males: one out of nine, none out of nine, none out of nine, eight out of nine; females: none out of ten, none out of ten, one out of ten, six out of ten, respectively) was observed in both sexes of the animals receiving the highest dose. The severity in females was graded as “minimal to slight”, while in males the severity was graded as “moderate” with one animal graded as “marked.” In the males, the affected tubules were frequently enlarged and contained proteinaceous casts and cellular detritus. The cytoplasm in centrilobular hepatocytes was denser in the liver of some males (five out of ten) in the group receiving the highest dose. This correlates histologically with a reduction in the cellular glycogen content of the hepatocytes.

The NOAEL was 1000 ppm (266.5 and 453.9 mg/kg bw per day in males and females, respectively) on the basis of the observation in both sexes of increased serum cholesterol, bilirubin and creatinine, decreased kidney weight, increased water consumption, increased food consumption (males), decreased food efficiency (males), renal tubular basophilia (both sexes),

renal protein casts and cellular detritus (males), and reduced glycogen content of hepatocytes (males) at 10 000 ppm, the highest dose tested (Eiben & Ruhl-Fehlert, 1993).

In a 90-day study of oral toxicity, groups of 20 male and 20 female Crl:CD1(ICR)BR mice were given diets containing fenhexamid (batch No. 898812004; purity, 97.5%) at a concentration of 0, 200, 2000, or 20 000 ppm (equal to 0, 32.5, 322.9, or 3416.8 mg/kg bw per day for males and 0, 54.8, 573.7 or 6145.4 mg/kg bw per day for females) mixed with 1% peanut oil (excipient). After 4 weeks of treatment,10 mice of each sex per dose were necropsied and the remaining animals were continued for up to 13 weeks. This study was conducted at the request of the Japanese National Agency. The primary objective of this study was to examine haematology, renal function and erythropoietin to identify a possible renal anaemia. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and public holidays. Body weights were determined before the initiation of the study, once a week during the study and at termination at the end of week 4 and week 13. Water and food consumption for individual mice were determined once a week for 4 weeks and 13 weeks. Ophthalmoscopic examinations and urine analyses were not performed. Blood was collected from all animals during week 4 and 13 for measurement of haematological and clinical parameters. All animals that died and those sacrificed on schedule (4 and 13 weeks) were subjected to gross pathological examination. Selected organs (only kidneys and spleen) were weighed and selected tissues (femur including bone marrow and knee joint, kidneys, spleen, sternum, tattooed ears) were collected for histological examination. This study was conducted in accordance with GLP.

Stability and homogeneity data indicated that the test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks before the start of the study. The analysis of test substance concentration indicated that the measured concentrations were in an acceptable range of the nominal concentrations except at the highest dose, probably due to partial nonhomogeneities.

No treatment-related deaths occurred. Seven animals died during or after blood sampling. No treatment-related clinical signs of toxicity were observed in the study. Food and water intake was increased for both sexes at 20 000 ppm. Body weight was not affected during the study. No treatment-related changes in erythrocyte counts, erythrocyte morphology, haemoglobin, or erythrocyte volume fraction were observed during the study. Mean corpuscular haemoglobin (MCH) was slightly decreased in females at 20 000 ppm at week 13, which was considered as incidental and within the range of historical control values. No treatment-related changes in differential blood counts were seen except for a slight increase in eosinophils in females at the highest dose at week 4 and 13. The concentration of creatinine and urea were statistically significantly increased in both sexes at 20 000 ppm. In addition, the concentration of calcium was increased in males at the highest dose at week 4. The activity of erythropoietin was statistically significantly lower in females at the highest dose than in the controls at the end of the study.

No gross pathological alterations were observed at 2000 ppm and below at week 4. Changes in kidneys were observed (deformation of right kidney in one animal and pale discolouration of both kidneys in one animal) in two males at 20 000 ppm. The surface of both kidneys was rough in two males at the highest dose at terminal necropsy. The absolute and relative kidney weights were decreased in both sexes of the group receiving the highest dose compared with controls at termination. The renal alterations manifested as dilated tubules, tubular casts and increase in basophilic tubules were observed in males and females at the highest dose at week 4 and 13. Males were more severely affected than females at the end of the treatment. An increase in splenic siderin storage was observed in females only at 2000 ppm and above at week 4 and 13. The incidence of siderosis was more frequent in treated females at week 4 but showed no dose–response relationship at week 13. There was no evidence of erythrocyte damage, the incidence of an increase in siderin in the spleen is considered to be most probably the result of the individual animal's reaction to blood sampling.

The NOAEL was 2000 ppm (equal to 322.9 and 573. mg/kg bw per day in males and females, respectively) on the basis of reduced kidney weights, macroscopic and microscopic renal alterations and increased creatinine, urea and food and water consumption at 20 000 ppm, the highest dose tested (Krotlinger, 1999a).

Rats

In a 28-day range-finding study, groups of 10 male and 10 female Bor:WISW(SPF) Wistar rats were given fenhexamid (purity, 97.8%; batch No. 17002/90) at a dose of 0, 100, 300 or 1000 mg/kg bw per day in 2% Cremophor EL in demineralized water by gavage. Control animals received vehicle only, by gavage. Animals received the test article once daily over 28 days. The gavage volume was 10 ml/kg bw. The dosing formulation was prepared daily. The stability and homogeneity were confirmed by analytical methods. Animals were observed daily for mortality, and clinical signs of toxicity. Individual body weight, food and water consumption were recorded weekly. Ophthalmoscopic examinations of the control and 1000 mg/kg bw per day groups were conducted at the initiation of the study and the end of the study. Ophthalmoscopic examinations were also performed on five animals of each sex in the 100 and 300 mg/kg bw dose groups. Liver tissue was taken from five rats of each sex per dose, and the following parameters were examined: *N*-demethylase, *O*-demethylase, cytochrome P450, and triglycerides. Haematological and clinical chemistry tests and necropsy were performed at the end of the study period. Urine analysis was performed on five rats of each sex per dose at termination. Gross necropsies were performed at the end of the study and selected organs were removed, weighed and histopathological examinations were performed. This study was conducted in accordance with GLP.

The homogeneity and stability data indicated that the compound was stable and homogenous. Analysis of the dosing solution indicated that the measured concentrations were in an acceptable range of the nominal concentrations.

There were no mortalities and no treatment-related effects on clinical signs of toxicity. No treatment-related effects on body weight, body-weight gain, or food consumption, no treatment-related ophthalmologic changes, and no treatment-related changes in urine analyses or haematological parameters were observed. There were no treatment-related changes in coagulation time or methaemoglobin content. No Heinz bodies were observed in the erythrocytes of treated animals.

There were slight increases in creatinine concentrations in the males at the highest dose. However, creatinine concentrations were decreased in females at the highest dose. There was slightly decreased serum albumin in males at the highest dose. There were slight decreases in urea, sodium and potassium concentrations in males at the highest dose. The thyroxine binding capacity was slightly increased in a non dose-related manner in males at the highest dose. Thyroxine was slightly increased in a non dose-related manner. The changes in clinical chemistry parameters were not considered as toxicologically relevant, since a dose–response relationship in clinical chemistry parameters over the wide dose range were not observed and the changes were sporadic and incidental in nature. Furthermore, these changes in clinical chemistry parameters were not corroborated by organ weight data, gross or histopathological observations. The liver tissue enzyme analysis revealed no biologically significant treatment-related changes, although *O*-demethylase activities were significantly decreased in females at both 100 and 1000 mg/kg bw per day. Since these changes were not dose-related and did not occur in the males, this finding is considered incidental. No dose-related changes in absolute or relative organ weights were observed. Some small changes in organ weights were seen in groups receiving the lowest and intermediate doses, but were not considered treatment-related. No treatment-related gross or microscopic pathological findings were observed.

On the basis of the study results, the NOAEL was 1000 mg/kg bw per day, the highest dose tested (Bomann, 1996; Bomann & Popp, 1994).

In a 90-day study of oral toxicity, groups of 10 male and 10 female Wistar rats [Bor:WISW(SPF Cpb)] were given diets containing fenhexamid (batch No. 4258/76; purity, 95.4%) at a concentration of 0, 2500, 5000, 10 000 or 20 000 ppm (equal to 0, 202, 415, 904, and 1904 mg/kg bw per day for males and 0, 270, 549, 1132 and 2824 mg/kg bw per day for females) mixed with 1% peanut oil (excipient) to minimize dust formation. An additional two groups dosed at 0 and 20 000 ppm for 13 weeks were observed for an additional 4 weeks on regular diet and served as a recovery group. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice daily for signs of toxicity and mortality and once per day on weekends and on public holidays. Body weights were determined before the initiation of the study, once a week during the study and at termination on week 13 or 17. Water and food consumption for individual rats was determined once a week for 13 or 17 weeks. Ophthalmoscopic examinations were performed on all control animals and animals at the highest dose at the start of the experiment and during week 12. Blood was collected from all animals in weeks 4 and 12/13, and week 17 for the recovery group for measurement of haematological and clinical parameters. Urine analysis was performed on all animals at week 12 or 17. At termination, liver tissue was taken from five rats of each sex per dose and the activity of the cytochrome P450 enzymes—the monooxygenases (ECOD, 7-ethoxycoumarin de-ethylation; EROD, 7-ethoxyresorufin de-ethylation; ALD, aldrin epoxidation) and phase II enzymes (EH, epoxide hydrolase; GS-T, glutathione transferase; GLU-T, UDP-glucuronyl transferase) were assayed. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed and tissues were collected for histological examination. Only the liver was examined microscopically in the recovery group. This study was conducted in accordance with GLP regulations.

The homogeneity and stability data indicated that the test substance was stable and homogenous in the diet. The analysis of test substance concentration indicated that the measured concentration ranged between 97% and 108% of the nominal concentrations. There were no mortalities and clinical signs of toxicity during the study. Opthalmoscopic examination revealed no treatment-related changes.

Females of the main groups showed no treatment-related changes in body weight. The male rats of the main study groups showed no treatment-related changes in body weight at doses up to 5000 ppm. There was a maximal 9% decrease in body weight of 10 000 ppm males in week 9 and a maximal 12–13% decrease in body weight of 20 000 ppm males in weeks 1 and 2. By the end of the study, body weight in both treatment groups had risen to about 7% below the control males. The recovery male group (20 000 ppm) showed a slight decrease in body weight (not statistically significant except at week 1) from week 1 to week 6. However, by week 10, the body weight of males in the recovery group had risen slightly above that of the controls. No treatment-related effects on body weight were seen in females. There was an increase in food consumption in both sexes at 10 000 ppm and above. The cumulative food intake was increased by 15% in males and 36% in females at 10 000 ppm compared with controls. The food consumption per kg bw was elevated by 27% and 38% in males and females, respectively. The total food consumption of the recovery group during the last week returned to control levels in males and was slightly elevated in females (6.4%). Males at 10 000 ppm and males and females at 20 000 ppm were less efficient in converting their daily food intake into body weight when compared with controls. It is possible that administration of high doses may have compromised the nutritional status of these animals by inhibiting absorption of nutrients from the intestinal tract through some unknown mechanism. There were no treatment-related effects on water consumption.

No treatment-related haematological changes were seen in the study. Isolated statistically significant deviations from controls with respect to erythrocyte parameters, leukocytes and platelets, and thromboplastin time are not considered to be toxicologically relevant since the differences were small, transient and not dose-dependent.

ALAT values were statistically significantly elevated in 10 000 and 20 000 ppm males at week 4, but were comparable to those for controls by 12/13 weeks, suggesting that adaptation had occurred. There were some statistically significant changes in ASAT activities in males at

4 weeks (increased) and 12/13 weeks (decreased). These changes fell within the range of normal biological variation as reported in the historical control data provided by the company report. Although concentrations of bilirubin and creatinine were decreased in males at 4 and 12/13 weeks, the changes also fell within the normal physiological range as provided by the company report. Therefore, these changes were of no toxicological concern. Any other changes in clinical chemistry parameters, in either males or females, were sporadic with no dose–response relationship, were transient, and fell within the normal range of biological variation. There were no treatment-related changes in cytochrome P450-dependent monooxygenases or in phase II enzymes. The results of urine analysis conducted after 12 weeks (main group) and 17 weeks (recovery group) did not reveal any significant differences between treated and control groups. The females at the two higher doses voided a higher volume of more dilute urine than the controls at the terminal sacrifice; however, in the absence of histological corroboration or of an effect in males, this change was not considered to be toxicologically relevant.

Liver weights (absolute and relative) in the main study groups were lower (without dose-dependence) in all treated males owing to two very high individual values in the control group, which made the treated animal values appear statistically lower. Absolute and relative liver weights of all treated males were still 10–12% lower than control values even after excluding the two high values in the control group. Both absolute and relative liver weights were 8–9% (statistically non-significant) lower in females at 20 000 ppm than in the controls. Because decreased liver weights were also associated with alterations in liver morphology for females at the highest dose (but not in males), the reduced liver weight was considered likely to be treatment-related. However, the liver alterations were reversible since liver parameters for all recovery animals were comparable. Isolated changes in the heart, adrenal, kidney and testes weights were noted; however, these changes were not considered treatment-related because they were not corroborated by clinical chemistry or histology. These changes were low in magnitude, and not seen in the recovery groups.

No treatment-related changes in gross pathological findings were seen in this study. There was an increased incidence of focal Kupffer cell proliferation (seven out of ten), slight degenerative changes in isolated hepatocytes showing a dark cytoplasm and nucleus (two out of ten) and condensed cytoplasm (three out of ten) in females at the highest dose. This is interpreted as a reduction of glycogen. Condensed cytoplasm was also seen in one female at 5000 ppm and one male at 2500 ppm, which were considered to be incidental findings. Histopathological examination of the livers of the recovery group animals did not reveal any evidence of changes attributable to treatment. Therefore, the liver changes seen in the females at the highest dose (main group) were proven to be reversible.

The NOAEL was 5000 ppm (415 mg/kg bw per day) in males and 10 000 ppm (1132 mg/kg bw per day) in females on the basis of decreased body weight and body-weight gain, increased food consumption, and decreased food efficiency in both sexes at the next higher dose (Eiben & Ruhl-Fehlert, 1994 and 1997).

In a 90-day study of oral toxicity, groups of 20 male and 20 female Wistar rats (Hsd Cpb:WU) were given diets containing fenhexamid (batch No. 898812004; purity, 97.5%) at a concentration of 0, 500, 5000, or 50 000 ppm (equal to 0, 38, 403.9, and 5585.1 mg/kg bw per day for males and 0, 47.4, 552.8, and 8100.8 mg/kg bw per day for females) mixed with 1% peanut oil (excipient). After 4 weeks of treatment, 10 rats of each sex per group were necropsied. Treatment of the remaining animals was continued for 13 weeks. This study was conducted at the request of the Japanese National Agency. The primary objective of this study was to examine haematology, renal function and erythropoietin in order to explain a possible renal anaemia. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and on public holidays. Body weights were determined before the initiation of the study, once a week during the study and at termination in week 13. Water and food consumption for individual rats were determined once per week for 13 weeks. Ophthalmoscopic examinations were not performed.

Blood was collected from all animals in week 4 and in week 13 for measurement of haematological and clinical parameters. Urine analysis was performed for all animals at week 4 and week 13. All animals that died and those sacrificed on schedule (4 and 13 weeks) were subjected to gross pathological examination and selected organs (kidneys and spleen) were weighed and tissues were collected for histological examination. This study was conducted in accordance with GLP.

Stability and homogeneity data indicated that the test article was homogenously distributed in the diet and was stable in the diet for at least 2 weeks. The analysis of test substance concentration indicated that the measured concentration ranged from 89% to 101% of the nominal concentrations. Increased excretion of urine (wet bedding) was observed in male and female rats at 5000 ppm and above. In addition, piloerection, and decreased motility and reactivity were noted in males and females at 50 000 ppm. There were no mortalities in the study. Food and water consumption were increased at or above 5000 ppm in both male and female rats. There was a slight but statistically significant decrease in body weight of males (94% of the controls) and females (91% of the controls) at 50 000 ppm.

No treatment-related effects on haematological parameters were observed in females at doses of up to 50 000 ppm. Reticulocyte counts in male rats were slightly decreased (week 4 and week 13) at 5000 ppm and above, and the erythrocyte counts were decreased and mean corpuscular volume and mean corpuscular haemoglobin values were slightly increased in males at 50 000 ppm. Leukocyte and lymphocyte counts were statistically increased in males at 50 000 ppm. No treatment-related effects were seen in clinical chemistry parameters in male or female rats at doses up to 5000 ppm. There were increases in creatinine, urea, and calcium concentrations in males at 50 000 ppm after 4 weeks and decreases in phosphate concentrations in males and increases in phosphate concentrations in females were also observed at 50 000 ppm at week 4. The concentration of calcium was increased in females at the end of the study. Urine analysis of animals in the group at 50 000 ppm showed a decrease in protein in males at week 4 and week 13. The electrophoresis of urine showed a decrease in pre-albumin in males at week 4 at 50 000 ppm and at week 13 at 5000 ppm and above. After 4 weeks of treatment at necropsy, the males at 50 000 ppm showed enlarged kidneys (four out of ten) and discoloured kidneys in two out of ten males. The kidneys were discoloured in one out of ten females at 5000 and 50 000 ppm at week 13. There were no effects on organ weights (absolute or relative) in females at doses of up to 50 000 ppm after 13 weeks of treatment. The absolute and relative kidney weights were increased in males at 50 000 ppm at week 4. After 4 and 13 weeks of treatment at 50 000 ppm, a number of animals of each sex showed a nephropathy characterized by basophilic tubules, tubular dilation and tubular casts. The dilated tubules showed a flattened epithelium with either a basophilic or an eosinophilic cytoplasm. Compared with findings at 4 weeks, the nephropathy showed no significant progression in terms of severity after 13 weeks of treatment. Spleen and bone marrow showed no treatment-related changes.

The NOAEL was 5000 ppm (403.9 and 552.8 mg/kg bw per day in males and females, respectively) on the basis of clinical findings, changes in erythrocytes and leukocytes (males only), kidney changes and nephropathy at 50 000 ppm (Krotlinger, 1999b).

Dogs

Groups of two male and two female beagle dogs were given diets containing fenhexamid (batch no. 17002/90; purity, 95.5%) at a concentration of 0, 50, 400, 3000 or 20 000 ppm (equivalent to 0, 1.25, 10.0, 75.0 or 500 mg/kg bw per day) for 4 weeks. The dogs were given physical, haematological, clinical chemistry and urine analysis tests on two occasions during the 2 weeks before initiation of dosing and a complete physical examination including direct ophthalmoscopy was performed on each dog the week before initiation of dosing. Body weights were determined once before dosing and weekly during the treatment. Food consumption was recorded daily and reported over weekly intervals. Animals were observed daily for mortality and clinical signs of toxicity. Urine analysis, haematology and clinical chemistry parameters were evaluated at week 2 of the study and at termination. Opthalmoscopy and physical examinations

were performed at termination. Gross necropsies were performed at the end of the study and selected organs were removed, weighed and histopathological examinations were performed. This study was conducted in accordance with GLP.

There were no mortalities or treatment-related clinical signs of toxicity. No treatment-related effects on body weight, body-weight gain, food consumption or ophthalmological effects were observed. Statistically significant variations occurred in a few clinical, haematological and urine analysis parameters, but the magnitude of the variations was small, there was a lack of a clear dose–response relationship, and values were within the range for historical controls. The only variation that appeared to be treatment-related was decreased methaemoglobin, without a clear dose–response relationship. No treatment-related effects on organ weights (absolute and relative to body weight) were observed. No treatment-related gross or microscopic pathological findings were observed.

The NOAEL was 20 000 ppm (equivalent to 500 mg/kg bw), the highest dose tested (Porter, Jasty & Hartnagel, 1991).

Groups of four male and four female beagle dogs were given diets containing fenhexamid (batch No. 4258/76; purity, 95.4%; moistened 1:1 with warm tap water) at a concentration of 0, 1000, 7000 or 50 000 ppm (equal to 0, 33.9, 239.1 or 1747.7 mg/kg bw per day for males and 0, 37.0, 261.0 or 1866.2 mg/kg bw per day for females) for 90 days. The dry food was mixed with the test substance once per week and the dry preparation was mixed with water daily before administration. Homogeneity, stability and dietary concentrations were determined analytically. Animals were observed daily during feeding, during maintenance and cleaning, and during exercise for clinical signs of toxicity. Reflexes (papillary, corneal, patellar, extensor, postural, and flexor), body temperature, and pulse were measured 2 weeks before dosing and again at weeks 6 and 13 of the study. Food and water consumption were determined individually for each animal daily. Body weights were measured 2 weeks before initiation of the study and once per week during the study. Eye examinations, electrocardiograms (ECG), blood pressure measurements and heart rates were conducted 2 weeks before initiation of the study and at weeks 6/7 and 13 during the study. Haematological, clinical chemistry and urine analysis parameters were measured 2 weeks before the study and at weeks 2, 6 and 13 of the study. Liver tissue was taken and activities of enzymes (*N*-demethylase., *O*-demethylase and cytochrome P450) and triglycerides were measured at termination. Triglyceride concentrations were also determined in the adrenal gland and muscle. Gross necropsies were performed and selected organs were removed, weighed and histopathological examinations were performed at study termination. This study was conducted in accordance with GLP.

Homogeneity and stability data indicated that that the compound was stable and homogeneously distributed in the diet. The measured concentrations of the test substance in the diet ranged from 98% to 104% of the nominal concentrations, indicating that the variance between nominal and actual dosage to the animals was acceptable.

No mortality or treatment-related clinical signs of toxicity were observed. No treatment-related changes in the reflexes, body temperature, blood pressure, pulse rate, ECG, and heart rates were observed. No treatment-related changes in body weight, body-weight gain, food consumption and water intake were observed between the controls and treated groups. No treatment-related changes were observed in the eyes.

There were slight decreases in erythrocyte counts, haemoglobin and erythrocyte volume fraction in males and females at the highest dose. A dose-related increase in Heinz bodies was observed in animals at the intermediate dose (three out of four males and four out of four females) and at the highest dose (four out of four males and females) (Table 5). Activities of alkaline phosphatase (ALP) were increased by 13 weeks in males at the highest dose (15%) and females (200%) when compared with their pretest values. In males, ALP activities at 13 weeks were 103, 126, 203 and 184 U/l for control to high dose, respectively. In females, ALP concentrations at 13 weeks were 135, 128, 233 and 586 U/l for control to high dose, respectively. ASAT was

increased in females at the highest dose at week 2 and ALAT was increased at weeks 2 and 3 owing to high activities of these two enzymes obtained from one animal. The activities of ASAT and ALAT were comparable to those for controls at week 13, therefore, these variations were considered to be incidental findings. Glutamate dehydrogenase (GLDH) was increased only in week 2 in females at the highest dose owing to very high activities of this enzyme in one female. GLDH activities decreased after week 2 in a dose-related manner in males (2.0, 2.2, 0.9, 0.5 U/l; control to high dose) and females (1.5, 2.7, 1.4, 0.9 U/l). The toxicological significance of decreased GLDH activity in this study is not clear; the decrease in GLDH is considered to be treatment-related but not adverse in the absence of any associated gross liver or histopathological findings. No treatment-related changes in urine analysis parameters were seen.

Mean absolute and relative liver weights were increased in males at the highest dose (20%) and in females at the intermediate and highest doses (19% and 37%). There was no evidence from liver histopathology to corroborate these findings.

Dose-related increases in absolute and relative spleen weights were seen in females at the highest dose. This increase in spleen weight is considered to be an adaptive response to haematotoxicity induced by the test substance and the removal of abnormal erythrocytes (with Heinz bodies) from the circulation. There were slight increases in mean absolute and relative uterus weights in females at the highest dose. This increase in uterus weight is not considered toxicologically relevant since it was caused by a very high uterus weight in one female only.

There was an increase in the liver enzyme *N*-demethylase in females at the highest dose (21%) compared with controls. This finding was considered to be toxicologically irrelevant in the absence of any liver histopathology. No treatment-related changes in gross or histopathology were observed. Cytoplasmic inclusions were observed in the liver of one control female animal and considerably more frequently in treated animals in a non-dose-related manner. The pathology report indicated that these types of cytoplasmic inclusions are common in beagle dogs and are the result of the method of sacrifice of the animals, and are particularly due to local increases of blood pressure. Hyperaemia/congestion of the liver also was often observed in these same animals. Focal cystic degeneration of the aortic media was found in a female receiving the highest dose. This was regarded as a spontaneous occurrence since it was observed in control animals in a previous study in dogs conducted by the company. Slight non-suppurative encephalitis was observed in the brain of a female at the intermediate dose. Karyomegaly observed in the proximal tubules of the kidneys in two animals at the intermediate dose (litter mates, one male and one female) was proposed to be genetic in origin.

The NOAEL was 1000 ppm (33.9 and 37 mg/kg bw per day in males and females, respectively) on the basis of significant increases in the number of Heinz bodies in males and females at 7000 ppm (Ruf, 1997a, 1996a, 1995).

Table 5. Mean number of Heinz bodies per 1000 erythrocytes at selected times[a]

Week	Dietary concentration (ppm)			
	0	1000	7000	50 000
Males				
6	0	0	0	11 ± 22.5
13	0	0	18 ± 14.8	50 ± 35.3
Females				
6	0	0	0	114 ± 21.7
13	0	1 ± 1.3	18 ± 11.8	94 ± 75.5

From Ruf (1997a, 1996a, 1995)

[a] Mean value of four animals ± standard deviation

In a 52-week study of toxicity, groups of four male and four female beagle dogs were given diets containing fenhexamid (batch No. 4258/76; purity, ≥ 94.6%; moistened 1:1 with warm tap water) at a concentration of 0, 500, 3500 or 25 000 ppm (equal to 0, 17.4, 124.3 or 917.8 mg/kg bw per day for males and 0, 19.2, 132.7, or 947.1 mg/kg bw per day for females). The dry food was mixed with the test substance once per week and the dry preparation was mixed with water daily before administration. Homogeneity, stability and dietary concentrations were determined analytically. Animals were observed daily during feeding, during maintenance and cleaning, and during exercise for mortality and clinical signs of toxicity. Reflexes (papillary, corneal, patellar, extensor, postural, and flexor), body temperatures, and pulse were measured 2 weeks before dosing and then at weeks 7, 13, 26, 39 and 52 of the study. Food and water consumption were determined daily. Body weight was measured once a week during the study. Eye examination, ECG, blood pressure and heart rates were measured 2 weeks before initiation of the study and at weeks 7, 13, 26, 39 and 52 during the study. Haematological, clinical chemistry and urine analysis parameters were measured 2–3 weeks before the study and at weeks 7, 13, 20, 26, 39 and 52 of the study. Liver tissue was taken at termination and activities of enzymes: cytochrome P450-dependent monooxygenases (ECOD, EROD, ALD), epoxide hydrolase (EH), and the conjugation enzymes (GS-T, GLU-T) were measured. Gross necropsies were performed at termination and selected organs were removed, weighed and histopathological examinations were performed. This study was conducted in accordance with GLP.

Homogeneity and stability data indicated that that the compound was stable and homogeneously distributed in the diet. The measured concentrations of the test substance in the diet ranged from 83% to 96% of the nominal concentrations, indicating that the variance between nominal and actual dosage to the animals was acceptable.

No pathological findings were noted in reflexes at any time during the study. There were no treatment-related effects on body temperature, pulse rate, blood pressure, heart rate or ECG. A slight reduction in nutritional state (i.e. animals appearing “thin”) was observed in the group receiving the highest dose compared with controls. There were no deaths in the study. An irregular heartbeat and watery faeces containing blood were observed in one female at the highest dose at week 26, deemed not to be toxicologically relevant since these symptoms were not noted at any other time-point. No other treatment-related clinical signs were noted in the other dose groups. Over the 52-week duration of the study there were treatment-related significant decreases seen in body-weight gain in both males and females at 25 000 ppm (–29% and –34%, respectively) versus the control animals. The observed decreases in body-weight gain of animals in the groups at 500 or 3500 ppm were not dose-related and, therefore, were not considered to be toxicologically relevant in this study as the gains were considerably lower at 500 ppm than those observed at the next highest dose of 3500 ppm. Food and water consumption were not affected by the treatment. No treatment-related ocular changes were detected.

Treatment-related changes in haematological parameters included: statistically significant decreases in erythrocytes, haemoglobin and erythrocyte volume fraction for both sexes at 25 000 ppm and in females at 3500 ppm; a marginal decrease (< 10%) in erythrocytes, haemoglobin and erythrocyte volume fraction in males at 3500 ppm; and a significant increase in number of Heinz bodies in both sexes at 3500 and 25 000 ppm (Table 6). These changes were apparent from week 7–13 and remained approximately stable in magnitude for the remainder of the study. At termination, the numbers of Heinz bodies per 1000 erythrocytes were 0, 1, 4, and 36 (males) and 0, 4, 30, and 46 (females) at 0, 500, 3500 and 25 000 ppm, respectively.

No treatment-related changes in clinical chemistry parameters were observed except for an increase in ALP activities in males and females at the highest dose (450 U/l compared with 154 U/l for controls at week 52). The increases in ALP values were largely due to high values in one male and one female receiving the highest dose. The increase in ALP values was considered to be toxicologically significant because seven out of eight animals (excluding outliers) in the group at the highest dose had ALP values at least that were 50% above the control mean, and no other animal in the other group had ALP values comparable to the two apparent outliers in the group receiving the highest dose. Thyroid hormone values were comparable for controls and

treated groups or exhibited normal variability. Urine analysis at various intervals did not reveal any treatment-related changes in any of the parameters examined.

No treatment-related gross pathological findings were observed. No treatment-related changes in organ weights (absolute and relative) were observed except for an increase in absolute and relative mean adrenal weight in the females at 3500 ppm and 25 000 ppm. Fenhexamid had only marginal effects on liver enzyme activities.

A statistically significant increase in EROD, EH, GS-T, and GLU-T activity in females of the 3500 ppm dose groups was observed. However, only a statistically significant increase in GS-T activities was observed in females at the highest dose. This increase in liver enzyme activity is not judged to be toxicologically significant in the absence of any gross or histopathology findings in the liver.

There was an increase in the number of intracytoplasmic vacuoles in the inner cortex of the adrenal glands in three out of four females at 3500 and 25 000 ppm (Table 7). The intracytoplasmic vacuoles were also seen in one of four females at 500 ppm and in two out of four control females.

The number of intracytoplasmic vacuoles in the cells of the adrenal cortex was greater in females at 25 000 ppm than in females at 3500 ppm. These histological findings were corroborated by the increased adrenal weights in females. Therefore, the increase incidence and/or severity of intracytoplasmic vacuoles in the inner cortex of the adrenal glands of females at 3500 and 25 000 ppm groups are considered to be treatment-related.

Focal hypertrophy of the zona fasciculata of the adrenal cortex was observed in two out of four males at 25 000 ppm; one of these males also had increased numbers ("few") of intracytoplasmic vacuoles. Another male had focal inflammation of the adrenal cortex. These findings were not observed in the control animals or in the other treatment groups. However, histopathological findings were not supported by any gross pathology or organ weight changes in the adrenal gland of the males at 25 000 ppm. There were no other treatment-related effects in any other of the organs examined microscopically.

Fenhexamid did not produce any evidence of neoplasias in dogs at doses up to and including 25 000 ppm given in the diet for 1 year.

The NOAEL was 500 ppm (17 mg/kg bw per day) on the basis of increased adrenal weight and the presence of intracytoplasmic vacuoles in the adrenal cortex of three out of four female dogs, and alterations in erythrocytes, haemoglobin and erythrocyte volume fraction and an increase in Heinz bodies at 3500 ppm and above (Ruf, 1997b, 1996b).

Table 7. Incidence of microscopic lesions in the adrenal glands[a]

	Dietary concentration (ppm)							
	Males				Females			
	0	500	3500	25 000	0	500	3500	25 000
Number of animals- Number examined[a]	4	4	4	4	4	4	4	4
Adrenal cortex:								
Focal inflammation	0	0	0	1	0	0	0	0
Focal hypertrophy	0	0	0	2	0	1	0	0
Vacuoles inner cortex	0	0	0	1	2	1	3	3

From Ruf (1997b, 1996b)

[a]Data extracted from table on page 496 (pathology report) of the study report.

[b] As per reported No. of examinations

Codes for the grading of histological lesions are as follows: 1 = very few; 2 = few; 3 = moderate number; 4 = many.

Table 6. Mean haematology values from week 52 ± standard deviation (percentage change from control)

Dietary concentration (ppm)	Erythrocytes		Haemoglobin (g/l)		Erythrocyte volume fraction (l/l)		Heinz bodies (No. per 1000 erythrocytes)	
	Male	Female	Male	Female	Male	Female	Male	Female
0	6.69 ± 0.790	7.04 ± 0.888	142 ± 13.4	153 ± 18.8	0.402 ± 0.037	0.434 ± 0.043	0	0
500	6.47 ± 0.506 (–3.3)	6.47 ± 0.237 (–8.1)	136 ± 9.0 (–4.2)	138 ± 6.3 (–9.9)	0.390 ± 0.028 (–3.0)	0.400 ± 0.012 (–7.9)	1 ± 0.6	4 ± 4.5
3 500	6.06 ± 0.383 (–9.4)	5.91 ± 0.648 (–16.5)	128 ± 7.9 (–9.9)	129 ± 17.6 (–15.7)	0.370 ± 0.042 (–8.0)	0.367 ± 0.047 (–15.4)	4 ± 3.7	30 ± 16.0
25 000	5.25 ± 0.764 (–21.5)	5.78 ± 0.413 (–17.9)	115 ± 14.1 (–19.0)	127 ± 5.9 (–17.0)	0.329 ± 0.038 (–18.0)	0.368 ± 0.030 (–15.2)	36 ± 12.8	46 ± 16.4

From Ruf (1997b, 1996b) From Table 19 (page 238) of the study report. No indication of statistical significance was provided.

(b) Dermal application

Rabbits

In a 21-day study of repeated dermal toxicity in New Zealand White rabbits (HC:NZW), groups of five male and five female rabbits received dermal applications of fenhexamid (batch No. 4258/76; purity, 95.4%) at a dose of 0 or 1000 mg/kg bw per day, for 6 h per day, 5 days per week for the first 2 weeks and 7 days per week for the third week. The test substance was formulated in sterile physiological saline containing 2% Cremophor EL. The backs and flanks of the rabbits were shaved 1 day before application and twice per week thereafter. With the aid of a syringe, the test substance formulation (2 ml/kg bw) was applied to a small pad consisting of four layers of gauze placed on the rabbit's back. The pad was then turned over and secured in place using adhesive dressing. The gauze pad was the same size as the application site i.e. 11 × 12 cm. The application area was stated to be more than 10% of the body surface area of the rabbit. After 6 h, the adhesive dressing and gauze pad were removed and the application area was washed with soap and water. The control group received the vehicle formulation minus the test substance and the application method was the same as the treatment group. Animals were observed daily for signs of mortality, toxicity, and the presence of dermal irritation. The animals were examined for signs of local skin irritation before the start of the study and 24 h after each application. The skin irritation was evaluated using the United States Department of Agriculture (USDA) and Draize methods. Animals were weighed before the start of treatment, then weekly to ensure accurate dosing. Body weights were recorded weekly. Food consumption for each animal over a week was determined after 7, 14 and 21 days, and the average daily intake was calculated. The skin-fold thickness was measured in the centre of the application area on each animal's back using a cutimeter in order to quantify swelling. In each case two measurements were taken at different sites in the application area. The mean of the two measurements was calculated. Haematological and clinical chemistry parameters were measured at the start of the study and from surviving animals at termination. Three enzyme determinations (*N*-demethylase, *O*-demethylase and cytochrome P450) were performed in the liver tissues. All animals were sacrificed on schedule and subjected to gross pathological examination. Selected tissues were collected for weighing and histological examination. This study was conducted in accordance with GLP.

No test animals died or had to be killed during the course of the study. The study authors stated that there were no treatment-related clinical signs of toxicity in appearance or behaviour in any of the animals. However, no summary or individual animal data were provided in the study

report. Body weights or body-weight gains for both sexes in all of the treatment groups were not significantly different to those of the controls. No treatment-related changes in food intake were observed. No evidence of erythema was observed on the skin of treated animals. No treatment-related changes were evident in the skin-fold thickness of the treated skin, indicating the lack of any evidence of swelling. No treatment-related changes were seen in any of the haematological or clinical parameters examined. No treatment-related changes were seen in the liver tissue parameters (liver enzymes and triglycerides). There were no treatment-related changes in organ weights, either in the absolute or relative weights. No treatment-related changes in gross pathology were observed. There were macroscopic findings in the liver and kidney (abnormal colouration) of the dosed animals, which were considered to be incidental and not toxicologically relevant as they were also observed in control animals and in the absence of any corroborative histopathology. There were no treatment-related histopathological changes in any organs or tissues. In view of the apparent low toxicity of the test substance, deficiencies in this study (individual animal and summary data for clinical findings were not provided; ophthalmological examinations were not performed; blood clotting measurements were not made) were considered to be minor.

The NOAEL was 1000 mg/kg bw per day (Vohr, Krotlinger & Rinke, 1995).

(c) Exposure by inhalation

Rats

In a study of toxicity after inhalation, 10 male and 10 female Bor:WISW (SPF-Cpb) Wistar rats were exposed to fenhexamid dust (batch No. 17002/90; purity, 97.8%) by head–nose-only exposure at achieved concentrations of 0, 11.8, 97.7 or 1092.6 mg/m^3 in the air for 6 h a day for five consecutive days. One half of the rats were sacrificed on the third day of the recovery period (study day 7) and the other half at the end of the 2-week follow-up period (study day 21). Control rats received the filtered humidified air by head–nose-only. Achieved dust concentrations were measured gravimetrically. Mass median aerodynamic diameter and number median aerodynamic diameter were determined. The mass fraction with an aerodynamic diameter ≤3 µm was regarded as capable of penetrating the alveoli. In the groups receiving the lowest dose, the intermediate dose and the highest dose, respectively, 73%, 70% and 17.3% of particles were determined to be ≤3 µm as measured in the breathing zone of the rat. Animals were observed for clinical signs of toxicity, behavioural changes and mortality several times during the exposure and twice daily thereafter. Body weights were determined before the first exposure, on the fourth and seventh day of the study, and at weekly intervals thereafter. Food and water consumption were not determined. The rectal temperature of all rats was determined about 20 min after exposure on days 0, 4, and 7. Haematological and clinical chemistry parameters were measured at the time of interim sacrifice (study day 7). Autopsies were performed on all animals in the interim sacrifice and follow-up groups (study day 21). Urine analysis and ophthalmoscopic examinations were not performed. Gross necropsies were performed and selected organs were removed and weighed at the end of the study. This study was conducted in accordance with GLP.

Achieved dust concentrations closely matched nominal levels. No mortalities or treatment-related clinical signs of toxicity were observed. Reflex testing and rectal temperature measurement did not reveal treatment-related effects. Statistically significant decreases in mean rectal temperature in the group at the lowest dose and the group at the highest dose were observed on day 7; however, this finding is not considered to be of toxicological significance in the absence of a clear dose–response relationship or significant treatment-related effects on body weight. No treatment-related effects on body weights or body-weight gains were observed. No treatment-related effects on haematological and clinical parameters were observed. A marginal but statistically significant decrease in total bilirubin was observed in males and females at the highest dose; however, this decrease was not considered to be of toxicological significance. Both absolute and relative lung weights were slightly increased in males and females at the highest dose sacrificed at day 7. Grey colouration of the lungs was noted at the intermediate dose (one out of

five males; two out of five females) and at the highest dose (five out of five males and females) at interim sacrifice. Animals sacrificed on day 21, exhibited grey colouration of the lung macroscopically in (three out of five) the group receiving the highest dose and (two out of five) females at the intermediate dose. No other treatment-related changes were observed in other organs or tissues.

The NOAEL was 97.7 mg/m^3 (0.098 mg/l), on the basis of marginal increases in lung weights and grey discolouration of lungs observed macroscopically at the next highest dose of 1092.6 mg/m^3 (1.1 mg/l; the highest dose tested) (Pauluhn, 1991b, 1996c).

In a study of toxicity after inhalation, 10 male and 10 female Hsd Cpb:WU [formerly Bor:WISW(SPF-Cpb)] Wistar rats were exposed to fenhexamid dust (batch No. 4258/76; purity, 95.4%) by head-nose only exposure at actual concentrations of 0, 10.2, 68.7 or 486.7 mg/m^3 in air for 6 h/day, 5 days per week for 4 weeks. Control rats received filtered humidified air only. Achieved dust concentrations were measured gravimetrically. Mass median aerodynamic diameter and number median aerodynamic diameter of the particles were determined. Animals were observed for clinical signs of toxicity, behavioural changes and mortality twice daily except once on exposure-free days. Reflexes and a functional observation battery were tested during weeks 1 and 4. Rectal temperatures were measured on days 0 and week 4.

Body weights were determined twice per week. Haematological and clinical chemistry parameters were measured at termination. Urine analysis was performed on all animals at the end of the study. Eye examinations were performed on five rats of each sex per group before the first exposure and at study termination. Liver tissues were analysed for *N*-DEM (*N*-demethylase, aminopyrin-*N*-demethylase), *O*-DEM (*O*-demethylase, *p*-nitroanisol-*N*-demethylase), cytochrome P450 and triglycerides. Gross necropsies were performed and selected organs were removed and weighed at the end of the study. Histopathological examinations of selected organs and tissues were performed. This study was conducted in accordance with GLP regulations.

Achieved fenhexamid dust concentrations closely matched nominal levels. The mass fraction with an aerodynamic diameter of ≤3 µm was regarded as capable of penetrating the alveoli. The analysis of the test atmosphere demonstrated between 56% and 68% of the dust generated had an aerodynamic diameter ≤3 µm, with the mass median aerodynamic diameter between 2.1 and 3.1 µm for the three exposure levels.

No treatment-related mortality or clinical signs of toxicity were seen. A statistically significant decrease in forelimb grip strength was observed in males at the intermediate dose and at the highest dose (769 and 744 versus 941 controls) and at the intermediate dose for females compared with controls (770 versus 891). Grip strength when measured for all paws appeared to be increased compared with controls, hence contradicting the fore-paw results which are therefore not considered adverse. A statistically significant decrease in foot splay reflex was observed in males at the intermediate dose and at the highest dose (7.3, and 6.6 versus 9.3 controls) and in females at the highest dose compared with controls (6.9 versus 8.8 controls). The toxicological significance of this finding is questionable in the absence of any other neurological findings. Rectal temperatures were within the normal range. Body-weight gain appeared slightly lowered during the course of the study among males at the highest dose (58 g compared with 67 g for controls).

Haematological parameters were largely unaffected, although a slight decrease in lymphocytes (< 10%) was seen in males and females at the highest dose. There was an approximately 80% increase in segmented neutrophils seen in males and females at the highest dose. This mild change in leukocytes may have been caused by mild inflammatory effects in the lung. No Heinz bodies were detected. ALP activity was slightly increased (25%) in females at the highest dose, but there was no histopathology to corroborate this finding. Liver biochemistry indicated a slight but statistically significantly increase in *O*-DEM and cytochrome P450 in males at the highest dose. Hepatic triglyceride concentrations were not affected by the treatment. Increased urinary volume was observed in females at the highest dose, which was associated with

increased water consumption during the sampling period. There was no pathology to corroborate this finding. Ophthalmoscopic examinations did not reveal treatment-related changes in the eye. The gross pathological examination did not reveal treatment-related changes except for an increased incidence of grey discolouration of the lungs and lung-associated lymph nodes which were thought to be associated with deposited test compound. There was a statistically significant increase in lung weights (absolute and relative) in males and females at the highest dose. An increased incidence of bronchoalveolar proliferations and pigment-laden alveolar macrophages, with a sinus histiocytosis in lung-associated lymph nodes were observed in the rats at the highest dose. These findings were suggested to be indicative of a particle overload situation at the highest dose.

The NOAEL was 68.7 mg/m^3 (0.069 mg/l), on the basis of multiple toxicological minor changes such as an increase in lung weights, grey discolouration of the lungs, bronchoalveolar proliferations, and pigment-laden alveolar macrophages, with a sinus histiocytosis in lung-associated lymph nodes (possibly owing to particle overload), increase in lung weight (absolute and relative) and increase in liver enzymes (*O*-DEM, cytochrome P450) at the highest dose tested (Pauluhn, 1996a, 1998).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study of carcinogenicity, groups of 50 male and 50 female B6C3F_1 mice were given diets containing fenhexamid (batch No. 4257/76; purity, 95.4%) at a concentration of 0, 800, 2400, or 7000 ppm (equal to 0, 247.4, 807.4 or 2354.8 mg/kg bw per day for males and 0, 364.8, 1054.5 or 3178.2 mg/kg bw per day for females) mixed with 1% peanut oil (excipient) for 2 years. An additional 10 mice of each sex per group were sacrificed at 52 weeks (interim sacrifice). Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and public holidays. Body weights were determined before the initiation of the study, and once per week until week 104. Food and water consumption for each animal was determined weekly in the first 13 weeks on the first 20 living animals per group, and every 4 weeks thereafter up to week 101. Urine analysis and ophthalmoscopic examinations were not performed. Blood was collected from 10 randomly selected animals from each group during weeks 52/53, 80 (differential blood count only) and 103/104. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed and tissues were collected for histological examination. This study was conducted in accordance with GLP regulations.

Stability and homogeneity data indicated that the test compound was stable in the diet for at least 1 week and homogenously distributed in the diet. Mean actual concentration as a percentage of the nominal dose was 100.5%, with a range of 99–102%, indicating that the variance between nominal and actual dosage to the animals was acceptable. There were no treatment-related clinical signs of toxicity in any of the dosed groups. No evidence of treatment-related effects could be deduced from the frequency, localization and time of appearance of palpable masses. Survival in all groups at 104 weeks was 70% or better. There was no indication of any treatment-related effect on mortality. There was no toxicologically relevant effect on the body weight of males at up to 2400 ppm and females at up to 7000 ppm. Body weights were statistically significantly lower in males in the 7000 ppm dose group (8% maximum at week 48) compared with control males. Body-weight gain of males at 7000 ppm was approximately 17% less than that of the control males. Food consumption was not affected by the treatment. Water consumption was increased (37–39%) in both sexes at 7000 ppm only. There were slight but statistically significantly elevated mean haemoglobin and erythrocyte volume fraction (week 103) in the group of males at 7000 ppm. These changes were not considered to be toxicologically significant since individual values for one male among the controls were below and for one male at 7000 ppm were above the range of historical controls. When these two outliers were excluded from the calculation of the

respective means, the haemoglobin and erythrocyte volume fraction of males at 7000 ppm were comparable to those of control males. No treatment-related changes in reticulocyte count or number of Heinz bodies were observed.

No toxicologically relevant changes were seen in serum biochemistry parameters at doses up to and including 7000 ppm. Creatinine concentrations were statistically significantly increased in males at the highest dose in week 53 (10.3%) and 104 (18.5%). This increase in creatinine concentrations appeared to be treatment-related and may reflect the renal pathology which was observed microscopically in this study. A statistically significant increase in serum bilirubin was observed in males at the highest dose at week 53 and a non-significant increase in the same group at week 104. Total bilirubin may be increased in the peripheral blood as a result of excessive haem turnover by the reticuloendothelial system or as a result of altered metabolism in the liver. Splenomegaly was not observed in this study. A statistically significant increase in serum albumin was observed in males at 2400 ppm (7.5%) and 7000 ppm (6.8%) at week 53, and a non-significant increase in males at 7000 ppm (10.7%) at week 104. This increase in serum albumin may be caused by dehydration since water consumption was increased in both sexes at 7000 ppm. Cholesterol concentrations were increased in males at termination (2.97, 3.40, 4.12, 4.59 mmol/l at 0, 800, 2400 and 7000 ppm, respectively), which was not seen in males at week 53. Cholesterol concentrations were unaffected in females. This increase in cholesterol concentrations was not considered to be toxicologically relevant since two males in the groups at 2400 and 7000 ppm had a higher value for this parameter while those of the rest of the animals in the respective groups were comparable to control values. These four males had significant deviations in other clinical chemistry parameters (plasma protein and albumin or ASAT activity) indicating a change in liver function. These changes in biochemical parameters were not considered to be caused by treatment since these four mice had developed hepatocellular tumours (not treatment-related).

No treatment-related macroscopic findings were observed at interim sacrifice. There were no treatment-related effects on organ weights (absolute and relative) at termination except those of the kidneys of male mice at 2400 ppm and above. The kidney weights were reduced in males at 2400 ppm (10% relative) and at 7000 ppm (20% absolute and 17% relative). A statistically significant increase in absolute liver weights was observed in females at the highest dose (9.7%); however, this was not reflected in the relative liver weights and hence was not considered to be treatment-related.

There were no toxicologically relevant effects at termination on organ weights (absolute and relative) at any dose except slightly decreased kidney weights in males at 2400 ppm (9.8%/8.3%) and also in males at the highest dose (23.5%/19.9%) and females (13.8%/13.7%). A slight increase in both absolute/relative liver weights in males at the highest dose (7.6%/10.3%) was not judged to be toxicologically significant since there was no alteration of biochemical parameters and liver histopathology.

Histopathological investigations revealed treatment-related non-neoplastic changes in the kidneys of both sexes. The incidence of basophilic tubules in the cortex was seen more frequently in females at 7000 ppm (22 out of 50), which was graded as “minimal to slight”. An increased incidence in chronic renal disease was observed in males at the highest dose (9 out of 50) which was graded as “slight”. A dose-dependent reduction of sex-specific vacuolation of the proximal tubules was seen in males at 2400 ppm (21 out of 50) and 7000 ppm (50 out of 50). The other organs/tissues showed degenerative and inflammatory changes in control and treatment groups with a similar incidence and degree.

There were no treatment-related neoplastic changes seen in either sex at interim or terminal sacrifice. There was a positive trend in the frequency of lung adenomas in females arising from the occurrence of the finding in 2 out of 50 females in the group at 7000 ppm and none at any other doses. These lung adenomas are not considered to be of toxicological concern because the incidence (4%) was within the range of historical controls for female mice (6%) and a negative trend in lung adenomas was observed in males.

The tumour spectrum in treated groups (the total number of animals with: neoplasms, benign or malignant tumours and benign and malignant tumours combined) was comparable to

that for controls. The majority of tumour-bearing treated and control animals were necropsied late in the experimental phase of the study. Fenhexamid was not carcinogenic in mice at doses up to and including 7000 ppm. There was no treatment-related increase in tumour incidence, tumour spectrum or latency when compared with controls.

The NOAEL was 800 and 2400 ppm in males and females, respectively (247.4 mg/kg bw per day in males and 1054.5 mg/kg bw per day for females) on the basis of decreased kidney weights in males, marginal increase in serum albumin in males and decrease in kidney weight in females, increase in water consumption in females, increase in incidence of basophilic cortical tubules in females, at the next higher dose of 2400/7000 ppm, males/females, respectively (807.4/3178.2 mg/kg bw per day, males/females). Fenhexamid was not carcinogenic in mice at doses of up to and including 7000 ppm in the diet for 2 years (Eiben & Rinke, 1996).

Rats

In a long-term study of combined toxicity/carcinogenicity, groups of 50 male and 50 female Wistar rats (Hsd/WIN:WU) were given diets containing fenhexamid (batch No. 4257/76; purity, 95.4%) at a dose of 0, 500, 5000, or 20 000 ppm (equal to 0, 28, 292 or 1280 mg/kg bw per day for males and 0, 40, 415 or 2067 mg/kg bw per day for females) mixed with 1% peanut oil (excipient) for 24 months. An additional 10 rats of each sex per dose were sacrificed after 52 weeks. Stability, homogeneity and dietary concentrations were confirmed analytically. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and public holidays. Body weights were determined before the initiation of the study, once per week for 13 weeks and then every 2 weeks thereafter up to week 105. Food and water consumption for individual rats were determined once a week for the first 13 weeks, and every 4 weeks thereafter, up to week 101. Ophthalmoscopic examinations were performed on all rats during the first week, and in week 53 and before the final necropsy of all rats that survived from the control group and the group at the highest dose. Urine analysis was performed for 10 rats of each sex per dose at weeks 27, 53, 79/80 and 105. Blood was collected from 10 randomly selected animals from each group during weeks 27, 53, 79/80 and 105. All animals that died and those sacrificed on schedule were subjected to gross pathological examination and selected organs were weighed and tissues were collected for histological examination. This study was conducted in accordance with GLP.

Stability and homogeneity data indicated that the test compound was stable in the diet for at least 1 week and homogenously distributed in the diet. Actual mean concentration as a percentage of the nominal dose was 100.5%, with a range of 91.3–102%, indicating that the variance between nominal and actual dose administered to the animals was acceptable.

There were no treatment-related clinical signs of toxicity in any of the dosed groups. No evidence of treatment-related effects could be deduced from the frequency, localization and time of appearance of palpable masses. Survival at 104 weeks was not affected by the treatment and was not less than 50% in any group. Cumulative mortality was 28, 18, 20 and 24% in males and 40, 38, 46, and 28% in females at 0, 500, 5000 and 20 000 ppm, respectively. No treatment-related changes in body weight were observed for males. A transient lower body weight ($\leq 6\%$) was observed in males in groups 5000 and 20 000 ppm during weeks 10-49, and therefore, was not considered toxicologically relevant. A statistically significant decrease in body weight was observed in females at the highest dose (10% in week 105) compared with controls. There was a slight retardation of body weight of females (7.5% in week 99) at 5000 ppm starting from week 60 onwards compared with controls. Food consumption was increased in males at the highest dose (14.2%) and females (27.7%) compared with controls averaged over 708 days. This is also reflected in weekly feed intake data, which showed a statistically significant increase in males and females at the highest dose. Feed efficiency ratios in males were comparable to controls. Females in the groups at 5000 and 20 000 ppm were less efficient in converting their daily food intake into body weight when compared with controls. Water consumption was increased (7.5–8%) in males at the highest dose and in females at the intermediate and highest doses compared with controls, which is not judged to be toxicologically significant.

There was no evidence of toxicologically relevant changes in erythrocyte parameters, haemoglobin, MCH, mean corpuscular haemoglobin concentration (MCHC) and erythrocyte volume fraction in any treated males and females throughout the study. Some statistically significant changes were observed in haematological parameters, but they were not considered to be toxicologically relevant since the changes were small, not dose-related or fell within the range of values for historical controls. The number of Heinz bodies was not altered in the treatment group. There was a slight increase in reticulocytes in males at the highest dose (46% increase over the controls; not statistically significant), while the increase in reticulocytes was statistically significant in females at the highest dose (155% increase over that of the controls). This increase was associated with increased spleen weights at termination in males and females at the highest dose; however, the histopathological examinations did not reveal any morphological changes in the spleen that could be attributed to treatment. There was no evidence of toxicologically relevant changes in leukocytes and differential counts in any treated males and females.

Treated animals did not show any toxicologically relevant changes in ASAT, ALAT, glucose, triglycerides, urea, creatinine or bilirubin. ALP activities were slightly elevated in females at the highest dose ($p < 0.05$) at week 53 and 105 and in males at the highest dose (not significant) in week 105. Lower glutamate dehydrogenase (GLDH) activities were seen in rats at the highest dose and occasionally in animals of both sexes at the intermediate dose, although it is unlikely that depression of the activity of this enzyme in blood is of toxicological significance. Albumin concentrations were elevated in males receiving 5000 ppm and above at termination and were lower in females at 20 000 ppm in week 27. These sporadic findings were considered to be indications of slight alterations in liver function as a consequence of metabolic adaptation rather than a toxic effect since there was no indication of treatment-related changes in liver morphology. There was a slight tendency for the excretion of higher volumes of more dilute urine in females at the highest dose, with these parameters achieving occasional statistical significance. There was a slight decrease in excretion of urinary protein and protein concentration in both sexes at 5000 and 20 000 ppm . This decrease in urinary protein is not judged to be a toxicologically relevant effect since there were no changes in other urinary parameters and no evidence of increased kidney weights or kidney morphology.

The incidence of male rats showing cataracts or exhibiting corneal turbidity was minimally increased (24 versus 16 eyes affected among controls) at the highest dose. The extent of lens opacity was described as being of “slight degree”. Fenhexamid is not considered to be an ocular toxicant, since these types of findings in the eyes are common to aged rats, and were not seen at the interim sacrifice, and no treatment-related effects were seen in the females at the highest dose.

No treatment-related macroscopic findings were observed in treated groups at interim sacrifice. No treatment-related findings were observed in gross pathology at terminal necropsy or in unscheduled deaths during the study. At interim and terminal sacrifice, there were no clear treatment-related effects observed on organ weights. Some differences in organ weights (liver, kidneys, heart) were statistically significant but were judged to be not treatment-related since changes were small, and observed in the absence of any obvious dose–response relationship. Relative spleen weights (organ to brain weight ratio) were increased in males at the highest dose (10.1%) and females (13.4%) compared with controls. At terminal sacrifice, an “enlarged” spleen was noted in males (incidence of 1, 1, 2, 4) and females (0, 2, 2, 3), which correlated with an increase in relative spleen weights in males and females at 20 000 ppm, along with increased splenic extramedullary haematopoiesis during the histopathological examination of the spleen of males at 5000 and 20 000 ppm. Therefore, the changes in spleen are considered to be treatment-related.

Histopathological examinations of organs and tissues at interim sacrifice did not reveal any test substance-related non-neoplastic lesions. Slight focal calcification of the arteries of the lung was noted in 7 out of 10 males at the highest dose compared with 2 out of 10 in control males. This finding in the lungs was not considered to be toxicologically relevant since such a finding was not noted at terminal sacrifice. At terminal sacrifice, there were no treatment-related non-neoplastic lesions observed in males at 500 ppm or in females at 5000 ppm. An increased

incidence of thyroid follicular cell hyperplasia was seen in males at the highest dose (4%); however, this increase was within the historical control values (0–4%) for this strain of rats. Treated males had a higher incidence of Leydig cell adenomas of the testes (0 out of 48, 0 out of 48, 2 out of 50, 2 out of 40), but this incidence (4–5%) was within the normal biological range of 14–50% for this age and strain of rat. Statistical analysis showed a positive trend ($p = 0.04$) for an increased incidence of splenic extramedullary haematopoiesis in males in the groups at 5000 and 20 000 ppm (0 out of 48, 0 out of 48, 4 out of 50, 4 out of 49). There was a dose-related increase in the cellularity of the bone marrow of the femur (14 out of 50, 12 out of 50, 19 out of 50, 22 out of 50) and sternum (15 out of 50, 13 out of 50, 18 out of 50, 23 out of 50) in females in the groups at 5000 and 20 000 ppm. Statistical analysis showed this to be a positive trend ($p = 0.007/0.0016$). This finding was corroborated by an increase in reticulocytes in peripheral blood in females at these doses. A statistically significant trend was not observed in cellularity of bone marrow of the femur and sternum in males. An increased incidence of glandular hyperplasia of the uterine mucosa was observed in females at the highest dose (17 out of 50, 24 out of 50, 18 out of 50, 28 out of 50). Although statistical analysis indicated a positive trend ($p = 0.03$) for uterine glandular hyperplasia, there were no corroborating findings of treatment-related neoplastic lesions of the uterus associated with uterine glandular hyperplasia in this study. A low incidence of caecal mucosal hyperplasia was noted in males at 5000 ppm (2 out of 48) and 20 000 ppm (4 out of 49) and in 1 out of 50 females at 20 000 ppm. This lesion was associated with distinct mucosal inflammation or severe periarteritis of adjacent vessels. Mucosal hyperplasia was likely to have resulted from regenerative proliferation of the epithelial cells after initial mucosal irritation by the test compound. Three of these cases showed necrotic changes and/or mineralization within the hyperplastic mucosa. There were no associated neoplastic lesions in the large intestine. An increased incidence and severity of colloid alterations in thyroid gland follicles were observed in the males and females at the highest dose. This finding was associated with a reduced follicle volume and the occurrence of blue-grey clumps. The authors speculated that an increased turnover of thyroid hormone could be the mode of action, possibly owing to increased food intake. However, thyroid hormone concentrations were not measured in this study. The male groups receiving the lowest dose and the intermediate dose showed incidences of colloid alterations that were comparable to the control values. The observed increased incidence of colloid alterations in females at the lowest dose and the intermediate dose was not deemed to be treatment-related as the number of animals affected were very similar and the severity of colloid alterations were determined to be greater in the females at the lowest dose when compared with that of the females at the intermediate dose. The incidence of follicular cell hyperplasia in males at 20 000 ppm (4.0%) fell within the range of normal biological variation for this age and strain of rat (up to 4.0%).

Histopathological examinations of the organs and tissues of the interim sacrifice group did not reveal treatment-related neoplastic lesions. At interim sacrifice, spontaneous neoplastic lesions observed that were not dose-related included: hepatocellular adenoma in one female rat of 500 ppm and in one male at 20 000 ppm; a benign adenoma of the pars distalis of the pituitary gland in one female dosed at 20 000 ppm; a benign stromal polyp of the uterus in one female in the control and one female at 20 000 ppm.

Histopathological examination performed at termination revealed no treatment-related neoplastic lesions. Neoplastic lesions common in all male groups were adrenal medullary tumours, pituitary adenomas and thyroid C-cell and follicular adenomas. Neoplastic lesions common in all female groups included pituitary adenomas, mammary adenomas, fibroadenomas, uterine adenocarcinomas and systemic tumours, i.e. histiocytic sarcomas. Incidences of the following neoplastic lesions were observed to be within the range of normal biological variability for this age and strain of rat (incidences for historical controls were supplied by the company) and, therefore, are regarded as spontaneous findings. Hepatocellular carcinomas were observed in males (0 out of 48, 0 out of 48, 0 out of 50, 2 out of 49). The incidence (4.1%) of this finding in males at the highest dose fell within the historical control range of 0–6.0%. A higher incidence (4%) of parathyroid gland adenomas was noted in the males at 20 000 ppm (0 out of 47, 0 out of

47, 0 out of 49, 2 out of 47) that fell within the historical control range of 0-6.4%. Additionally, there was no dose-related increase of pre-neoplastic lesions (i.e. hyperplasia, 1 out of 47, 2 out of 47, 0 out of 49, 2 out of 47). The pooled incidences of squamous cell carcinomas of the skin and ear, although somewhat higher in treated males (0 out of 48, 1 out of 48, 1 out of 50, 2 out of 49), fell within the historical control range of 0–4% for this age and strain of rat. There was a slightly higher incidence of Leydig cell adenomas of the testes (0 out of 48, 1 out of 48, 3 out of 50, 1 out of 49) in males at the intermediate dose. Statistical analysis did not reveal a positive trend and the incidence fell within the range for normal biological variability (6–22%) for this age and strain of rat. Endometrial stromal polyps were noted at higher frequency in the uteri of females receiving the intermediate dose and the highest dose (6 out of 50, 4 out of 50, 7 out of 50, 11 out of 50) and statistical analysis showed this to be a significant positive trend ($p = 0.025$). Historical control data supplied by the company indicated that endometrial stromal polyps occurred spontaneously in up to 30% of female Wistar rats of the same strain and age. Therefore, in the current study the incidences of 14% and 22% of this finding in the females at 5000 ppm and 20 000 ppm fell within the range of normal biological variation for this age and strain of rat. All other neoplastic and hyperplastic lesions occurred with equal frequency among all dose groups or were found to be spontaneously occurring lesions common to this age and strain of rat.

In all treated females and in males at 500 and 5000 ppm, the total numbers of animals with neoplasms of all kinds, total numbers of animals with benign (only) and malignant (only) tumours, and the total number of animals having both benign and malignant tumours were comparable to control values. The males at 20 000 ppm had more malignant tumours (5 out of 48, 4 out of 48, 8 out of 50, 9 out of 49). There were more males at 20 000 ppm that had both benign and malignant tumours (2 out of 48, 2 out of 48, 4 out of 50, 7 out of 49). However, the malignant tumours occurred in several different tissues or organs with a very low frequency. Of the total of eleven malignant tumours (in nine animals), there were three systemic tumours (two lymphomas, one histiocytic sarcoma), two hepatic carcinomas, two squamous cell carcinomas, two haemangiomas (one heart, one mesenteric lymph node), one adrenal medullary carcinoma and one schwannoma (heart). None of these tumours were detected before week 88 of the study and therefore, were likely to be an age-related phenomenon. Since the total number of benign tumours was reduced in the males at 20 000 ppm, the overall incidence of tumours did not differ from that of control animals.

The majority of tumour-bearing treated and control animals were necropsied late in the experimental phase of the study. There was no shift in the tumour spectrum, no increase in the total number of tumours and no increase in the incidence of animals with tumours that could be attributed to treatment with fenhexamid.

The NOAEL was 500 ppm (28 mg/kg bw per day) on the basis of decreased body weight, body-weight gain, food consumption and food efficiency in females, increased incidence of caecal mucosal hyperplasia in males, increased cellularity of the bone marrow in females and the presence of splenic extramedullary haematopoiesis in males at the next highest dose of 5000 ppm (292 mg/kg bw). There was no evidence of carcinogenicity at doses up to and including 20 000 ppm (1280 mg/kg bw per day; Eiben et al., 1996).

2.4 Genotoxicity

The results of studies of genotoxicity with fenhexamide are summarized in Table 8.

In an assay for reverse gene mutation in bacteria, strains TA1535, TA100, TA1537, and TA98 of *Salmonella typhimurium* were exposed to fenhexamid (batch No. 17002/90; purity, 95.5%) in dimethylsulfoxide (DMSO) at concentrations of up to 5000 µg/plate, with or without metabolic activation. Solvent controls were not included. This study was conducted in accordance with GLP. Compound insolubility and excessive cytotoxicity was observed at concentrations of ≥ 2000 µg/plate. Bacteriotoxic effects were observed at doses of > 125 µg/plate. There was no evidence of induced mutant colonies over background with or without metabolic activation (Herbold, 1991).

In a test for unscheduled DNA synthesis, primary rat hepatocyte cultures were exposed to fenhexamid (batch No. 17002/90; purity, 95.5%) in DSMO at concentrations of up to 40.0 µg/ml for 18–24 h in one trial. Cytotoxicity was evident at 40 µg/ml. The viability of primary hepatocytes was slightly below acceptance criteria for the laboratory (74% instead of > 80%), but the assay was deemed acceptable owing to the stable cell number and normal cell morphological appearance. At test article concentrations of < 40 µg/ml, the mean gross nuclear grain values and the percentage of nuclei in repair were not significantly different from the vehicle controls. There was no evidence that unscheduled DNA synthesis was induced by fenhexamid (Brendler, 1992).

In an assay for mammalian cell gene mutation at the HGPRT locus using Chinese hamster lung (V79) cells cultured in vitro, fenhexamid (batch No. 4258/76; purity, 95.4%) (DMSO as vehicle) was applied at concentrations ranging from 2.4 to 625 µg/ml for the preliminary test and up to 150 µg/ml in a definite test; with or without metabolic activation. Fenhexamid revealed a slightly dose-dependent cytotoxicity up to 78.1 µg/ml and marked cytotoxicity at concentrations > 156.3 µg/ml both in the presence and absence of metabolic activation (0% viability relative to vehicle control). There was no evidence of a concentration-related positive response of induced mutant colonies over background with or without metabolic activation (Brendler-Schwaab, 1994).

In an assay for mammalian cell chromosomal aberration, Chinese hamster ovary (CHO) cells were cultured in vitro and exposed for 4 h to fenhexamid (batch No. 4258/76; purity, 95.4%) in DMSO at concentrations of up to 120 µg/ml in the presence or absence of metabolic activation. The cells were harvested 8, 24 and 30 h later. Fenhexamid was tested up to cytotoxic concentrations (± S9). Under the conditions of this study in vitro there was no evidence that fenhexamid induced chromosomal aberrations over background values. Therefore, fenhexamid demonstrated no clastogenic effects in cultured mammalian cells, either with or without metabolic activation (Gahlmann, 1995).

In an assay for gene mutation, *S. typhimurium* strains TA1535, TA100, TA1537, and TA98 and *E. coli* WP2 *uvrA* were exposed in separate trials to fenhexamid (batch No. 4258/76; purity,

Table 8. Results of studies of genotoxicity with fenhexamid

Test system	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* [a] TA 98, 100, 1535, 1537	62.5–2000 µg/plate	95.5	Negative	Herbold (1991)
Unscheduled DNA synthesis	Rat primary hepatocytes[a]	2.5–40 µg/ml	95.5	Negative	Brendler (1992)
Mammalian cell gene mutation (HGPRT locus)	Chinese hamster lung cells (V79) [a]	25–150 µg/ml	95.4	Negative	Brendler-Schwaab (1994)
Chromosomal aberration	Chinese hamster ovary cells [a]	2–120 µg/ml	95.4	Negative	Gahlmann (1995)
Reverse mutation	*S. typhimurium*[a] TA98, TA100, TA1535, TA1537 and *E. coli WP2 uvrA*	43.8–700 µg/plate	95.8	Negative	Ohta (1995)
Mammalian cell DNA repair (Rec assay)	*Bacillus subtilis* strains H17 (Rec+) and M45 (Rec-) [a]	6.25–100 µg/disk	96.1	Negative	Watanabe (1997)
In vivo					
Micronucleus formation	Mice (males and females)	750 mg/kg, intraperitoneal	95.4	Negative	Herbold (1993)

[a] With and without metabolic activation (S9)

95.4 %) in DSMO at concentrations of up to 700 µg/plate (±S9). There was no evidence of induced mutant colonies over background (Ohta, 1995).

In a rec assay, spores of *Bacillus subtilis* H17 strain with recombinant repair ability (Rec^+) and M15 strain without this ability (Rec^-) were exposed to fenhexamid (batch No. 170034/94; purity, 96.1%) in DMSO at concentrations of up to 200 µg/disk, in the presence and absence of metabolic activation. No evidence of growth inhibition was observed at doses ranging from 6.25 to 50 µg/disk in the presence or absence of metabolic activation. Under the conditions of the study, it can be concluded that fenhexamid had no DNA-damaging effects, either with or without metabolic activation (Watanabe, 1997).

In an assay for micronucleus formation in mouse bone marrow, groups of five NMRI mice of each sex were treated intraperitoneally with a single dose of fenhexamid (batch No. 4258/76; purity, 95.4%; in 0.5% aqueous Cremophor) at a dose of 750 mg/kg bw. Bone marrow cells were harvested 16, 24, and 48 h after treatment.

Clinical signs of toxicity noted at 750 mg/kg bw were apathy, roughened fur, staggering gait, sternal recumbency, spasm and difficulty in breathing; 1 out of 40 treated animals died during the test period. Treatment-related cytotoxic effects were noted in the bone marrow. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time (Herbold, 1993).

2.5 Reproductive toxicity

(a) Multigeneration study

Rats

In a two-generation study of reproductive toxicity (one litter/generation), groups of 30 male and 30 female Sprague-Dawley rats were given diets containing fenhexamid (batch No. 4258/76; purity, 93.8–95.2%) at a concentration of 0, 100, 500, 5000 or 20 000 ppm (equal to 0, 7.6, 38.2, 406 or 1814 mg/kg bw per day for males and 0, 9.0, 44.8, 477 or 2043 mg/kg bw per day for females determined for the 10-week premating period). After a 10-week pretreatment period, each female was co-housed with one male for up to 21 consecutive days. Insemination was determined by the presence of a vaginal plug and/or presence of sperm in a daily vaginal lavage. The F_1 pups selected to be parents for the F_2 generation were placed on treated feed after weaning. Approximately 2 weeks after the weaning of the last litter, these animals received treated feed for an additional 10 weeks before mating. Homogeneity, stability and concentrations were determined analytically. Animals were observed twice daily and once during weekends and holidays for moribundity, mortality, and clinical signs. Body weight was measured once a week for each male during the study. During the 10-week period before the P and F_1 matings, body weight and food consumption were measured once a week for each female. Body weight and food consumption were measured for dams during gestation and lactation as follows: body weight: days 0, 6, 13, and 20 during gestation and days 0, 4, 7, 14, and 22 during lactation; food consumption: once per week during gestation, twice per week during week 1 of lactation and once per week during weeks 2 and 3 of lactation. Blood was taken via orbital bleeding from ten P and F_1 adult rats of each sex per dose during premating weeks 9 or 10, and just before sacrifice and selected clinical chemistry parameters were evaluated. The following litter observations were made on days 1, 4 (before and after culling), 7, 14 and 21; number of live pups or stillborn pups, pup weights, external alterations, and sex of each pup. Each litter was randomly culled on day 4 to yield eight pups, including, if possible, four males and four females. Excess pups were sacrificed and subjected to a gross necropsy examination. Adult males were sacrificed after completion of the mating phase. Adult females were sacrificed after each dam's pups were weaned/died, when day 24 of gestation was reached, or 24 days after the last day of co-housing. All adults were

necropsied. For females, uteri were examined for implantation sites. Terminal body weights and kidney, liver and gonad weights were recorded from all P and F_1 adults. Selected tissues were preserved for histology. This study was conducted in accordance with GLP.

Stability and homogeneity data indicated that the test article was homogenously distributed and was stable in the diet. The test substance concentration analysis indicated that the variance between the nominal and actual dosage to animals was acceptable.

No treatment-related clinical signs were observed in adults. The clinical observation of dehydration which was recorded for all thirty control females of the P generation on study day 28 can be attributed to a problem with the automatic watering system. No treatment-related deaths were observed. Five rats died during the study and two rats were sacrificed early. There were treatment-related reduced weights observed for P and F_1 males and females at 20 000 ppm, and for P females at 5000 ppm. For the P males and females, statistically significant lower body weights of 7–15% and 6–10%, respectively, below the control group were observed, beginning on day 7 of the premating phase for the males and day 14 of the premating phase for the females. The body weights on day 28 were excluded owing to water system malfunction. For F_1 males and females, statistically significant lower body weights ranging from 6–16% and 8–10%, respectively, below the control group were observed beginning on day 0 of the premating phase. For the F_1 males, the greatest weight decrement was observed on day 0 (–16%) and decreased during the study to a non-statistically significant difference in weight by the termination of the study. However, for females the weight decrement remained constant throughout the premating phase. For the F_1 males and females, the lower body weights were attributable to the low body weight of the F_1 adults as pups, rather than a reduction in post-weaning weight gain (premating weight gain of 155 g versus 175 g for the males in the control group and at 2000 ppm, and 70 g versus 63 g for the females in the control group and at 20 000 ppm). For the P females at 5000 ppm, lower body weight (4% and 5% below the control group) began on day 14 with statistically significant decreases observed on day 21 and days 49–63. A treatment-related increase in food consumption was observed for P males (12–26% above controls) and F_1 males (12–21%) and females (5–11%) at 20 000 ppm.

During gestation, statistically significant and compound-related lower body weights and weight gain were observed in the animals in the P and F_1 generations at 20 000 ppm. The body weights for the P and F_1 animals were 7% and 8%, and 7% and 9%, respectively, lower than the control group. The weight gain during gestation was 12% lower than the control group for both the P and F_1 animals. During the lactation phase, statistically significant and compound-related lower body weights were observed in the P and F_1 generation at 20 000 ppm and in the P generation at 5000 ppm. The body weights for the P and F_1 animals at 20 000 ppm were 8–12% and 7–11%, respectively, lower than the control group. The body weights for the P generation at 5000 ppm were 5% lower than the control group.

For both the P and F_1 generations, there were no treatment-related effects on estrus cycle length and periodicity, time to insemination, the mating, fertility, gestation indices, gestation length, implantation sites or birth index.

Urea nitrogen and creatinine concentrations showed a tendency to increase for both sexes at 20 000 ppm. The only significant urea nitrogen increase over control values was F_1 females at 20 000 ppm at termination. There were slight increases in creatinine concentrations in P males and F_1 females. There is no evidence to allow determination of whether this was a direct effect of the compound on the kidney or an indirect effect caused by dehydration or high protein/nitrogen metabolism. ALP activities were often highest at 5000 and 20 000 ppm for both sexes and in both generations, but this was significant only in the F_1 females. There was also a concomitant increase in gamma-glutamyl transpeptidase (GGT) activities in P females at 5000 and 20 000 ppm and in P males at 20 000 ppm. This increase in both ALP and GGT activities suggests a treatment-related effect as they are both microsomal membrane-bound enzymes which may increase in the presence of bile stasis (cholestasis) or drug treatment. All other differences in clinical chemistry parameters were minor and incidental in occurrence and/or did not occur in both generations.

There were no compound-related macroscopic or microscopic findings in males and females of the P or F_1 generations. Terminal body weights were significantly decreased in rats of both sexes in the P generation at 20 000 ppm rats and in F_1 females. Terminal weights were also decreased in 20 000 ppm F_1 males, but the change was not statistically significant.

A compound-related effect was suggested in the liver: in males, absolute and relative hepatic weights were significantly decreased at 5000 and 20 000 ppm in animals in the P generation. Absolute hepatic weights were significantly decreased in P females at 20 000 ppm, possibly a result of the decreased terminal body weight noted above. Absolute hepatic weights were also significantly decreased in F_1 males at 5000 ppm, but not in F_1 males at 20 000 ppm. Relative hepatic weights were significantly decreased from control values in all groups of P males, but there were no other instances of relative weight changes in other generations or sexes. The decreases in liver weight, when taken with the clinical chemistry findings, are again suggestive of an equivocal hepatic effect at 5000 and 20 000 ppm. Statistically significant decreases were also observed in relative and absolute kidney weights in P and F_1 females at 20 000 ppm (change in relative weight not statistically significant) and in F_1 males at 5000 and 20 000 ppm. The findings, when taken with the clinical chemistry findings, are again suggestive of a renal effect of unknown origin. Absolute but not relative ovary weights were lower (15–20%) in P females at 20 000 ppm.

For F_1 pups kept after weaning to be F_1 parents, there was an increase in the number of pups that died in the group receiving 20 000 ppm when compared with the control group (0 out of 66, 2 out of 68, 0 out of 68, 0 out of 68, and 10 out of 78). The increase in the number of deaths of pups at 20 000 ppm was attributed to the small size of the pups at weaning. There were no treatment-related effects on pup sex, litter size, live birth, and viability indices. There was no compound-related effect on birth weight. There was a statistically significant and compound-related decrease in pup weights at 5000 and 20 000 ppm, which occurred on days 7 to 21. No histopathology was performed, and no organ weight data was collected on F_1 or F_2 pups. Necropsy revealed no treatment-related effects in either the F_1 or F_2 weanlings sacrificed at postnatal day 21.

The NOAEL for the reproductive toxicity for fenhexamid was 20 000 ppm, the highest dose tested; 1814 mg/kg bw for males and 2043 mg/kg bw per day for females). The NOAEL for neonatal toxicity was 500 ppm (38.2 mg/kg bw per day for males, and 44.8 mg/kg bw per day for females) on the basis of decreased pup body weights at 5000 and 20 000 ppm. The NOAEL for parental toxicity was 500 ppm (38.2/44.8 mg/kg bw per day, males/females, respectively) on the basis of lower adult male and/or female premating body weights in 20 000 and 5000 ppm, lower gestation body weights at 20 000 ppm, lower lactation body weights at 5000 and 20 000 ppm, statistically significant changes in clinical chemistry parameters, terminal body weight, and organ weights in the 5000 and/or 20 000 ppm (Eigenberg, 1996, 1997).

(b) Developmental toxicity

Rats

Groups of 30 female Sprague-Dawley rats were given fenhexamid (batch No. 898805001; purity, 97.7%) at a dose of 0, 300, 1000 or 2000 mg/kg bw per day by gavage as a suspension in aqueous 0.5% carboxymethylcellulose and 0.4% Tween 80 from days 6 to 15 of gestation. Analytically confirmed concentrations were 0, 300, 1080 or 2160 mg/kg bw per day. Animals were examined twice daily for clinical signs of toxicity and once daily on weekends and holidays. Food consumption was determined over days 0–6, 6–11, 11–16, and 16–20 of gestation. Body weights were determined on day 0, 6–16, and 20 of gestation. Females were sacrificed on day 20 of gestation and gross external and internal examinations were performed. The liver, thyroid, gravid uterus and ovaries were removed and weighed. The corpora lutea were counted, and pregnancy was determined. The uterus was opened for determination of resorptions, and placentas were weighed, the number of live and dead fetuses and the sex of each fetus was determined and weighed. Fetuses were removed and examined for external malformations, and visceral malformations and skeletal variations according to the modified Wilson technique. The fetuses

were also evaluated for the occurrence of findings in abdominal, pelvic and thoracic organs as well as skeletal findings by the Staples technique.

Homogeneity and stability data indicated that that the compound was stable for at least 30 days and homogeneously distributed. The analytically confirmed concentrations of fenhexamid in the dosing suspensions were 0, 300, 1080, and 2160 mg/ml, which corresponds to doses of 0, 300, 1080, and 2160 mg/kg bw. The only observation seen consistently throughout the dosing period was tan-coloured stools, observed in five animals at 300 mg/kg bw, two animals at 1000 mg/kg bw, and six animals at 2000 mg/kg bw. This finding is not considered to be toxicologically meaningful because it disappeared within 2 days after stopping the treatment and a clear dose–response relationship was not observed, nor was this finding directly associated with any other effects. The study authors suggested that the tan-coloured stool may have been caused by the brown colour of the test material. No other treatment-related clinical signs were noted. There were no mortalities in the study, except that one dam was sacrificed in a moribund condition on day 12 of gestation. Necropsy observations indicated that the animal had been improperly gavaged. Body weights were not affected in animals in the group receiving fenhexamid at 300 mg/kg bw. Statistically significant decreases in body weight were observed in the groups at 1000 and 2000 mg/kg bw on days 7–16 and 7–14 of gestation, respectively. The decrease in body weight observed in these two groups ranged from only 4% to 5% compared with controls, with no dose–response relationship. No dose–response relationship was observed for any body-weight gain intervals. Food consumption was not affected during the dosing period. After the dosing period, a statistically significant increase in food consumption was observed in the groups at 300 (6.5%) and 2000 (7.3%) mg/kg bw during days 16 to 20 of gestation. However, no statistically significant effect on food consumption was observed at 1000 mg/kg bw. No treatment-related maternal necropsy findings were observed in animals sacrificed at the termination of the study. No treatment-related effects were seen on reproductive indices for controls and treated groups.

No treatment-related effects were seen in the mean number of corpora lutea, implantation sites, pre- and postimplantation loss, or early and late resorptions. There were no statistically significant differences in litter size, number of fetuses, number of implantations, mean fetal weight, or mean placental weight. Slight but statistically significant differences in the percentage of male fetuses/implantation in the group at 2000 mg/kg bw, 40.8% compared with 47.4% in the control group was observed; however, it was not considered to be treatment-related because it was within the historical control range (38.2%–56.5%) for this finding in Sprague-Dawley rats.

There were no statistically significant effects on the fetal or litter incidences of external malformations or variations at any dose. One fetus from one litter in the group at 1000 mg/kg bw had multiple malformations (exencephaly, protruding tongue, spina bifida, and a curly tail). No findings were observed in the group at 2000 mg/kg bw.

No treatment-related effects were seen on visceral malformations or variations. The incidence of malformations was independent of treatment, and included: transposition of great vessels, observed in the control group and the group at 1000 mg/kg bw; heart reduced in size, observed in the control group and the group at 1000 mg/kg bw; and anophthalmia observed in the group at 300 mg/kg bw (one fetus from one litter). The incidence of variations, which was observed in all groups, was independent of treatment, and included: left-sided umbilical artery and hydroureter (which was statistically significantly decreased at 2000 mg/kg bw). No treatment-related effects were seen in skeletal malformations or variations. The incidence of skeletal malformations was independent of treatment, and included: missing ribs; extra, fused, or missing thoracic arches; and missing lumbar arches or centra. One fetus in the group at 1000 mg/kg bw exhibited multiple malformations and variations. Fetal skeletal variations were observed in all fetuses from all groups. Skeletal variations that were statistically significantly affected included: incompletely ossified parietal bones, caudal arches, xiphoid bones, and hyoid bodies and enlarged sagittal sutures and posterior fontanelles. However, all skeletal variations, including those that were statistically significant, were either within the historical control range or did not demonstrate

a dose–response relationship, or both. No sex-related differences were observed on the incidences of external, visceral, or skeletal malformations.

The NOAEL for maternal toxicity was 2000 mg/kg bw per day, the highest dose tested. The lowest-observed-adverse-effect level (LOAEL) for maternal toxicity was not identified. No fetal toxicity or teratogenic effects were observed at doses of up to and including 2000 mg/kg bw per day. Therefore, the NOAEL for developmental toxicity was 2000 mg/kg bw per day. The study authors stated that this study was conducted to address two concerns of the Ministry of Agriculture, Fisheries, and Forestry, of Japan, following review of a previous developmental toxicity study with fenhexamid (Astroff, 1994; not submitted to JMPR for evaluation). These concerns were the potential relationship between the test compound and maternal food consumption and the incidence of fetal cranio-facial malformations observed in one litter in the original study. On the basis of the findings of the present study, which included a dose that was twofold greater than in the previous study, neither the food consumption nor the fetal effects observed in the original study were test compound-related (Young & Astroff, 1998).

Rabbits

Groups of female Russian CHBB:HM rabbits were given fenhexamid (batch No. 4258/76; purity, 95.4%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage as a suspension in aqueous 0.5% Tylose from days 6 to 18 of gestation. Animals were examined twice daily for clinical signs of toxicity and once daily on weekends and holidays. Food consumption was determined over days 0–6, 6–10, 10–14, 14–19, 19–24 and 24–29 of gestation. Body weights were determined on days 0, 6–18, and 29 of gestation. Females were sacrificed on day 29 of gestation and the ovaries were removed, corpora lutea were counted, and pregnancy was determined. The uterus was removed, weighed and opened for determination of resorptions, and placentas were weighed, the number of live and dead fetuses and the sex of each fetus was determined, and the fetus was weighed. Fetuses were removed and examined for external malformations, and visceral malformations and variations according to the modified Wilson technique. The fetuses were also evaluated for the occurrence of findings in abdominal, pelvic and thoracic organs as well as skeletal findings by the Staples technique.

Homogeneity and stability data indicated that that the compound was stable for at least 1 week at room temperature, and homogeneously distributed. The measured concentrations of the test substance indicated that the variance between nominal and actual dosage to the animals was acceptable.

There were no mortalities during the study. No treatment-related clinical signs of toxicities were observed. One doe in each of the groups receiving the intermediate or highest dose aborted on day 26 and 23 of gestation, respectively. Reduced food consumption was noted particularly within the first few days of dosing at 300 and 1000 mg/kg bw per day. There was evidence of a rebound in food consumption at the highest dose tested. Reduced water intake was noted in all groups before and during the treatment, and hence was not considered to be treatment-related. There was also a significant impairment of body-weight gain during the dosing period at 300 and 1000 mg/kg bw per day. This body-weight gain impairment started during the first week of the treatment period was compensated after the treatment was finished.

One doe at 300 mg/kg bw per day that aborted had a discoloured (light-brown) and firm liver and firm stomach contents. One doe at 1000 mg/kg bw per day that aborted had a slightly discoloured (light) liver, slightly congested gallbladder, and friable and firm fatty tissue in the abdomen. No other pathological findings were noted either in the treatment groups or controls. No treatment-related effects were noted in the fertility rate, the number of corpora lutea, preimplantation losses and implantations. Two does in the group at the highest dose had total litter resorptions. One doe at 300 and at 1000 mg/kg bw per day had abortions which affected gestation indices for these two groups. However, the abortions and resorptions seen in these groups were not considered to be treatment-related since they were within the historical range for this strain of rabbits. A treatment-related statistically significant decrease in placental weights at 300 and 1000 mg/kg bw per day (8.7% and 9.8%, respectively) and decrease in the mean body weights of

male fetuses (4.8%) at 1000 mg/kg bw per day were observed. There was no treatment-related effect on the incidence or type of malformations at doses up to and including 1000 mg/kg bw per day. A higher incidence of delayed ossifications at a few sites (e.g. fifth sternal segments, fifteenth caudal vertebrae) was observed in fetuses of the group at 1000 mg/kg bw per day. A number of fetuses showing arthrogryposis (persistent flexure or contracture of a joint) were increased at 300 and 1000 mg/kg bw per day; however, there was no dose–response relationship. External malformation (arthrogryposis) was not considered to be treatment- related because it was also observed in controls and arthrogryposis is considered as the spontaneous malformation that occurs very commonly in this strain of rabbits, which was verified in the historical control data.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of body-weight loss and reduced placental weights at the next higher dose. The NOAEL for developmental toxicity was 300 mg/kg bw per day on the basis of marginally decreased male fetal body weights and evidence of delayed ossification at 1000 mg/kg bw per day. Fenhexamid was not teratogenic in rabbits at doses of up to and including 1000 mg/kg bw per day (Kolb, 1995, 1996).

2.6 Special studies

(a) Plasma kinetics

An 8-week study of oral bioavailability was conducted in Wistar rats (Hsd/Win:WU). The objective of this study was to investigate the bioavailability of fenhexamid, since no significant toxicity was observed in a previous short-term study of toxicity in rats given fenhexamid at doses of up to 20 000 ppm that would fulfil the criteria for MTD (maximum tolerated dose). This study was designed to investigate whether there is saturation of intestinal absorption of fenhexamid given as a dietary dose in the range of 10 000 to 20 000 ppm. Groups of 10 male and 10 female rats were given diets containing fenhexamid (batch No. 4258/76; purity, 95.4%) at a dose of 0, 1000, 5000, 10 000, 15 000 or 20 000 ppm (equal to 0, 57.5, 284.7, 575.7, 943.8 or 1217.1 mg/kg bw per day for males and 0, 78.0, 407.1, 896.5, 1492.5 or 1896.7 mg/kg bw per day for females). Concentrations of fenhexamid were determined in samples of plasma and urine after a treatment period of 3 or 4 weeks, when steady-state conditions were expected. Fenhexamid containing 1% peanut oil (excipient) was mixed into the diet once per week. Animals were inspected at least twice per day for signs of toxicity and mortality and once per day on weekends and public holidays. Body weights were determined before the initiation of the study, once per week during the study and at termination on week 8. Water and food consumption for individual rats was determined once per week for 8 weeks. Urine samples were collected from the first five rats per group in week 3 (males) and week 4 (females). A second sample of urine was taken in week 5 because the measurement performed on the first sample in males revealed very low concentrations of fenhexamid. It was decided to repeat the urine analysis after an enzymatic cleavage of the conjugated compound had been performed. An enzymatic cleavage of the urinary conjugates with beta glucuronidase/arylsulfate was carried out. Blood was collected from all rats in week 3.

Stability and homogeneity data indicated that the test article was homogenously distributed in the diet and was stable in the diet for at least 1 week. The analysis of test substance concentration indicated that the variance between the nominal and actual dosage to animals was acceptable.

Two males died during the study, one in the group at 1000 ppm (week 2) and one in the group at 5000 ppm (week 3). Deaths were not considered to be caused by the treatment since no mortalities were observed at higher doses. No treatment-related clinical signs of toxicity or body-weight changes were observed during the study. Slight increases in food consumption were observed in males at doses higher than 10 000 ppm and in females at higher than 5000 ppm; however, there were no correlations with dose. The greatest differences in food consumption compared with controls were observed for males (10%) and females (20%) in the group at 15 000 ppm. Water consumption was not affected by the treatment. Food efficiency in males and females treated at the higher doses of fenhexamid was decreased in comparison to that in controls.

Plasma concentrations (without enzymatic cleavage) at week 3 were always below the detection limit (data were not presented in the report). In males and females at 15 000 ppm, maximum urinary fenhexamid concentrations were generally lower than in males at 20 000 ppm. Faecal excretion was not measured in this study probably because the major component in faeces was the parent compound. An increase in urinary excretion of conjugated fenhexamid was observed with ascending dose. From these data and considering the wide variation of urine volume, the renal elimination of conjugated fenhexamid was calculated to be approximately < 5.0% of the theoretically ingested compound (Eiben & Schmidt, 1994). Urine samples showed measurable excretion of conjugated fenhexamid indicating intestinal absorption in the dose range examined. In addition, there was a dose-related excretion of fenhexamid in both sexes indicating that the intestinal absorption was not saturated at doses of up to 20 000 ppm. Therefore, the observed absence of toxicity at doses up to and including 20 000 ppm in the short-term study of toxicity in rats is not owing to lack of bioavailability of fenhexamid.

These results are further corroborated by the results of the study of metabolism in rats in which rapid absorption and elimination of fenhexamid was observed (Anderson & Bornatsch, 1996). It should be noted that the study of metabolism in rats indicated that fenhexamid is rapidly and almost completely absorbed, conjugated with glucuronic acid, and excreted predominantly in the bile and ultimately in the faeces. Enterohepatic recirculation and a pronounced liver clearance rate observed with this compound would be anticipated to substantially reduce potential blood concentrations in the wider systemic circulation. This expectation was confirmed in rats given diets containing fenhexamid at 20 000 ppm for 56 days, where blood concentrations after 3 or 4 weeks of treatment were below the limit of detection (Eiben & Schmidt, 1994).

(b) Physiological functions

This study was conducted to evaluate the acute toxicity of fenhexamid, with a view to determining what clinical treatment might be required in the event of cases of human intoxication. Fenhexamid (lot No. 17003/94; purity, 95.8%) was suspended in 2% Cremophor EL saline. Animals used in this study were: mice: ICR strain, Crj:CD-l (SPF); rats: Sprague-Dawley strain, Crj:CD (SPF); and rabbits: Japanese native strain, Kbl:JW (SPF). Mice, rats and rabbits were dosed with fenhexamid by gavage at 0, 2500 or 5000 mg/kg bw. Various physiological parameters were assessed at various time intervals after gavage.

No notable changes were observed in the general condition of mice at 30 min, 1, 2, 4, 6 and 24 h after oral administration. In some treated mice, depressed motor activity was observed but disappeared within 30 min. No notable changes were observed in the general condition, eyes, snout and auricles of rabbits at 30 min, 1, 2, 4, 6 and 24 h after oral administration. Spontaneous movement both in vertical and horizontal directions were significantly suppressed in treated mice compared with controls. No treatment-related changes in rectal temperature were observed in rabbits. Pupillary size of rabbits did not change after oral administration. No treatment-related changes in respiration, blood pressure, heart rate or ECG were observed in rabbits after oral administration. Motor coordination and muscle force was not altered in mice due to fenhexamid treatment. No significant differences in charcoal transit time through the gastrointestinal tract were observed between treated and vehicle control mice. Urine collection at 6 h showed an increase in excretion of potassium in rats caused by the treatment; however, urinary volume, sodium and chloride excretion, and pH were not affected. Coagulation parameters prothrombin time (PT) and activated partial thromboplastin time (APTT) were not affected by fenhexamid treatment in rats. No treatment-related effects were seen in haemolysis in both in vivo and in vitro tests in rats.

The study author concluded that single oral doses of fenhexamid at high concentration have no marked effects on the general condition, behaviour, the central nervous system, the autonomic nervous system, the respiratory and circulating system, the somatic nervous system, the digestive system, renal function or the haemopoietic system. Thus fenhexamid at doses of up to 5000 mg/kg bw caused no acute toxic symptoms under the conditions of the present study (Ohara, 1996).

(c) Study of acute neurotoxicity in rats

In a study of acute neurotoxicity, 12 male and 12 female Wistar rats (Hsd Win:WU) were given a single oral dose of fenhexamid (batch No.4258/76; purity, 95.4%) at a dose of 0, 200, 630 or 2000 mg/kg bw by gavage in 2% Cremophor EL in demineralized water (10 ml/kg bw). The rats were observed for 14 days. Functional observation battery and motor activity testing were performed 7 days before dosing, approximately 20 min to 3 h after dosing, and on days 7 and 14. Motor activity was measured by testing animals individually for 70 min in ten figure-eight mazes. The figure-eight maze was selected as an established and widely-used automated activity measuring device that can be used to detect increases and decreases in activity. Each maze consisted of a series of inter-connected alleys (approximately 10×10 cm in cross-section) converging on a central arena and covered by transparent acrylic plastic.

A non-GLP study was performed to measure the concentration of fenhexamid in plasma of fasted rats to determine the time of peak plasma concentration, in order to determine a suitable time to begin behavioural testing on the day of dosing. Two groups of three male Wistar rats were orally dosed with fenhexamid by gavage at 2000 mg/kg bw. Blood samples were collected from the orbital plexus at 20 min, 40 min and 1 h (group 1) and 2 h, 4 h and 8 h (group 2). The results indicated that the peak plasma concentration was reached 20 min after dosing and lasted for approximately 1–2 h.

In the study of neurotoxicity, animals were inspected twice daily and once during the weekend and holidays for mortality and signs of toxicity. Detailed physical examinations were performed daily. Animals were weighed once before the test, on day 0 and weekly during the study and at termination. Food consumption was not measured. All animals were subjected to a complete gross necropsy on day 15 or 16 after dosing. Six rats of each sex per dose were anaesthetized and perfused via the left ventricle with a fixative in phosphate buffer. The brain was removed and weighed. Tissues from controls and animals at the highest dose and all tissues were microscopically examined.

Homogeneity and stability analysis indicated that the test substance was homogeneous and stable for 3 days under the test conditions. Analysis of the dosing solutions indicated that test concentrations corresponded to the nominal concentrations. The actual measured doses were 197, 619 and 1903 mg/kg for the nominal doses of 200, 630 and 2000 mg/kg bw, respectively.

No mortality or treatment-related clinical signs of toxicity were observed in the study. No treatment-related effects were observed on body weight during the study. No treatment-related effects were noted in the functional observation battery parameters in males in the groups at 200 and 630 mg/kg bw or in any of the groups of dosed females. The only effect which was treatment-related was a marginal decrease of body temperature on day 0 in the males treated at 2000 mg/kg bw (38.5, 38.5, 38.4, and 38.2 °C, for 0, 200, 630 and 2000 mg/kg bw, respectively). Observations considered to be incidental and not related to treatment included a slight decrease in the incidence of rearing movements in the open field on day 0 in males at 2000 mg/kg bw (−16%) and 630 and 2000 mg/kg females (−10%). These minimal and non-statistically significant observations were not treatment-related since they fell within the range of normal variability. Grip strength and foot splay were not affected in either sex at any dose. There were no treatment-related effects on measures of motor activity, locomotor activity or habituation.

There were no treatment-related macroscopic morphologic abnormalities observed at any dose. The statistically significant decrease in absolute brain weight in the males at 2000 mg/kg bw (1.73 g versus 1.83 g controls; 5.5% decrease compared with controls) is not considered to be treatment-related because a corresponding statistically significant decrease in relative brain weight was not observed and a similar decrease was not observed in females. There were no treatment-related microscopic lesions at 2000 mg/kg bw and therefore the tissues from animals in the groups at 200 and 630 mg/kg bw were not examined.

Decreased body temperature may be a sign of acute general systemic toxicity or may possibly be caused by a central nervous system-mediated (neurotoxic) effect of the test substance

on the brain, since the brain controls temperature regulation in the body. Since the decrease in body temperature was marginal at most and only seen on day 0 at the highest dose, this effect was not considered to be a toxicologically significant neurotoxic effect. Therefore, the NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested (Dreist & Popp, 1996).

2.7 Studies with metabolites

No studies of toxicity with metabolites were available. A review by Diesing & Brauner (2004) states that no studies have been performed because plant metabolites are identical to metabolites formed in animals.

3. Observations in humans

No reports on adverse health effects from employees handling fenhexamid in large-scale production or operators handling large volume are available. No epidemiological studies are available on health effects of fenhexamid in the general population. No specific antidote therapy is available or required for the treatment of poisoning (Diesing & Brauner, 2004).

Comments

Biochemical aspects

In toxicokinetic studies in rats given single doses (1.0 or 100 mg/kg bw) or repeated doses (1.0 mg/kg bw per day for 14 days) by gavage, radiolabelled fenhexamid was rapidly and completely absorbed from the gastrointestinal tract (> 97%). A peak in plasma concentrations of radioactivity was observed 5–10 min after dosing at 1.0 mg/kg bw. Approximately 96% of the administered dose was eliminated in excreta within 48 h; the major route of excretion was in the faeces (62–81% of the administered dose) with 15–36% of the administered dose being recovered in the urine. Approximately 60% of the administered dose was excreted in bile in the first hour and > 97% within 48 h, primarily as the glucuronide conjugate of the parent compound. A pronounced first-pass effect and extensive enterohepatic circulation was observed with hydrolysis of the glucuronide in the gastrointestinal tract and reabsorption of the parent compound. Only 0.3% of the administered dose was detected in the body at 72 h, with the gastrointestinal tract, kidney and liver having the highest concentrations of radioactivity. The main pathway of biotransformation in rats was conjugation of the aromatic hydroxyl group with glucuronic acid. Limited hydroxylation of the 2, 3 and 4 positions of the cyclohexyl ring also occurred with excretion of these compounds as glucuronide or sulfate conjugates. The main compound detected in excreta was the unchanged parent compound (62–75% of the administered dose). The glucuronic acid conjugate of the parent ranged from about 4% to 23% of the administered dose. Excretion, distribution and metabolite profiles were essentially independent of dose, pre-treatment and sex.

Toxicological data

Fenhexamid has low toxicity when administered by the oral, dermal or inhalation routes. LD_{50} values after oral administration were > 5000 mg/kg bw in rats and mice. The LD_{50} in rats treated dermally was > 5000 mg/kg bw. LC_{50}s in rats treated by inhalation (nose only) was > 0.32 mg/l (aerosol) and > 5.1 mg/l (dust). Fenhexamid was not a skin or eye irritant. Fenhexamid was not a skin sensitizer in guinea-pigs (Buehler test) or in the local lymph node assay, and showed equivocal skin sensitizing potential in a Magnusson & Kligmann (maximization) test in guinea-pigs.

In short-term studies in mice, rats and dogs, very high doses of fenhexamid produced minimal systemic toxicity. In longer-term studies, the major target organ was the kidney in rats

and mice and the haematopoietic system in dogs. Slight evidence of liver toxicity was also observed in rats, mice and dogs.

No systemic toxicity was seen in a 28-day study in rats given fenhexamid at doses of up to 1000 mg/kg bw per day by gavage. A 28-day dietary study in dogs given fenhexamid at doses of up to 20 000 ppm (equivalent to 500 mg/kg bw per day) did not produce systemic toxicity.

In a 90-day dietary study of toxicity in mice, increased cholesterol, bilirubin, creatinine, water and food consumption, decreased kidney weights, increased renal protein casts and cellular detritus, and renal tubular basophilia were observed at 10 000 ppm (equal to 3283 mg/kg bw per day). In a second study, similar toxicity in the kidney was observed at the highest dose of 20 000 ppm (equal to 3417 mg/kg bw per day). The lowest NOAEL in these two studies was 1000 ppm (equal to 266.5 mg/kg bw per day).

In a 90-day dietary study of toxicity in rats, decreased body weight and body-weight gains, increased food consumption, reduced food conversion efficiency and decreased liver weights in males (reversible within 4 weeks) were observed at 10 000 ppm (equal to 904 mg/kg bw per day). In females, these findings, plus an increased incidence of mild to moderate focal Kupffer cell proliferation in females, were observed at 20 000 ppm (equal to 2824 mg/kg bw per day). The NOAEL was 5000 ppm (equal to 415 mg/kg bw per day). In a second 90-day dietary study in rats, nephropathy was seen at 50 000 ppm (equal to 5585 mg/kg bw per day). The NOAEL in this study was 5000 ppm (equal to 404 mg/kg bw per day).

In a 90-day study of toxicity in dogs, increases in the number of Heinz bodies were seen at 7000 ppm (equal to 239 mg/kg bw per day) and increases in ALP activity were measured at the highest dose of 50 000 ppm (equal to 1748 mg/kg bw per day). The NOAEL was 1000 ppm (equal to 33.9 mg/kg bw per day). In a 52-week study of toxicity in dogs, increases in the number of Heinz bodies and decreases in erythrocyte count, concentration of haemoglobin, and erythrocyte volume fraction were seen at 3500 ppm (equal to 124 mg/kg bw per day) with increases in ALP activity, adrenal weights and intracytoplasmic vacuoles in females at the highest dose of 25 000 ppm (equal to 918 mg/kg bw per day). The NOAEL was 500 ppm (equal to 17.4 mg/kg bw per day).

No systemic toxicity was seen in a 28-day study of dermal toxicity in rats at 1000 mg/kg bw per day, the highest dose tested. Five-day and 28-day studies of toxicity suggest that high doses administered by inhalation were well tolerated by rats. The NOAEC in the 28-day study was 0.069 mg/L on the basis of an increase in lung weights, grey discolouration of lungs, pigment-laden alveolar macrophages and an increase in liver enzymes seen at the lowest-observed-adverse-effect concentration (LOAEC) of 0.487 mg/l.

Fenhexamid gave negative results with or without metabolic activation in an adequate range of studies of genotoxicity in bacteria and cultured mammalian cells in vitro, and in a test for micronucleus formation in mice in vivo.

The Meeting concluded that fenhexamid is unlikely to be genotoxic.

In long-term studies of toxicity and carcinogenicity in mice and rats, there were no treatment-related neoplastic findings. In male mice, decreased kidney weights were observed at 2400 ppm (equal to 807 mg/kg bw per day). Additional effects observed at the highest dose of 7000 ppm (2355 mg/kg bw per day) in males included increased water consumption, increased serum concentrations of creatinine, bilirubin and albumin, decreased body weight, decreased body-weight gain. In females at 7000 ppm (equal to 3178 mg/kg bw per day, the highest dose tested), increased water consumption, decreased kidney weights and increased basophilic cortical tubules in the kidney were observed. The NOAEL for systemic toxicity in mice was 800 ppm (equal to 247 mg/kg bw per day). In rats, only mild treatment-related effects such as increased splenic extramedullary haematopoiesis, increased caecal mucosal hyperplasia, decreased body weights, decreased body-weight gains, decreased food conversion efficiency, and bone marrow hyperplasia were observed. The NOAEL for systemic toxicity was 500 ppm (equal to 28 mg/kg bw per day). Fenhexamid was not carcinogenic in mice or rats.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that fenhexamid is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproduction in rats, reproductive parameters were not affected at the highest dose tested (20 000 ppm, equal to 1814 mg/kg bw per day). The NOAEL for parental systemic toxicity was 500 ppm (equal to 38 mg/kg bw per day) on the basis of lower pre-mating weights, increases in ALP activity and decreases in liver weights and kidney weights in males only. The NOAEL for offspring toxicity was 500 ppm (equal to 38 mg/kg bw per day) on the basis of decreases in body weights during lactation. Fenhexamid was not teratogenic at doses of up to 2000 and 1000 mg/kg bw per day in rats and rabbits, respectively. No systemic toxicity, embryotoxicity or fetotoxicity was observed in the study of developmental toxicity in rats at doses of up to 2000 mg/kg bw per day. At the highest dose tested in rabbits (1000 mg/kg bw per day), a slight decrease in fetal weight of males and delayed ossification (fifth sternal segments, fifteenth caudal vertebrae) was observed in the presence of maternal toxicity. The NOAEL for developmental toxicity in rabbits was 300 mg/kg bw per day.

The Meeting concluded that fenhexamid is not teratogenic nor a reproductive toxicant.

In a study of acute neurotoxicity in rats, doses of up to 2000 mg/kg bw did not produce any systemic toxicity, neurotoxicity or neuropathology findings. There were no treatment-related effects on measures of motor activity, locomotor activity or habituation.

In a study evaluating clinical parameters and physiological functions in rats, mice, and rabbits given fenhexamid as single doses at up to 5000 mg/kg bw by gavage, fenhexamid did not produce marked effects on general condition, behaviour, the nervous system, the respiratory system, the circulatory system, haematopoietic parameters or renal function.

The Meeting concluded that the metabolites of fenhexamid are likely to be less toxic than fenhexamid because the major metabolites are polar glucuronide or sulfate conjugates that are rapidly excreted. Hydrolysis of the glucuronic acid conjugate of the parent can occur in the gastrointestinal tract, with subsequent reabsorption of the parent.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw on the basis of a NOAEL of 17.4 mg/kg bw per day for increased adrenal weight and the presence of intracytoplasmic vacuoles in the adrenal cortex of females and haematopoietic effects (increase in the number of Heinz bodies, decrease erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) seen at higher doses in both sexes in a 52-week study in dogs fed with fenhexamid, and a 100-fold safety factor.

The Meeting concluded that the establishment of an ARfD for fenhexamid was not necessary on the basis of its low acute toxicity, the absence of development toxicity in rats and rabbits, the lack of neurotoxicity after single exposures, and the absence of any other toxicological end-point that would be elicited by a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	104-week study of toxicity and carcinogenicity[a]	Toxicity	800 ppm, equal to 247 mg/kg bw per day	2400 ppm, equal to 807 mg/kg bw per day
		Carcinogenicity	7000 ppm, equal to 2355 mg/kg bw per day[c]	—

Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 28 mg/kg bw per day	5000 ppm, equal to 292 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 1280 mg/kg bw per day[c]	—
	Multigeneration study[a]	Parental toxicity/offspring toxicity	500 ppm, equal to 38 mg/kg bw per day	5000 ppm, equal to 406 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	2000 mg/kg bw per day[c]	—
		Embryo- and fetotoxicity	2000 mg/kg bw per day[c]	—
	Acute neurotoxicity[b]	Neurotoxicity	2000 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw per day	1000 mg/kg bw per day
Dog	1-year study [a]	Toxicity	500 ppm, equal to 17.4 mg/kg bw per day	3500 ppm, equal to 124 mg/kg bw per day

[a] Dietary administration

[b] Gavage administration

[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion, and metabolism in mammals	
Rate and extent of oral absorption	Rapid; maximum reached in blood by 5–10 min; later at higher doses. About 97% absorbed within 48 h
Distribution	Extensive enterohepatic recirculation; highest concentrations in gastrointestinal tract, liver, and kidney
Potential for accumulation	No evidence of significant accumulation; about 0.3% of the total administered dose found in tissues after 72 h
Rate and extent of excretion	Excretion was rapid; approximately 96% excreted in urine (15–36%) and faeces (62–81%) within 48 h.
Metabolism in animals	Extensive; metabolic pathways include conjugation of the aromatic hydroxyl group with glucuronic acid and sulfate.

	Hydroxylation of the cyclohexyl ring on positions 2, 3 and 4 also occurred. Unchanged fenhexamid in faeces (49–69% of the administered dose).
Toxicologically significant compounds (animals, plants and environment)	Fenhexamid and its glucuronide conjugate
Acute toxicity	
Rat LD_{50} oral	> 5000 mg/kg bw
Rat LD_{50} dermal	> 5000 mg/kg bw
Rat LC_{50} inhalation	> 0.32 mg/l (aerosol) and > 5.1 mg/l (dust) (4-h exposure, nose only)
Rabbit, dermal irritation	Not an irritant
Rabbit, eye irritation	Not an irritant
Skin sensitization (test method used)	Not a skin sensitizer in guinea-pigs (maximization test, Buehler test and local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Haematopoietic system/increase in Heinz bodies and adrenal effects
Lowest relevant oral NOAEL	17.4 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEL	0.069 mg/l (6 h/day for 5 days per week for 4 weeks; in rats)
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Decreases in body weights, body-weight gains, food consumption and food conversion efficiency, increases in cellularity of bone marrow and the presence of splenic extramedullary haematopoiesis
Lowest relevant NOAEL	28 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	No evidence of neurotoxicity at doses of up to 2000 mg/kg bw (rats)
Other toxicological studies	
Physiological functions	No acute effects after single doses at up to 5000 mg/kg bw in mice, rats and rabbits.
Medical data	
	Limited data; no adverse health effects reported

Summary	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw	Dog, 1-year study of toxicity	100
ARfD	Unnecessary	—	—

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