Document Title

Tier 2 Summary
of Toxicological and Toxicokinetic Studies
of the active substance Fenhexamid (KBR 2738)

(Specification No.: 102000006806)

Substance(s)

FENHEXAMID
(Annex I renewal)

Data Requirements

Regulation EC/1141/2010
on the renewal of the inclusion of AIR2 active substances

in conjunction with

Directive 91/414/EEC and **Regulation EC/1107/2009**

According to OECD format guidance for industry data submissions
(SANCO/10387/2010 rev. 8 - on the renewal of active substances included in Annex I)

Annex II
Document M
Section 3, Point 5

Date

2012-02-03

Author(s)

[redacted]

Bayer CropScience

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer CropScience. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer CropScience.

TABLE OF CONTENTS

IIA 5 Toxicological and Toxicokinetic Studies on the Active Substance

Comments with respect to the Annex I renewal process

This delta dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of fenhexamid and were therefore not evaluated during the first EU review of this compound. The summaries on the different toxicological endpoints from the baseline dossier were supplemented and adapted with the new information. In order to facilitate discrimination between new information and original paragraphs, the new information is written in bold italic letters. All other studies, which were already submitted by Bayer for the first Annex I inclusion, are contained in the Monograph and in the baseline dossier provided by Bayer CropScience.

A synonymous name for fenhexamid used at several locations in this delta dossier is KBR 2738.

The following table provides an overview on the batches of fenhexamid used in all toxicological studies on this compound. Studies not evaluated during the first EU review are written in bold italic letters.

Table 5-1: Fenhexamid batches used in the toxicological studies belonging to this Annex II

Annex Point	Report No.	Study	Fenhexamid – Batch No.	Purity [%]
KIIA 5.2.1	20640	KBR 2738 - Study for acute oral toxicity in rats	17002/90	95.5
KIIA 5.2.1	20638	KBR 2738 – Study for acute oral toxicity in mice	17002/90	95.5
KIIA 5.2.2	20639	KBR 2738 - Study for acute dermal toxicity in the rat	17002/90	95.5
KIIA 5.2.3	20369	KBR 2738 - Studies of the acute inhalation toxicity in rats	17002/90	95.5
KIIA 5.2.4 KIIA 5.2.5	19884	KBR 2738 - Study for skin and eye irritation / corrosion in rabbits	17002/90	95.5
KIIA 5.2.6	20973	KBR 2738 - Studies on skin sensitizing effect in guinea pigs (Buehler Test)	17002/90	97.5
KIIA 5.2.6	25538	KBR 2738 - Studies for the skin sensitiziation effect in guinea pigs (Guinea pig maximization tests according Magnusson and Kligman)	study 1: 4258/76* study 2: 17003/94	94.6 96.3
KIIA 5.2.6	***29748***	***KBR 2738 - Local lymph node assay in mice (LLNA/IMDS)***	***H0003***	***98.8***
KIIA 5.2.6	***R7847***	***Examination of Fenhexamid (KBR 2738) in the skin sensitisation test in guinea pigs according to Magnusson and Kligman (maximisation test)***	***H0003***	***98.8***
KIIA 5.3.1	23160	KBR 2738 - Subactute oral toxicity study on Wistar rats (administration per gavage over 28 days)	17002/90	97.8
KIIA 5.3.1	R5319	Safety evaluation of KBR 2738: Four-week dietary toxicity study in dogs	17002/90	95.5
KIIA 5.3.2	23579	KBR 2738 - Investigations of subchronic toxicity in Wistar rats (feeding study over 13 weeks with a subsequent recovery period over 4 weeks)	4258/76 *	95.4
KIIA 5.3.2	***28583***	***KBR 2738 - Study for subchronic oral toxicity in rats (feeding study over 13 weeks)***	***898812004***	***97.5***

* : mixed batch

Annex Point	Report No.	Study	Fenhexamid – Batch No.	Purity [%]
KIIA 5.3.2	22332	KBR 2738 - Range-finding subchronic toxicological investigation for a 2-year feeding study with B6C3F1 mice (administered in feed over approx. 14 weeks)	17002/90	97.8-97.5
KIIA 5.3.2	***28580***	***KBR 2738 - Study for subchronic oral toxicity in mice (feeding study over 13 weeks)***	***898812004***	***97.5***
KIIA 5.3.3	23979	Subchronic toxicity study in Beagle dogs (13 week feeding)	4258/76 *	95.4
KIIA 5.3.4	25618	KBR 2738 - Chronic toxicity study in beagle dogs (52 week feeding study)	4258/76 *	94.6-95.8
KIIA 5.3.5	20224	KBR 2738 - Preliminary investigations for a subacute inhalation toxicity study in the rat (5 x 6h exposures)	17002/90	97.8
KIIA 5.3.5	25489	KBR 2738 (Fenhexamid) - Study on subacute inhalation toxicity in rats exposure: 5x6 hrs/week for 4 weeks) according to OECD protocol 412	4258/76 *	95.4
KIIA 5.3.7	23715	KBR 2738 - Subacute dermal toxicity study on rabbits	4258/76 *	95.4
KIIA 5.4.1	20307	KWG 4168 - Salmonella/microsome test	17002/90	95.5
KIIA 5.4.1	***RA95006***	***KBR 2738 - Reverse mutation assay (Salmonella typhimurium and Escherichia coli)***	***4258/76 ****	***95.8***
KIIA 5.4.1	***NR96660***	***KBR 2738 - DNA repair test in bacterial system***	***17003/94***	***96.1***
KIIA 5.4.2	24405	KBR 2738 - In vitro mammalian chromosome aberration test with Chinese hamster ovary (CHO) cells	4258/76 *	95.4
KIIA 5.4.3	23529	KBR 2738 - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro	4258/76 *	95.4
KIIA 5.4.3	21312	KBR 2738 - Mutagenicity test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro	17002/90	95.5
KIIA 5.4.4	22625	KBR 2738 - Micronucleus test on the mouse	4258/76 *	95.4
KIIA 5.5.1 KIIA 5.5.2	25522	KBR 2738 - Study on chronic toxicity and carcinogenicity in Wistar rats (administration in the diet over 2 years)	4258/76 *	94.6-95.8
KIIA 5.5.3	25523	KBR 2738 - Oncogenicity study in B6C3F1 mice (administration in diet over 2 years)	4258/76 *	94.6-95.8
KIIA 5.6.1	BC8008	A two-generation dietary reproduction study in rats using technical grade KBR 2738	4258/76 *	93.8-95.2
KIIA 5.6.10	***BC7438***	***A developmental toxicity study with KBR 2738 technical in the Sprague-Dawley rat***	4258/76 * ***898805001***	95.4 ***97.7***
KIIA 5.6.11	23733	KBR 2738 - Developmental toxicity study in rabbits after oral administration	4258/76 *	95.4
KIIA 5.7.1	24745	KBR 2738 - Acute oral neurotoxicity screening in Wistar rats	4258/76 *	95.4
KIIA 5.10	20642	KBR 2738 - Study on acute intraperitoneal toxicity in rats	17002/90	95.5
KIIA 5.10	23578	KBR 2738 - Determination of the test substance in plasma and urine after subchronic feeding to Wistar rats over 8 weeks	4258/76 *	95.4

* : mixed batch

IIA 5.1 Absorption, distribution, excretion and metabolism in mammals

A rat metabolism study conducted with [phenyl-UL-14C]Fenhexamid was submitted with the original EU dossier and was evaluated during the Annex I listing. Since the metabolisation and excretion of fenhexamid is well known, no additional rat metabolism studies were considered necessary to be conducted. No cleavage products were found in the rat and the total identification rate was high. No further rat metabolism study was considered necessary because all metabolites were and would be detected unchanged with the cyclohexyl label.

The metabolic pathway was similar in rat and goat, with no accumulation observed in fat, meat, and milk. No species-specific metabolites were found.

IIA 5.1.1 Toxicokintic studies - Single dose, oral route, in rats

No additional toxicokinetic studies than those presented and evaluated during the EU process for Annex I listing, were necessary. Please refer to the statement above (IIA 5.1).

IIA 5.1.2 Toxicokinetic studies - Second single dose, oral route, in rats

No additional toxicokinetic studies than those presented and evaluated during the EU process for Annex I listing, were necessary. Please refer to the statement above (IIA 5.1).

IIA 5.1.3 Toxicokinetic studies - Repeated dose, oral route, in rats

No additional toxicokinetic studies than those presented and evaluated during the EU process for Annex I listing, were necessary. Please refer to the statement above (IIA 5.1).

IIA 5.2 Acute toxicity

Summary of acute toxicity, irritation and sensitisation studies

Table 5.2-1: Summary of acute toxicity, irritation and sensitisation studies

Species	Sex	Vehicle	NSD/NSC [mg/kgbw]	LLD/LLC [mg/kg bw]	LD/LC_{50} [mg/kg bw]	Reference
Acute oral toxicity						
rat	male female	water/ cremophor 2%v/v	5000 5000	> 5000 > 5000	> 5000 > 5000	[redacted], 1991; 20640
mouse	male female	water/ cremophor 2%v/v	2500 2500	> 5000 > 5000	> 5000 > 5000	[redacted], 1991; 20638
Acute percutaneous toxicity						
rat	male female	NaCl solution	5000 5000	> 5000 > 5000	> 5000 > 5000	[redacted], 1991; 20639

Acute inhalation toxicity						
rat	male female	polyethylene-glycol E 400 / ethanol (1:1)	5057 5057	> 5057 > 5057	> 5057 > 5057	[redacted]; 1991; 20369

NSD/NSC: no-symptoms dose/concentration; LLC/LLD: lowest lethal dose/concentration

Species	**Sex**		**Vehicle**	**Result**	**Reference**
Irritation					
rabbit	female	skin eye	moistened with water none	not irritating not irritating	[redacted], 1996; 19884
Sensitisation					
guinea pig	male	Buehler test	Cremophor® EL (2%v/v) / phys. saline solution	not sensitising	[redacted], 1996; 20973
guinea pig	male	maximisation test	Cremophor® EL (2%v/v) / phys.saline solution	not sensitising	[redacted], 1997; 25538
guinea pig	***male***	***maximisation test***	***sesame oil***	***not sensitising***	[redacted], ***2000; R7847***
mouse	***female***	***LLNA-IMDS***	***DAE 433 (mixture of 40% dimethylacetamide, 30% acetone and 30% ethanol***	***not sensitising***	[redacted], ***2000; 29748***

Fenhexamid has no significant acute toxicity after oral, dermal and inhalatory administration. The compound is neither a skin nor an eye irritant. No skin-sensitising potential was found in ***three*** different test systems.

The additionally conducted tests, a guinea pig maximisation test and a local lymph node assay in mice, confirmed the results of the former studies (a Buehler test and a guinea pig maximisation test) that fenhexamid is no skin sensitiser. In the newly conducted guinea pig maximisation test a concentration of 10% fenhexamid did not cause any skin effects, neither at dose range finding nor at challenge, while a concentration of 15% already caused skin irritation at dose range finding. These findings are in line with the results of the first maximisation test, in which a concentration of 12% fenhexamid caused skin irritation (in 1 of 4 or 3 of 4 guinea pigs in the preliminary studies as well as in 6 of 10 animals at challenge) and a concentration of 6% fenhexamid was neither irritating nor sensitising. In the new local lymph node assay fenhexamid was also not sensitising up to the highest concentration of 30%. In conclusion, the data show that fenhexamid has no skin sensitising properties.

IIA 5.2.1 Acute oral toxicity

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.2.2 Acute percutaneous toxicity

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.2.3 Acute inhalation toxicity

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.2.4 Skin irritation

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.2.5 Eye irritation

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.2.6 Skin sensitization

In addition to the two studies on skin sensitization already available in the Monograph and baseline dossier a new guinea pig maximization test and a local lymph node assay were conducted in 1999 in order to support non-classification of fenhexamid regarding skin sensitisation at ECB.

Report:	**KIIA 5.2.6/03, [redacted] 2000, (amended 2000)**
Title:	Examination of Fenhexamid (KBR 2738) in the skin sensitisation test in guinea pigs according to Magnusson and Kligman (maximisation test)
Report No.: Document No.:	R7847 M-043503-02-1
Dates of work:	1999-11-17 to 1999-12-27
Guidelines:	EC guideline B.6; OECD 406 Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: Fenhexamid (KBR 2738)

Article no.:	05386861
Description:	light-brown powder
Lot/Batch no:	H0003
Purity:	98.8%
Stability of test compound:	guaranteed for study duration; expiry date: 2000-03-30

2. Vehicle: sesame oil (DAB)

3. Test animals:

Species:	guinea pig
Strain:	Dunkin-Hartley
Age:	52 days
Weight at dosing:	313 g – 374 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	Altromin 3022 ([redacted], Germany), ad libitum
Water:	tap water, ad libitum
Housing:	in MAKROLON type IV cages, 2 animals/cage bedding: granulated textured wood ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment:

Dose:		
Intradermal induction:	5% fenhexamid	
Topical induction:	80% fenhexamid	
Challenge:	10% fenhexamid	
Application route:	intradermal, dermal	
Application volume:	intradermal induction:	0.1 mL/injection
	topical induction, challenge:	2 mL/patch
Duration:	topical induction: 48 hours, challenge: 24 hours	
Group size:	33 males (control: 5, test item: 10, range-finding: 18)	
Observations:	mortality, clinical signs, skin effects, body weight (at beginning and termination of study)	

II. Results and discussion

A. Findings

Preliminary investigations revealed no skin irritation after topical application (with depilation) of a 5 or 10% suspension of fenhexamid in sesame oil, while an erythema grade 1 (discrete or patchy erythema) was observed after application of a concentration of 15% and higher concentrations.

In the main study, after intradermal induction as well as after the topical induction all vehicle control

as well as all fenhexamid treated animals exhibited an erythema grade 1 (discrete or patchy erythema) to 2 (moderate and confluent erythema).
The challenge with 10% fenhexamid revealed no sensitising properties of fenhexamid to the depilated skin.
The vehicle control animals treated with sesame oil in the same way during the inductions and with fenhexamid for challenge exhibited also no skin reactions.
There was no mortality and the behaviour of all animals remained unchanged during the study.
The body weight gain of the animals treated with fenhexamid was within the range of the vehicle control.

Table 5.2.6-1: Number of animals exhibiting skin effects

Animal No.	intradermal induction		topical induction		challenge					
	Hours after start of treatment									
	shoulder				flanks					
	25	48	49	72	24		48		72	
					left	right	left	right	left	right
vehicle control										
1	1	2	2	2	0	0	0	0	0	0
2	1	1	1	1	0	0	0	0	0	0
3	1	2	2	2	0	0	0	0	0	0
4	1	2	2	2	0	0	0	0	0	0
5	1	2	2	2	0	0	0	0	0	0
fenhexamid										
6	1	2	1	2	0	0	0	0	0	0
7	1	1	2	2	0	0	0	0	0	0
8	1	2	2	1	0	0	0	0	0	0
9	1	2	2	2	0	0	0	0	0	0
10	1	2	2	2	0	0	0	0	0	0
11	1	2	2	1	0	0	0	0	0	0
12	1	2	2	2	0	0	0	0	0	0
13	1	2	2	2	0	0	0	0	0	0
14	1	2	2	2	0	0	0	0	0	0
15	1	1	2	2	0	0	0	0	0	0

III. Conclusion

Fenhexamid revealed no sensitising properties in the maximisation test according to [redacted].

Classification/labelling according to Commission Directive 1999/45/EEC as amended:

none

Report:	**KIIA 5.2.6/04; [redacted]., 2000**
Title:	KBR 2738 - Local lymph node assay in mice (LLNA/IMDS)
Report No.:	29748
Document No.:	**M-030058-01-1**
Dates of work:	1999-11-29 to 1999-12-02
Guidelines:	OECD 406; US-EPA OPPTS 870.2600 (draft); guideline draft the Local Lymph Node Assay (LLNA)

	Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: KBR 2738

- Article no.: 05386861
- Description: light brown powder
- Lot/Batch no: H0003
- Purity: 98.8%
- Stability of test compound: guaranteed for study duration; expiry date: 2000-03-30

2. Vehicle: DAE 433 formulation, a mixture of 40% dimethylacetamide, 30% acetone and30% ethanol

3. Test animals:

- Species: NMRI mouse
- Strain: Hsd Win:NMRI
- Age: not stated
- Weight at dosing: not stated
- Source: [redacted], Germany
- Acclimatisation period: at least 5 days
- Diet: Altromin™ 1324 maintenance diet for rats and mice ([redacted] Germany), ad libitum
- Water: tap water, ad libitum
- Housing: adaptation period: in Makrolon™ type III cages, up to 10 mice per cage; study period: in Makrolon™ type II cages, up to 6 mice per cage. bedding: low-dust wood granulate type S 8/15 ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment:

- Dose: 0%-3%-10%-30%
- Application route: epicutaneously onto the dorsal part of both ears
- Application volume: 25 µL/ear
- Duration: three consecutive days (d0, d1 and d2)
- Group size: 6 females/group
- Observations: ear swelling, ear weight, local lymph node weight and cell count determination

II. Results and discussion

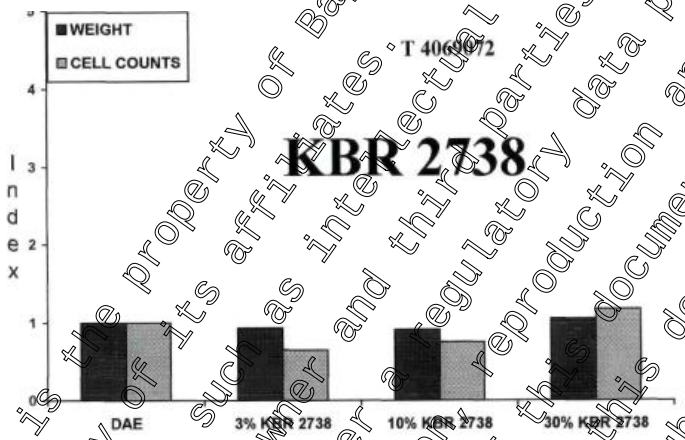
A. Findings

Stimulation indices (local lymph node weight and cell count)

The NMRI mice did not show any significant dose-dependent increase in the stimulation indices for

the local lymph node weight or cell counts (Appendix 11.1) or ear swelling or ear weights. Also the statistical analysis revealed no significant effect for the test compound fenhexamid.
These results in mice are corroborated by the findings in the FACScan analyses.
FACScan analyses
All analyses of T-cell surface markers and B-cell (B220) and macrophage activation markers (I-A) did not reveal any substance-related changes after treatment with fenhexamid.
All differences measured were not dose-dependent, nor did they exceed the normal variance of the marker, and were thus not significant or of biological relevance.

Figure 5.2.6-1 Stimulation indices of local lymph node weight and cell counts after daily topical application of 0, 3, 10 and 30% fenhexamid for 3 consecutive days to the ears of mice



III. Conclusion

Fenhexamid has no skin sensitizing potential in the local lymph node assay in mice. Moreover, no hint for a substance specific or non-specific activation of the cells of the immune system *via* dermal application was found in this study.

Classification/labelling according to Commission Directive 1999/45/EEC as amended:

none

IIA 5.2.7 Potentiation/interactions of multiple active substances or products

No EC data requirement. The OECD point concerned is not covered by or part of an EC point according to Council Directive 91/414/EEC or Regulation 1107/2009.

IIA 5.3 Short-term toxicity

Summary of short-term toxicity studies

Table 5.3-1: Summary of short-term toxicity studies

Type of study	Species	Dose range tested	NOEL	Reference
oral, 4 weeks, gavage	rat	0-100-300-1000 mg/kg bw/day	1000 mg/kg bw/day	[redacted], 1994, ***amended 1996***; 23560
oral, 13 weeks, feed	rat	0-2500-5000-10000-20000 ppm ***(M/F: 0/0-202/270-415/549-904/1132-1904/2824 mg/kg bw/day)***	5000 ppm ***(M/F: 415/549)***	[redacted], 1994, ***amended 1997***; 23579
oral, 13 weeks, feed	***rat***	***0-500-5000-50000 ppm (M/F: 0/0-38.0/47.4-403.9/552.8-5585.1 /8100.8 mg/kg bw/day)***	***500 ppm (M/F: 38.0/47.4 mg/kg bw/day)***	[redacted], ***1999; 28583***
oral, 14 weeks, feed	mouse	0-100-1000-10000 ppm ***(M/F: 0/0-26.52-267/454-3284/5151 mg/kg bw/day)***	1000 ppm ***(M/F: 267/454 mg/kg bw/day)***	[redacted], 1993; 22332
oral, 13 weeks, feed	***mouse***	***0-200-2000-20000 ppm (M/F: 0/0-32.5/54.8-322.9/573.7-3416.8/6145.4 mg/kg bw/day)***	***2000 ppm (M/F: 322.9/573.7 mg/kg bw/day)***	[redacted], ***1999; 28580***
oral, 4 weeks, feed	dog	0-30-400-3000-20000 ppm ***(M+F combined: approx. 0-1.25-10-75-500 mg/kg bw/day)***	20000 ppm ***(M+F combined: approx. 500 mg/kg bw/day)***	[redacted], 1991; R5319
oral, 13 weeks, feed	dog	0-1000-7000-50000 ppm ***(M/F: 0/0-33.9/37.0-239.1/261.0-1747.7/ 1866.2 mg/kg bw/day)***	1000 ppm ***(M/F: 33.9/37.0 mg/kg bw/day, M+F combined: 35.5 mg/kg bw/day)***	[redacted], 1995, ***amended 1996 & 1997***; 23979
oral, 52 weeks, feed	dog	0-500-3500-25000 ppm ***(M/F: 0/0-33.9/37.0-239.1/261.0-1747.7/ 1866.2 mg/kg bw/day)***	500 ppm ***(M/F: 17.4/19.2 mg/kg bw/day, M+F combined: 18.3 mg/kg bw/day)***	[redacted]., 1996, ***amended 1997***, 25618
dermal, 3 weeks	rabbit	0-1000 mg/kg bw/day	1000 mg/kg bw/day	[redacted]., 1995; 23715
inhalation, 1 week	rat	0-11.8-97.7-1092.6 mg/m³ air	97.7 mg/m³ air	[redacted], 1991, ***amended 1996***: 20224
inhalation, 4 weeks	rat	0-10.2-68.7-486.7 mg/m³air	68.7 mg/m³ air	[redacted], 1996, ***amended 1998***; 25489

After repeated oral administration of high doses of fenhexamid, no evidence of cumulative toxicity was seen in rats, mice and dogs. The oral administration of 1000 mg/kg bw/day for 4 weeks by gavage did not induce any toxic effects in rats. In feeding studies unspecific signs of toxicity such as reduced body weight development, increased feed and water intake were seen in rats and mice. The liver turned out to be the main toxicological target organ in the three species tested. ***In addition, effects on the kidneys were observed in rats and mice at very high dose levels (effects on kidney weight, increased incidences of basophilic tubules, dilated tubules, tubular casts, increased plasma creatinine and urea).***

In dogs effects on the red blood system (appearance of Heinz' bodies, reduction of no. of red blood cells) were the primary finding.

The red blood system was ***also*** the main toxicological target in dogs following 12 months administration of high dose levels. When comparing the intensity of the effects with those after 3 months, no enhancement was observed indicating that the dogs' organisms are able to compensate these blood effects adequately.

No systemic and local toxicological effects occured in rabbits following daily dermal application of 1000 mg/kg bw/day over a period of 17 days.

In a sub-acute inhalation study the highest concentration tested (486.7 mg/m³ air) did not result in pecific toxic effects but in particle overload response with regard to the clearance mechanisms of the respiratory tract. 68.7 mg/m³ air was the NOEL.

IIA 5.3.1 Oral 28-day toxicity

See Monograph and baseline dossier.

IIA 5.3.2 Oral 90-day toxicity (rodents)

An additional subchronic dietary study was conducted each in rats and mice to address specific japanese requirements. In these studies particular emphasis was given to examine hematology, renal function and erythropoietin in order to explain a possible renal anaemia.

Report:	**KIIA 5.3.2/03; [redacted], 1999**
Title:	KBR 2738 - Study for subchronic oral toxicity in rats (13 weeks feeding study)
Report No.: Document No.:	28583 **M-010247-01-1**
Dates of work:	1998-10-14 to 1999-01-15
Guidelines:	OECD 408; Directive 87/302/EEC, Part B; JMAFF 59 NohSan No.4200; US-EPA §82-1 Deviation(s): Deviating from study protocol the evaluation of bone marrow smears was not done in house. This deviation did not limit the assessment of results.
GLP:	yes

I. Materials and methods

A. Materials

1. Test material:	KBR 2738
Article no.:	05292344
Description:	brown powder
Lot/Batch no:	898812004
Purity:	97.5 %
Stability of test compound:	guaranteed for study duration, expiry date: 1999-03-25
2. Vehicle:	diet with 1% peanut oil
3.Test animals:	
Species:	Wistar rat
Strain:	Hsd Cpb:WU
Age:	approximately 5 – 6 weeks
Weight at dosing:	males: approx. 152 – 162 g females: approx. 131 – 138 g
Source:	[redacted]
Acclimatisation period:	at least 7 days
Diet:	fixed-formula standard diet (Altromin® 1321 meal, [redacted], Germany), ad libitum
Water:	tap water, ad libitum
Housing:	adaptation period: group housed in type III polycarbonate cages (5-6 rats of one sex per cage); treatment period: individually under conventional conditions in type IIA polycarbonate cages bedding: low-dust wood granulate ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment:

Dose:	0-500-5000-50000 ppm equivalent to: 0-38.0-403.9-5585.1 mg/kg bw/day (males) 0-47.4-552.8-8100.8 mg/kg bw/day (females)
Duration:	13 weeks, 4 weeks (interim sacrifice)
Application route:	oral (diet)
Group size:	20 rats/sex/group (interim sacrifice / terminal sacrifice: 10 / 10 rats/sex/group)
Observations:	mortality, clinical signs, body weight, food/water intake, haematology, clinical chemistry, urinalysis, gross necropsy, organ weight, histopathology

II. Results and discussion

A. Mortality

There were no mortalities up to a concentration of 50000 ppm.

B. In life observations

No treatment-related abnormalities of body orifices, general behaviour, posture and respiration were observed at 500 ppm in males and females.
At 5000 ppm and above increased urine excretion (wet bedding) was observed in males and females.
At 50000 ppm additionally piloerection as well as decreased motility and reactivity occurred. Loss of hair was seen in one male control rat and one female rat at 500 ppm.
Due to blood sampling via retroorbital plexus, in some animals of the control and dose groups injury of the eyes was observed.

C. Body weight

At 5000 ppm and below no toxicologically significant effects on body weights or on body weight development were observed. At 50000 ppm, in males and females the body weight development was slightly but partly statistically significantly retarded. At the end of the treatment, the mean body weights of this group were about 94% for males and about 91% for females of the corresponding control animals body weight. The mean body weights are presented in the following table.

Table 5.3.2-1: Mean body weight (g)

Dose (ppm)	0	500	5000	50000	0	500	5000	50000
Week	Males				Females			
0	152	152	156	162++	131	131	132	138+
1	194	195	195	181++	147	146	148	142
2	234	236	231	204++	161	163	162	154
3	262	263	257	229++	173	172	171	163
4	280	284	280	251++	178	180	178	173
5	311	309	322	295	188	190	196	187
6	328	324	339	308	198	195	204	189
7	338	337	351	319	201	201	208	191
8	349	348	363	329	206	205	212	193
9	361	360	375	341	211	209	216	195
10	371	369	388	351	217	213	220	200
11	382	382	396	361	222	220	225	203
12	382	384	394	362	221	221	224	204
13	384	390	391	360	224	222	225	204

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

D. Food and water consumption

At 5000 ppm and above the feed and water intake in g/animal and in g/kg body weight were dose relatedly increased in male and female animals. The increased feed intakes were interpreted by the authors as an effort of the animals to compensate the delayed body weight development, the increased water intake as most probably related to the increased feed intake.

Table 5.3.2-2: Mean food and water consumption

Mean food consumption									
Dose [ppm]	Days	Males				Females			
		g/animal		g/kg body weight		g/animal		g/kg body weight	
		total	per day	total	per day	total	per day	total	per day
0	91	2011	22.1	7018	77.1	1789	19.7	9767	107.3
500	91	1997	21.9	6912	76.0	1584	17.4	8629	94.8
5000	91	2143	23.5	7351	80.8	1872	20.6	10061	110.6
50000	91	2643	29.0	10165	111.7	2579	28.3	14743	162.0
Mean water consumption									
Dose [ppm]	Days	Males				Females			
		g/animal		g/kg body weight		g/animal		g/kg body weight	
		total	per day	total	per day	total	per day	total	per day
0	91	2506	27.5	8717	95.8	1782	19.6	9647	106.0
500	91	2507	27.5	8732	96.0	1817	20.0	9858	108.3
5000	91	2736	30.1	9379	103.1	2075	22.8	11132	122.3
50000	91	3078	33.8	11908	130.9	2371	26.1	13595	149.4

E. Laboratory investigations

Hematology

In females, no significant or dose related effects on erythrocyte counts, erythrocyte morphology and indices as well as on hematocrit values were detected up to 50000 ppm. In males, at 5000 ppm and above the reticulocyte counts were slightly decreased (week 3 and 12). At 50000 ppm the counts of erythrocytes were decreased and the MCV and MCH values were increased (week 12). With exception of the reticulocyte counts in week 3 and the MCV value in week 12 (at 50000 ppm), all individual values were in the historical control range.

Table 5.3.2-3: Hematology

Dose ppm	ERY 10E12/L	HB g/L	HCT L/L	MCV fl	MCH pg	MCHC g/L ERY	RETI 0/00	HEINZ 0/00	THRO 10 E9/L	Aniso-cytos	Micro-cytos	Hypo-chrom
	Males (week 3)											
0	7.71	145	0.453	58.8	18.8	320	33	0	1214	0	0	0
500	7.67	144	0.445	58.2	18.8	323	31	0	1121	0	0	0
5000	7.64	146	0.453	59.3	19.1	322	29+	0	1216	0	0	0
50000	8.01	150	0.457	57.1	18.7	328++	27+	0	1306	0	0	0
	Females (week 3)											
0	7.98	145	0.436	54.6	18.2	334	22	0	1135	0	0	0
500	7.90	141	0.439	55.6	18.6	335	21	0	1149	0	0	0
5000	7.94	147	0.448	56.5+	18.5	329	23	0	1077	0	0	0
50000	7.70	142	0.425	55.3	18.5	335	20	0	1283+	0	0	0
	Males (week 12)											
0	9.07	152	0.488	53.8	16.8	313	22	0	1111	0	0	2
500	8.96	155	0.492	54.9	17.3	315	21	0	1039	0	0	2
5000	9.08	154	0.489	53.9	16.9	314	19+	0	1139	0	0	2
50000	8.81+	155	0.491	55.7+	17.6++	315	19+	0	1147	0	0	2
	Females (week 12)											
0	8.57	150	0.474	55.3	17.5	316	18	0	1104	0	0	0
500	8.54	147	0.471	55.2-	17.3	313	20	0	1206+	0	0	1
5000	8.46	149	0.468	55.3-	17.6	317	18	0	1081	0	0	0
50000	8.43	148	0.470	55.7	17.5	315	17	0	1143	0	0	0

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

With exception of the statistically significantly increased mean values of leucocyte and lymphocyte counts in the 2s-range of historical controls in males at 50000 ppm fenhexamid in the diet, the differential blood count showed no changes during the treatment. The respective individual values showed distinctly higher values for the leucocyte and lymphocyte counts of two males. These individual values only exceeded the upper limit of the historical control range.

Table 5.3.2-4: Differential blood count

Dose ppm	LEUCO 10E9/L	NEUTRO 10E9/L	LYM 10E9/L	MONO 10E9/L	EOS 10E9/L	BASO 10E9/L	ATYP 10E9/L
	Males (week 3)						
0	8.45	0.76	7.29	0.22	0.09	0.02	0.08
500	9.08	0.65	8.04	0.20	0.08	0.02	0.09
5000	9.88	1.11	8.22	0.30	0.11	0.02	0.12
50000	11.65++	0.93	10.24++	0.21	0.13	0.04	0.10
	Females (week 3)						
0	7.30	0.56	6.46	0.15	0.07	0.02	0.05
500	6.69	0.68	5.69	0.17	0.09	0.01	0.04
5000	8.70	0.85	7.45	0.20	0.11+	0.02	0.07
50000	9.00	0.64	7.99	0.19	0.10	0.02	0.07
	Males (week 12)						
0	10.48	0.78	9.15	0.27	0.16	0.03	0.10
500	10.24	0.91	8.88	0.20	0.14	0.03	0.09
5000	9.77	0.76	8.55	0.23	0.13	0.02	0.07
50000	10.98	0.78	9.75	0.19	0.15	0.03	0.08
	Females (week 12)						
0	7.39	0.49	6.56	0.19	0.09	0.02	0.05
500	8.12	0.65	7.15	0.17	0.08	0.02	0.05
5000	7.22	0.43	6.48	0.15	0.10	0.02	0.05
50000	8.39	0.59	7.48	0.14	0.10	0.02	0.05

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

Clinical chemistry

There were no dose-related effects on the concentration of total protein, albumin, sodium, potassium, chloride and erythropoietin up to 50000 ppm fenhexamid in the diet as well as on the concentration of creatinine, urea, calcium and phosphate up to 5000 ppm.
At 50000 ppm after 4 weeks of treatment, the concentrations of creatinine, urea and calcium were increased in males and the concentrations of phosphate decreased in males and increased in females. However, all these values were in the 2s- or 3s-range of historical controls. At the end of the study, the concentration of calcium was increased in females. This value was just above the upper range of the 3s-range of historical controls. The increased concentrations of creatinine and urea in males at 50000 ppm are probably due to the described nephropathic changes in the kidneys.

Table 5.3.2-5: Clinical chemistry

	Males				Females			
Dose (ppm)	**0**	**500**	**5000**	**50000**	**0**	**500**	**5000**	**50000**
	Week 4							
Creatinine (µmol/L)	39	38	37	49++	40	39-	40-	44-
Urea (mmol/L)	7.78	7.60	7.33	9.45++	7.20	6.89	6.79	7.92
Calcium (mmol/L)	2.67	2.70	2.70	2.73+	2.68	2.66	2.66	2.73
Phosphate (mmol/L)	2.42	2.38	2.35	1.88++	1.67	2.07+	1.95	2.10+
	Week 13							
Creatinine (µmol/L)	44	42	43	46	44	46-	46	47
Urea (mmol/L)	6.70	6.84	6.22	7.17	6.34	6.94	6.83	7.19
Calcium (mmol/L)	2.61	2.58	2.59	2.68	2.64	2.71	2.69	2.80++
Phosphate (mmol/L)	1.53	1.39	1.62	1.47	1.65	1.60	1.72	1.61

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

Urine analysis

The determinations of volume and density showed no remarkable differences to the controls in any dose group. The concentrations of protein and protein per sampling period were lower in males at 50000 ppm, the differences became statistically significant for protein at week 12 and for protein per sampling period at week 4.

The semi-quantitatively determined protein, glucose, blood, bilirubine, ketone bodies and uribilinogen concentrations did not reveal toxicologically significant effects in any dose group. With exception of the determination at week 12 in females, the pH values were - not dose relatedly - higher at 500 ppm and above in both genders. However, despite the factor of 100 between the lowest and the highest dose levels, there was no dose effect relationship visible. Second, a pH variation could have been triggered by the relatively high counts for bacteria in the urines although the urine was cooled during the sampling periods. No treatment effect can be construed from this data.

The sediments showed no abnormalities.

The electrophoresis of urine showed for both time points in males lower values of pre-albumine (week 3: 50000 ppm, week 12: 5000 ppm and above).

At week 12, in both genders lower percentages for β-globulin (5000 ppm and above in males, 500 ppm and above in females) as well as corresponding higher α1-globulin percentages (statistically significantly different for all treated female groups) were noticed. In no case a dose-effect relationship could be demonstrated despite the wide range of selected dose levels. Therefore, these differences are considered as incidental.

Also the differences in percentage for γ-globulin measured only in the male groups treated with 5000 or 50000 ppm are not considered as treatment-related. They are confined to one gender and time point and show again no dose effect relationship.

F. Organ weight

In 50000 ppm males the absolute and relative weights of the kidneys were increased. Apart from this finding, there were no further effects on organ weights (including spleen weight) in this study.

Table 5.3.2-6: Organ weight

Dose (ppm)	Absolute kidney weights (mg) Males	Females	Relative kidney weights (mg/100 g) Males	Females
1st interim sacrifice				
0	1879	1204	673	659
500	1920	1275	663	692
5000	1760	1167	640	687
50000	2150 +	1170	882++	674
terminal sacrifice				
0	2266	1390	588	633
500	2282	1354	590	622
5000	2263	1367	571	610
50000	2284	1269	633	613

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

G. Gross necropsy

At the necropsy after 4 weeks of treatment, no treatment-related gross pathological alterations were detected up to 5000 ppm. At 50000 ppm enlargement of the kidneys was observed in 4/10 males, discoloration of the kidneys in 2/10 males.
At terminal necropsy after 13 weeks of treatment the kidneys at 5000 and 50000 ppm were discolored in 1/10 females each.

H. Micropathology

At 50000 ppm, after 4 and 13 weeks of treatment, a nephropathy characterized by basophilic tubules, tubular dilation and tubular casts was found in a number of animals of both sexes. The dilated tubules showed a flattened epithelium with either a basophilic or an eosinophilic cytoplasm. In the later the brush border was preserved. Compared to the findings of the interim sacrifice, the nephropathy showed no significant progression in terms of severity and dose levels affected after 13 weeks of treatment.
Spleen and bone marrow showed no test compound-related changes.
Kidney histopathology after 4 and 13 weeks of treatment is summarized in the following table.

Table 5.3.2-7: Kidney histopathology: Incidences and average grading

Dose (ppm)	0	500	5000	50000	0	500	5000	50000
	Males (n = 10/group)				Females (n = 10/group)			
	4 week treatment							
Basoph. Tub./Cort.								
Incidence	8	6	5	10	1	4	4	9
Average Grading	1.0	1.0	1.0	2.3	1.0	1.0	1.0	1.2
Basoph. Tub./Med.								
Incidence	0	0	0	7	0	0	0	2
Average Grading	0	0	0	2.0	0	0	0	1.5
Tubular Dilation								
Incidence	1	1	2	10	0	0	0	5
Average Grading	1.0	1.0	1.0	2.4	0	0	0	1.8
Tubular Casts								
Incidence	0	0	0	10	0	0	0	4
Average Grading	0	0	0	2.4	0	0	0	1.8
	13 week treatment							
Basoph. Tub./Cort.								
Incidence	7	9	8	10	2	0	1	7
Average Grading	1.1	1.1	1.4	2.3	1.0	0	1.0	1.3
Basoph. Tub./Med.								
Incidence	0	1	1	7	0	1	0	2
Average Grading	0	1.0	1.0	2.6	0	1.0	0	2.5
Tubular Dilation								
Incidence	2	3	0	9	0	0	0	5
Average Grading	1.0	1.0	0	2.4	0	0	0	1.6
Tubular Casts								
Incidence	0	4	2	8	0	0	0	6
Average Grading	0	1.3	1.0	2.3	0	0	0	1.5

Basoph. Tub./Cort.: cortical basophilic tubules
Basoph. Tub./Med.: medullar basophilic tubules

III. Conclusion

NOEL: 500 ppm
equivalent to: 38.0 / 47.4 mg/kg bw/day (males/females)

based on slight effects on red and white blood and on kidney effects at 5000 and 50000 ppm

Report:	**KIIA 5.3.2/04; [redacted]., 1999**
Title:	KBR 2738 - Study for subchronic oral toxicity in mice (feeding study - 13 weeks)
Report No. Document No.	28580 **M-009771-01-1**
Dates of work	1998-10-20 to 1999-02-12
Guidelines:	OECD 408; Directive 87/302/EEC, Part B; JMAFF 59 NohSan No.4200; US-EPA§82-1 Deviation(s): Deviating from study protocol the evaluation of bone marrow smears was not done in house. This deviation did not limit the assessment of results.
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: KBR 2738

Article no.:	05292344
Description:	brown powder
Lot/Batch no:	898812004
Purity:	97.5%
Stability of test compound:	guaranteed for study duration; expiry date: 1999-03-25

2. Vehicle: diet with 1% peanut oil

3.Test animals:

Species:	mouse
Strain:	Crl:CD-1(ICR)BR, SPF-bred
Age:	approximately 6 – 8 weeks
Weight at dosing:	males: approx. 30 – 32 g females: approx. 22 – 24 g
Source:	[redacted], Germany
Acclimatisation period:	at least 6 or 7 days
Diet:	fixed-formula standard diet (Altromin® 1321 meal; [redacted], Germany), ad libitum
Water:	tap water, ad libitum
Housing:	adaptation period: male mice: individually in type II polycarbonate cages; female mice: in groups of 5 mice in type III polycarbonate cages; treatment period: individually under conventional conditions in type II polycarbonate cages bedding: low-dust wood granulate ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment:

Dose:	0 - 200 - 2000 - 20000 ppm equivalent to 0-32.5-322.9-3416.8 mg/kg bw/day (males) 0-54.8-573.7-6145.4 mg/kg bw/day (females)
Duration:	13 weeks
Application route:	oral (diet)
Group size:	20 mice/sex/group
Observations:	mortality, clinical signs, body weight, food/water intake, haematology, clinical chemistry (blood), gross necropsy, organ weight, histopathology

II. Results and discussion

A. Mortality

There were no test substance-related mortalities up to the high dose of 20000 ppm fenhexamid in the diet. Seven animals of the 4 week treatment groups and six animals of the 13 week treatment groups died during or after blood sampling.

B. In life observations

No treatment-related abnormalities of body orifices, general behaviour, posture and respiration were observed up to 20000 ppm fenhexamid.

C. Body weight

No toxicologically significant effects on body weights or on body weight development were observed.

D. Food and water consumption

At 20000 ppm the feed and water intake in g/animal and in g/kg body weight were dose relatedly increased in male and female animals. Mean food and water consumption in males and females are summarized in the table below.

Table 5.3.2-8: Mean food and water consumption

Mean food consumption					
Dose (ppm)	**Days**	**g/animal**		**g/kg bw**	
		total	**per day**	**total**	**per day**
Males					
0	91	481	5.3	13920	153.0
200	91	532	5.8	14806	162.7
2000	91	540	5.9	14691	161.4
20000	91	561	6.2	15546	170.8
Females					
0	91	640	7.0	24636	270.7
200	91	652	7.2	24926	273.9
2000	91	698	7.7	26102	286.8
20000	91	737	8.1	27961	307.3

Mean water consumption					
Dose (ppm)	**Days**	**g/animal**		**g/kg bw**	
		total	**per day**	**total**	**per day**
Males					
0	91	503	5.5	14613	160.6
200	91	581	6.4	16385	180.1
2000	91	586	6.4	16132	177.3
20000	91	944	10.4	26078	286.6
Females					
0	91	525	5.8	20342	223.5
200	91	527	5.8	20184	221.8
2000	91	581	6.4	21914	240.8
20000	91	947	10.4	35925	394.8

E. Laboratory investigations

Hematology

In 20000 ppm females, the concentration of MCH was statistically significantly decreased in week 12. Since the value was well within the 2s-range of historical control data, this isolated finding is

considered as incidental. Thus, no toxicologically significant effects on erythrocyte counts, erythrocyte morphology and indices as well as on hemoglobin concentration and on hematocrit were detected.

With exception of the increased mean values of the counts of eosinophiles (20000 ppm females, week 3 and 12) also the differential blood count showed no change during the treatment.

Table 5.3.2-9: Hematology

Dose (ppm)	0	200	2000	20000	0	200	2000	20000
	Males				Females			
	week 3							
ERY (10E12/L)	9.12	8.96	9.35	8.88	9.11	9.52	9.12	8.98
HB (g/L)	144	144	148	140	145	149	146	144
HCT (L/L)	0.429	0.417	0.440	0.406	0.422	0.439	0.411	0.438
MCV (fl)	47.0	46.6-	47.4	45.7	46.5	46.2	45.1	48.9
MCH (pg)	15.8	16.0	16.0	15.8	16.0	15.6	16.0	16.1
MCHC (g/L ERY)	338	346	338	346	345	339	356	331
RETI (0/00)	21	23	24	20	23	22	28	23
Poikilo	0	0	0	0	0	0	0	0
Nucl ERY (#/100WBC)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nucl Sh. (#/100WBC)	19.7	24.1	18.1	15.0	20.9	28.8	24.1	26.0
Norm ERY	1	1	1	1	1	1	1	1
	week 12							
ERY (10E12/L)	9.15	9.22	9.07	9.19	9.06	9.12	9.51	9.24
HB (g/L)	139	138	138	136	145	143	147	140
HCT (L/L)	0.417	0.410	0.397	0.410	0.415	0.424	0.429	0.407
MCV (fl)	45.6	44.5	43.9	44.7	45.8	46.5	45.2	44.1
MCH (pg)	15.2	15.0	15.2	14.8	16.0	15.7	15.5	15.2++
MCHC (g/L ERY)	335	337	346	332	349	339	344	344
RETI (0/00)	23	24	25	28	25	22	21	23
Poikilo	0	0	0	0	0	0	0	0
Nucl ERY (#/100WBC)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nucl Sh. (#/100WBC)	21.4	21.3	16.9	15.9	25.4	25.9	30.7	32.6
Norm ERY	1	1	1	1	1	1	1	1

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

Table 5.3.2-10: Differential blood count

Dose (ppm)	0	200	2000	20000	0	200	2000	20000
	Males				Females			
	week 3							
LEUCO (10E9/L)	3.6	3.0	3.4	3.7	4.7	5.2	3.8	5.2
LYM (total 10E9/L)	3.09	2.48	2.99	3.00	3.80	4.36	3.25	4.32
SEGM (total 10E9/L)	0.43	0.41	0.34	0.63	0.71	0.67	0.44	0.60
EOS (total 10E9/L)	0.03	0.04	0.05	0.03	0.06	0.04	0.06	0.14+
MONO (total 10E9/L)	0.07	0.08	0.06	0.06	0.04	0.05	0.05	0.07
BAND (total 10E9/L)	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.01
PLAS (total 10E9/L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ATYP LYM (total 10E9/L)	0.01	0.010	0.00	0.01	0.03	0.01	0.02	0.02
	week 12							
LEUCO (10E9/L)	3.5	2.7	2.3	2.6	4.6	4.7	4.7	5.6
LYM (total 10E9/L)	2.88	2.19	1.78	2.10	3.74	3.95	3.89	4.74
SEGM (total 10E9/L)	0.44	0.36	0.46	0.40	0.80	0.57	0.66	0.63
EOS (total 10E9/L)	0.08	0.05	0.04	0.05	0.04	0.09	0.08	0.12
MONO (total 10E9/L)	0.04	0.03	0.06	0.02	0.02	0.06	0.08	0.06
BAND (total 10E9/L)	0.01	0.00	0.01	0.00	0.01	0.01	0.01	0.01
PLAS (total 10E9/L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ATYP LYM (total 10E9/L)	0.02	0.02	0.01	0.01	0.01	0.00	0.03	0.00

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

Clinical chemistry

Up to 2000 ppm fenhexamid in the diet no treatment-related effects of the concentration of creatinine, urea, total protein, albumin, sodium, potassium, calcium, phosphate and chloride were detected.
At 20000 ppm the concentrations of creatinine and urea were - mostly statistically significant - increased in both genders. However, all mean values were in the 2s-range of historical controls. Additionally, the concentration of calcium was slightly increased in 20000 ppm males in week 4 only.
The activity of erythropoietin was not influenced in males up to 20000 ppm, while it was statistically significantly lower in females at 2000 and 20000 ppm in comparison to the control group at the end of the study. However, despite the factor of 10 between the dose levels, there was no dose effect relationship visible. Additionally, this effect only occurred in females, and not in males, in which distinctly stronger kidney effects were observed. Therefore, the lower erythropoietin activities in female mice at 20000 ppm were not considered to be a treatment related effect.

Table 5.3.2-11: Clinical chemistry

Dose (ppm)	0	200	2000	20000	0	200	2000	20000
	Males				Females			
	Week 3							
CREA (µmol/L)	27	27	27	32++	25	25	26	29++
UREA (mmol/L)	10.96	11.14	11.16	13.99++	9.33	9.49	9.81	10.30
PROT (g/L)	56.7	56.5	56.4	57.8	54.1	56.0	53.8	55
ALBUMIN (g/L)	27.5	26.7	27.3	27.0	27.5	27.3	28.2	27.2
Na (mmol/L)	151	151	151	149	148	147	148	147
K (mmol/L)	4.2	3.7+	4.0	3.9	3.5	3.6	3.4	3.4
Ca (mmol/L)	2.33	2.32	2.34	2.40+	2.34	2.29	2.30	2.34
Cl (mmol/L)	108	109	109	109	110	109	110	110
P (mmol/L)	2.03	2.13	2.02	1.96	1.83	2.17	2.09	1.94
EPO (U/L)	*	*	*	*	*	*	*	*
	Week 12							
CREA (µmol/L)	25	24	24	30+	28	28	28	32
UREA (mmol/L)	10.66	11.16	10.50	12.96+	9.42	9.56	9.90	9.55
PROT (g/L)	58.7	59.8	59.4	59.0	60.9	58.6	59.3	58.9
ALBUMIN (g/L)	26.6	26.0	26.9	26.5	28.4	27.8	29.1	27.9
Na (mmol/L)	150	148	150	149	147	147	148	147
K (mmol/L)	3.8	3.7	3.7	4.0	3.3	3.1	3.2	3.2
Ca (mmol/L)	2.33	2.29	2.30	2.39	2.28	2.28	2.29	2.33
Cl (mmol/L)	107	105	108	107	106	107	108	106
P (mmol/L)	1.90	1.72	1.85	1.92	1.90	1.84	1.96	1.84
EPO (U/L)	42.1	40.9	37.9	47.3	50.1	46.6	29.2++	35.3+

* : The determination could not be performed because of problems with calibration. The sample size was not sufficient to allow repeat analysis.

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

F. Organ weight

No statistically significant effects on absolute or relative organ weights were observed up to 2000 ppm. At 20000 ppm, the kidney weights were slightly lower than in the controls. These differences became statistically significant for the relative weights in males.

Table 5.3.2-12: Organ weight

Dose (ppm)	Absolute kidney weights (mg)		Relative kidney weights (mg/100 g)	
	Males	Females	Males	Females
1st interim sacrifice after 4 weeks of treatment				
0	530	340	1637	1352
500	584	349	1687	1387
5000	588	330	1634	1223
50000	527	336	1578	1291
terminal sacrifice				
0	586	377	1583	1323
500	595	349	1521	1232
5000	592	349	1466	1242
50000	544	348	1414+	1261

Significantly different from control: +: $p < 0.05$ ++: $p < 0.01$

G. Gross necropsy

At the interim necropsy after 4 weeks of treatment, no gross pathological alterations were observed up to 2000 ppm fenhexamid in the diet. At 20000 ppm changes in kidneys were observed in two males (no. 61: deformation on right kidney; no. 62: pale discoloration of both kidneys).
At terminal necropsy, the surface of both kidneys was rough in two males of the 20000 ppm group (no. 70 and 80).

H. Micropathology

After 4 and 13 weeks of treatment, at 20000 ppm renal alterations, i.e. dilated tubules, tubular casts and an increase in basophilic tubules, were observed in both genders. In week 13, males were more severely affected than females. An overview on renal alterations is given in Table 5.3.2-13 below.
An increase in splenic siderin storage was observed in females at 200 ppm and above after 4 weeks of treatment and at 2000 ppm and above at the end of the study. At week 4 the incidence of siderosis was more frequent in treated animals than in controls, while there were no intergroup differences at week 13. At week 13 the average grade was slightly increased in females at 2000 ppm and above.
In contrast, male mice showed no differences in siderin storage. With respect to hematopoiesis here were no clear intergroup differences in males nor in females.
The degree of siderin storage in the spleen primarily depends on the given activation of hematopoiesis. In mice, the spleen plays an important role in normal hematopoiesis. Appropriate stimuli (e.g. blood sampling) can lead to a considerable increase in extramedullary hematopoietic foci in the spleen. As a rule, an increased hematopoiesis is accompanied by a decreased amount of siderin in the spleen. Almost all animals found dead after blood sampling had relatively more siderin whereas the hematopoietic activity was comparably low. In contrast, animals necropsied at term had survived blood sampling and thus demonstrated only few siderin granules and a stimulated hematopoiesis. These data show that blood sampling in mice has a major influence on the amount of siderin in the spleen.
Hematology did not provide any evidence of damage on red blood cells, in particular there was no indication of treatment-related hematologic effects. Hence, the observation of differences in the quantity of siderin in the spleen in females is considered to be most probably the result of the individual animals' reactions to blood sampling and not an effect triggered by fenhexamid treatment. The histopathological findings in the spleen are presented in Table 5.3.2-14.

Table 5.3.2-13: Kidney histopathology: Incidences and average grading

Dose (ppm)	0	200	2000	20000	0	200	2000	20000
	Males (n = 10/group)				Females (n = 10/group)			
	4 week treatment							
Basoph. Tubules								
Incidence	7	4	3	9	0	2	1	10
Average Grading	1.3	1.3	1.3	3.0	0	1.5	1.0	2.2
Tubular Dilation								
Incidence	0	0	0	8	0	0	0	6
Average Grading	0	0	0	1.6	0	0	0	1.5
Tubular Casts								
Incidence	0	0	0	8	0	0	0	5
Average Grading	0	0	0	1.0	0	0	0	1.0
	13 week treatment							
Basoph. Tubules								
Incidence	8	8	6	10	1	2	3	8
Average Grading	1.3	1.3	1.3	3.0	2.0	1.0	1.0	2.1
Tubular Dilation								
Incidence	0	1	0	8	0	0	0	4
Average Grading	0	1.0	0	2.1	0	0	0	1.5
Tubular Casts								
Incidence	0	0	0	7	0	0	0	2
Average Grading	0	0	0	1.4	0	0	0	1.0

Basoph. Tubules.: basophilic tubules

Table 5.3.2-14: Spleen histopathology: Incidences and average grading

Dose (ppm)	0	200	2000	20000	0	200	2000	20000
	Males (n = 10/group)				Females (n = 10/group)			
	4 week treatment							
Activ. hematopoiesis								
n examined	9	9	8	9	9	10	9	10
Incidence	9	9	8	9	9	10	9	10
Average Grading	3.4	3.6	3.4	3.3	3.1	3.0	2.9	3.1
unscheduled deaths*								
n examined	1	1	2	1	1	0	1	0
Incidence	0	0	0	0	1	0	0	0
Average Grading	0	0	0	0	1.0	0	0	0
Siderosis								
n examined	9	9	8	9	9	10	9	10
Incidence	0	0	0	0	4	4	4	6
Average Grading	0	0	0	0	1.0	1.3	1.0	1.0
unscheduled deaths*								
n examined	1	1	2	1	1	0	1	0
Incidence	1	0	1	1	1	0	1	0
Average Grading	2.0	0	2.0	1.0	2.0	0	2.0	0
	13 week treatment							
Activ. hematopoiesis								
n examined	10	10	9	10	8	10	8	9
Incidence	10	10	9	10	8	10	8	9
Average Grading	3.4	3.4	3.2	3.3	2.5	2.3	2.6	2.1
unscheduled deaths*								
n examined	0	0	1	0	2	0	2	1
Incidence	0	0	1	0	2	0	2	1
Average Grading	0	0	1.0	0	1.0	0	1.0	1.0
Siderosis								
n examined	10	10	9	10	8	10	8	9
Incidence	5	4	5	7	7	9	6	8
Average Grading	1.0	1.3	1.2	1.1	1.0	1.2	1.7	1.6
unscheduled deaths*								
n examined	0	0	1	0	2	0	2	1
Incidence	0	0	0	0	2	0	2	1
Average Grading	0	0	2.0	0	3.0	0	2.5	3.0

Activ. hematopoiesis: activated hematopoiesis

*: animals died during or after blood sampling

III. Conclusion

NOEL: 2000 ppm

equivalent to: 322.9 / 573.7 mg/kg bw/day (males/females)

based on renal alterations and slightly lower kidney weight at 20000 ppm.

IIA 5.3.3 Oral 90-day toxicity (dog)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.3.4 Oral 1 year toxicity (dog)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.3.5 28-day inhalation toxicity (rodents)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.3.6 90-day inhalation toxicity (rodents)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.3.7 Percutaneous 28-day toxicity (rodents)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.3.8 Percutaneous 90-day toxicity (rodents)

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing.
Please refer to the Monograph and the baseline dossier of fenhexamid.

IIA 5.4 Genotoxicity

Summary of genotoxicity testing

Table 5.4-1: Summary of genotoxicity testing

Test system	Test object	Concentration	Purity (%)	Result	Reference / report no.
Salmonella microsome test	S.typhimurium TA98, TA100, TA1535, TA1537*	62.5-2000 µg/plate	95.5	negative	[redacted], 1991; 20307
Reverse mutation assay	***S.typhimurium TA98, TA100, TA1535, TA1537, E.coli WP2/uvrA****	***43.8-700 µg/plate***	***95.8***	***negative***	[redacted], ***1995; RA95006***
HGPRT-Test	Chinese hamster lung cells (V79)*	25-150 µg/mL	95.4	negative	[redacted], 1994; 23529
Cytogenetic study	Chinese hamster ovary cells*	3-120 µg/mL	95.5	negative	[redacted], 1995; 24405
Unscheduled DNA synthesis test	rat primary hepatocytes*	2.5-40 µg/mL	95.5	negative	[redacted], 1992; 21312
DNA repair test in bacterial system (Rec assay)	***Bacillus subtilis : H17 (Rec+), M45 (Rec-)***	***6.25-200 µg/plate***	***96.1***	***negative***	[redacted], ***1997; NR96660***
Micronucleus test	mice (males & females)	750 mg/kg bw	95.4	negative	[redacted], 1993; 22625

* with and without metabolic activation (S9-mix)

The genotoxic action of KBR 2738 was studied in bacteria and mammalian cells with the aid of in-vitro test systems and in vivo by means of the micronucleus test. None of the test systems used revealed any evidence of a genotoxic potential of fenhexamid. ***This is also true for two additional in vitro studies, a reverse mutation assay and a DNA repair test in bacteria (Rec assay), which were conducted in 1995 and 1997 for the registration of fenhexamid in Japan.***

The different types of mutagenicity tests conducted and the results obtained in testing for the various genotoxic endpoints are presented in the table above.

IIA 5.4.1 *In vitro* genotoxicity - Bacterial assay for gene mutation

Report:	**KIIA 5.4.1/02; [redacted] 1995**
Title:	KBR 2738 - Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*)
Report No.: Document No.:	RA95006 **M-010423-01-1**
Dates of work:	1995-04-17 to 1995-04-27
Guidelines:	JMAFF 59 Nohsan No. 4200; OECD (1983); EPA-FIFRA (1991) Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material:	KBR 2738
Article no. / Development no.:	not stated
Description:	dark brown powder
Lot/Batch no:	4258/76
Purity:	95.8%
Stability of test compound:	guaranteed for study duration
2. Vehicle and/or positive control:	Vehicle: dimethyl sulfoxide (DMSO) Positive controls: 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) 2-Aminoanthracene (2AA) Sodium azide (NaN_3) 9-Aminoacridine (9-AA)
3. Test system:	Salmonella typhimurium TA98, TA100, TA1535, TA1537; Escherichia coli WP2uvrA
metabolic activation:	S9 mix from phenobarbital and 5,6-benzoflavone induced rat liver homogenate

B. Study design and methods

1. Treatment:	
Dose:	0-43.8-87.5-175-350-700 µg/plate +/- S9 mix positive control AF-2: 0.1 µg/plate (TA98, –S9 mix), 0.01 µg/plate (TA100, WP2/uvrA, -S9 mix) NaN_3: 0.5 µg/plate (TA1535, -S9 mix) 9-AA: 80 µg/plate (TA1537, -S9 mix) 2-AA: 0.5 µg(plate (TA98, + S9 mix), 1µg/plate (TA100, + S9 mix), 2 µg/plate (TA1535, TA 1537, + S9 mix) 10 µg/plate (WP2uvrA, + S9 mix) For each test solution or control n = 3 plates were used.
Application volume:	0.1 mL/plate
Incubation time/ temperature:	48 hours, 37°C

II. Results and discussion

In both tests, bacterial growth inhibition was observed for all test strains at the concentration of 700 µg/plate with and without S9 mix. At the other concentrations, no dose dependent increase of revertant colonies was observed for any of the test strains with or without S9 mix.
In contrast, in cases of the positive control substances, AF-2, NaN_3, and 9-AA without S9 mix, and 2-AA with S9 mix, a marked increase in the revertant colonies was observed for each strain. These results in the positive controls confirmed that the test system employed in this study was appropriate for detecting a mutagenic effects.

Table 5.4.1-1: Result of the first experiment (mean values of n = 3 plates per test solution /control)

Compound	S9 mix	Concentr. (µg/plate)	Revertants /plate				
			Base-pair substitution type			Frameshift type	
			TA100	TA1535	WP2uvrA	TA98	TA1537
DMSO	-	0	90	8	17	27	8
KBR 2738	-	700	0*	0*	0*	0*	0*
	-	350	76	9	13	29	7
	-	175	85	10	15	26	8
	-	87.5	76	8	13	31	7
	-	43.8	82	8	16	31	9
Positive control							
AF-2	-	0.01	391		180		
NaN3	-	0.5		137			
AF-2	-	0.1				431	
9-AA	-	80.0					487
DMSO	+	0	97	16	21	37	15
KBR 2738	+	700	0*	0*	0*	0*	0*
	+	350	85	9	21	34	13
	+	175	102	13	24	32	15
	+	87.5	115	15	26	33	12
	+	43.8	107	14	21	35	16
Positive control							
2-AA	+	1.0	478				
	+	2.0		116			86
	+	10.0			393		
	+	0.5				185	

*: killing (bacterial growth inhibition)

Table 5.4.1-2: Result of the second experiment (mean values of n = 3 plates per test solution/control)

Compound	S9 mix	Concentr. (µg/plate)	Revertants /plate				
			Base-pair substitution type			Frameshift type	
			TA100	TA1535	WP2uvrA	TA98	TA1537
DMSO	-	0	82	8	16	23	10
KBR 2738	-	700	0*	0*	0*	0*	0*
	-	350	82	6	10	22	6
	-	175	78	7	11	27	9
	-	87.5	80	6	13	24	11
	-	43.8	76	7	14	22	10
Positive control							
AF-2	-	0.01	441		175		
NaN3	-	0.5		154			
AF-2	-	0.1				373	
9-AA	-	80.0					447
DMSO	+	0	86	12	13	33	13
KBR 2738	+	700	0*	0*	0*	0*	0*
	+	350	80	9	10	23	10
	+	175	88	10	13	34	13
	+	87.5	95	8	13	39	13
	+	43.8	90	13	12	34	12
Positive control							
2-AA	+	1.0	469				
	+	2.0		131			114
	+	10.0			355		
	+	0.5				190	

*: killing (bacterial growth inhibition)

III. Conclusion

Fenhexamid does not have any mutagenic activity in the tested S. typhimurium and E. coli strains TA98, TA100, TA1535, TA1537 and WP2uvrA with and without S9 mix.

Report:	**KIIA 5.4.1/03; [redacted] 1997**
Title:	KBR 2738 - DNA repair test in bacterial system
Report No.: Document No.:	MR96660 **M-009215-01-1**
Dates of work:	1996-12-03 to 1996-12-05
Guidelines:	JMAFF 59 Nohsan: No. 4200, 1984 Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: KBR 2738

Article no. / Development no.:	not stated
Description:	brown powder
Lot/Batch no:	17003/94
Purity:	96.1%
Stability of test compound:	guaranteed for study duration

2. Vehicle and/or controls:

Vehicle: dimethyl sulfoxide (DMSO) for solvent control, KBR 2738 and positive control 2-AA
distilled water for positive control MMC and negative control KM

Positive control:
Mitomycin C (MMC),
2-aminoanthracene (2-AA)

Negative control:
Kanamycin sulfate (KM)

3. Test system: Bacillus subtilis : H17 (Rec$^+$), M45 (Rec$^-$)

metabolic activation: S9 mix from phenobarbital and 5,6-benzoflavone induced rat liver homogenate

B. Study design and methods

1. Treatment:

Dose:	0-6.25-12.5-25-50-100-200 µg/disk (+/- S9 mix)
	Mitomycin C (MMC): 0.005-0.01 µg/disk (- S9 mix)
	2-Aminoanthracene (2-AA): 5-20 µg/disk (+/- S9 mix)
	Kanamycin sulfate (KM): 0.5-1 µg/disk (- S9 mix)
Application volume:	20 µL/disk
Incubation time / temperature:	24 hours, 37°C

II. Results and discussion

At concentrations of 100 and 200 µg/disk slight growth inhibition was observed in both strains with and without metabolic activation. However, the differences of the diameter of growth inhibition between both strains were 0-1 mm, which is within the 5 mm range, which does not indicate DNA damaging effects. In the concentration range from 6.25 to 50µg/disk there was no growth inhibition in both strains with or without metabolic activation.

These results show that with or without metabolic activation KBR 2738 has no DNA-damaging effect in the Rec$^{+/-}$ assay.

In contrast, in case of the positive controls Mitomycin C (-S9 mix) and 2-aminoanthracene (+S9 mix) growth inhibition was not observed in H 17 (Rec$^+$), but in M 45 (Rec$^-$), indicating the DNA-damaging activity of both compounds.

In case of the negative control Kanamycin sulfate (-S9 mix) growth inhibition was observed in both strains, but the difference of the diameter of the growth inhibition zone with 1 and 3 mm was less than 5 mm. Accordingly, this result shows that the negative control substance also does not possess DNA-damaging properties.

Table 5.4.1-3: Result of the Rec$^{+/-}$ assay on Bacillus subtilis strains H17 (Rec^{+}) and M45 (Rec^{-})

Substance	Concentr. (µg/disk)	S-9 (-) G.I. zone (mm) H17	S-9 (-) G.I. zone (mm) M45	S-9 (-) Difference* (mm)	S-9 (+) G.I. zone (mm) H17	S-9 (+) G.I. zone (mm) M45	S-9 (+) Difference* (mm)
KBR2738	6.25	0	0	0	0	0	0
	12.5	0	0	0	0	0	0
	25	0	0	0	0	0	0
	50	0	0	0	0	0	0
	100	3	4	1	6	6	0
	200	4	4	0	5	6	1
2-AA	5	0	0	0	0	10	10
	20	0	0	0	0	12	12
MMC	0.005	0	14	14	/	/	/
	0.01	1	18	17	/	/	/
KM	0.5	10	11	1	/	/	/
	1	12	15	3	/	/	/
DMSO	20 µL	0	0	0	0	0	0

G.I. zone: growth inhibition zone

* : DNA damaging effects are indicated by a difference of growth inhibition zones between H17 and M45 of > 5 mm.

III. Conclusion

Fenhexamid has no DNA damaging activity in the Rec$^{+/-}$ assay on Bacillus subtilis strains H17 (Rec^{+}) and M45 (Rec^{-}) with or without metabolic activation.

IIA 5.4.2 *In vitro* genotoxicity - Test for clastogenicity in mammalian cells

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.4.3 *In vitro* genotoxicity - Test for gene mutation in mammalian cells

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.4.4 *In vivo* genotoxicity (somatic cells) - Bone marrow or micronucleus

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.4.5 *In vivo* genotoxicity (somatic cells) - DNA repair or mouse spot tests

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.4.6 *In vivo* studies in germ cells

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC

IIA 5.5 Long-term toxicity and carcinogenicity

Summary of long-term toxicity studies

Table 5.5-1: Summary of long-term toxicity studies

Type of study	Species	Dose range tested	NO(A)EL	Reference
chronic/carcino-genicity, oral	rat	0-500-5000-20000 ppm ***(M/F: 0/0-28/40-202/415-1280/2067 mg/kg bw/day)***	500 ppm (M/F: 28/40 mg/kg bw/day)	[redacted]., 1996; 25522
oncogenicity, oral	mouse	0-800-2400-7000 ppm ***(M/F: 0/0-247.4/364.8-807.4/1054.5-2354.8/3178.2 mg/kg bw/day)***	800 ppm (M/F: 247.4/ 364.8 mg/kg bw/day)	[redacted], 1996; 25523

M: male F: female

The limit concentration of 20000 ppm (equivalent to ~ 1000 mg/kg bw/day) was tolerated by rats over a period of 24 months without increased mortality or major organ damage. In addition to unspecific signs of toxicity, such as reduced body weight development (although the feed intake was increased) and higher water intake, only slight effects on liver and thyroid were established. Some mucosal hyperplasia in the caecum of the rats might be linked to the continuous stimulation by the mild irritant action of fenhexamid. Following long-term administration of high dose levels to mice, again the kidneyswere the target organ in this species. The main effects were a decrease of kidney weights and an increased incidence of common morphological findings such as basophilic tubuli and chronic renal disease.

No evidence of an oncogenic potential of fenhexamid was found in either the rat or mouse long-term feeding studies.

IIA 5.5.1 Long-term (2 years) oral toxicity in the rat

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.5.2 Carcinogenicity study in the rat

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.5.3 Carcinogenicity study in the mouse

Information presented and evaluated during the EU Annex I listing process. Please refer to Monograph and baseline dossier of fenhexamid.

IIA 5.5.4 Mechanism of action and supporting data

Please refer to the Monograph and baseline dossier of fenhexamid.

IIA 5.6 Reproductive toxicity

Summary of reproductive toxicity studies

Table 5.6-1: Summary of reproductive toxicity studies

Type of study	Species	Dose range	NOEL	Reference
2-generation, oral	rat	0-100-500-5000-20000 ppm	parental: 500 ppm ***(38.2/44.8 mg/kg bw/day (m/f)*** neonatal: 500 ppm ***(76.6 mg/kg bw/day (lactation))*** reproduction: 20000 ppm ***(1814.0/1867.3/3754.8 mg/kg bw/day (premating/gestation /lactation))***	[redacted] 1996 / BC8008
teratogenicity, oral	rat	0-1000 mg/kg bw	maternal: 1000 mg/kg bw/day developmental: 1000 mg/kg bw/day	[redacted], 1994 / BC7438
teratogenicity, oral	***rat***	***0-300-1000-2000 mg/kg bw***	***maternal: 300 mg/kg bw/day*** ***developmental: 2000 mg/kg bw/day***	[redacted] ***1998 / BC7438 (supplemental submission)***
teratogenicity, oral	rabbit	0-100-300-1000 mg/kg bw/day	NOAEL maternal/ intrauterine 100 mg/kg bw/day	[redacted], 1995 / 23733

M: male f: female

The limit concentration of 20000 ppm was tolerated by rats in a 2-generation study without adverse effects on reproductive behaviour and efficiency. Reduced pup weights which occured during the first three weeks post partum at high dose levels are attributed to a deficiency of the neonate rat for glucuronidation and, as a consequence, for the excretion of fenhexamid. The reduced pup -weights coincided with general toxic effects on the parent animals such as reduced body and organ weights.

In the first developmental toxicity study in rats the limit concentration of1000 mg/kg bw/day did not induce any adverse effects on intrauterine development; no signs of toxicity were established in the dams. ***In an additionally conducted study employing fenhexamid doses of 300, 1000 and 2000 mg/kg bw/day slightly decreased body weights were observed in dams at 1000 and 2000 mg/kg fenhexamid. No developmental effects occurred up to the high dose of 2000 mg/kg bw.***

In rabbits there was a correlation between slight developmental effects (decreased gestation rate, decreased placental and fetal weights, delayed ossification) and distinct maternal toxicity. No malformations were observed, neither in rats nor in rabbits.

Overall, it can be concluded that fenhexamid has no primary reproductive toxicity.

IIA 5.6.1 Two generation reproductive toxicity in the rat

Please refer to the Monograph and baseline dossier of fenhexamid.

IIA 5.6.2 Separate male and female studies

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.3 Three segment designs

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.4 Dominant lethal assay for the male fertility

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.5 Cross-matings of treated males with untreated females and *vice versa*

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.6 Effects on spermatogenesis

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.7 Effects on oogenesis

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.8 Sperm motility, mobility and morphology

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.9 Investigation of hormonal activity

Not required according to Regulation 1107/2009/EEC or/and Directive 91/414/EEC.

IIA 5.6.10 Teratogenicity test by the oral route in the rat

This supplemental developmental study was conducted in order to address concerns which were raised by the Ministry of Agriculture, Forestry and Fisheries of Japan after review of the original developmental toxicity study with KBR 2738. The original study was conducted with doses of 0 and

1000 mg/kg bw KBR 2738 and revealed no test compound-related effects. The concerns of the Japanese Authorities were whether or not the increased food intake seen in the 1000 mg/kg group was actually treatment related and whether malformed fetuses found in one litter from one dam were caused by the compound.

Report:	**KIIA 5.6.10/03; [redacted]., 1998**
Title:	A supplemental developmental toxicity study with KBR 2738 technical in the Sprague-Dawley rat
Report No.: Document No.:	BC7438 **M-010222-02-1**
Dates of work:	1998-05-19 to 1998-07-09
Guidelines:	OECD 414, US-EPA Guidelines for Developmental Toxicity Risk Assessment, Federal Register Volume 56, Number 234, 1991; US-EPA-TSCA, Health Effects Testing Guidelines, 40 CFR Section 798.4900; JMAFF 59 NohSan No. 4200; Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: KBR 2738DN Technical

- Description: brown powder
- Lot/Batch no: 898805001
- Purity: 97.7%
- Stability of test compound: guaranteed for study duration

2. Vehicle: 0.5% (w/v) aqueous carboxymethylcellulose and 0.4% (v/v) Tween 80 (CMC) solution

3. Test animals:

- Species: rat
- Strain: Sprague-Dawley
- Age: approximately 15 weeks
- Weight at dosing: approximately 250 g. 260 g
- Source: [redacted], USA
- Acclimatisation period: at least 6 days
- Diet: [redacted], USA), ad libitum
- Water: tap water, ad libitum
- Housing: individually (except during mating period) in suspended stainless steel cages with bedding trays with deotized animal cage board (males and females prior to being declared sperm-positive) or in plastic cages with corn cob bedding (females during gestation)

B. Study design and methods

1. Animal assignment and treatment:

Dose:	0-300-1000-2000 mg/kg bw/day
Duration:	daily on gestation days 6 to 15
Application route:	oral (gavage)
Application volume:	10 mL/kg bw
Group size:	30 dams/group
Observations:	mortality, clinical signs, body weight, food consumption, gross pathology, organ weights (liver, thyroid, uterus), reproductive data, fetal data

II. Results and discussion

A. Maternal data

Clinical signs

One dam (no. 73) of the 1000 mg/kg dose group was sacrificed moribund on gestation day 12. Necropsy observations showed that the animal had been improperly gavaged.

The only observation seen consistently throughout the dosing period was tan stool, which occurred only in KBR 2738 treated animals. An overview is given in the following table.

Table 5.6.10-1: Incidence summary of tan stool

Dose (mg/kg bw/day)		0	300	1000	2000
No. of dams evaluated		24	26	26	27
Gestation day 6-15 (treatment phase)	**No. of dams with tan stool**	0	5	2	6
	Occurrence (no. of days)	0	1-4	2	1-7
Gestation day 16-20	**No. of dams with tan stool**	0	0	0	2
	Occurrence (no. of days)	0	0	0	1-2

Tan stool was observed coincident with the dosing period and ceased within two days following administration of the compound. A clear dose response relationship was not observed nor was this finding directly associated with any other effects. Moreover, the relationship between the test compound and tan stool may have simply been related to the brown colour of KBR 2738. Therefore, this finding is not considered to be toxicologically meaningful.

Body weight

No statistically significant effects on body weight were seen in the 300 mg/kg dose group, but a statistically significant decrease of body weight was observeded in both the 1000 and 2000 mg/kg dose groups on each of gestation days 7-16 and 7-14, respectively. The decrease in body weight observed in these two groups ranged from only 4 to 5% compared to control, with no dose response relationship. No dose-response relationship was observed for any body weight gain endpoints. Particular consideration of the entire gestation phase demonstrates that not only is the body weight gain unaffected, but the body weight gain noted in the highest dose level is practically identical to that of control, 140.9 compared to 140.7 g.

Table 5.6.10-2: Mean maternal body weights during gestation (grams)

Dose (mg/kg bw/day) Gestation day	0	300	1000	2000
0	258.5	259.7	256.6	252.3
6	291.6	289.6	286.5	285.0
7	294.4	287.1	281.5*	280.4**
8	297.5	288.3	285.9*	281.9**
9	303.6	294.5	288.7**	284.7**
10	306.3	299.7	293.4*	290.7**
11	311.2	305.1	296.4**	294.6**
12	315.8	310.2	300.7**	299.0**
13	319.4	312.8	305.0**	304.8*
14	325.1	317.8	311.0*	309.3*
15	330.3	325.6	316.3*	319.6
16	340.6	337.5	327.9*	327.5
20	399.1	403.6	390.1	393.2
6-16: body weight gain	49.1	47.9	41.5	42.5
0-20: body weight gain	140.7	144.0	133.5	140.9
Terminal body weight without uterus	326.0	324.8	315.4	317.2
Body weight gain (without uterus weight)	67.6	65.1	58.8	64.9

Significantly different from control: *: $p < 0.05$ **: $p < 0.01$

Food consumption

No statistically significant effects on food consumption were seen in any dose group during gestation days 0 through 16. Following the dosing period, a statistically significant increase in food consumption was observed in the 300 (6.5 %) and 2000 (7.3 %) mg/kg dose levels during days 16 through 20. However, no statistically significant effect was observed in the 1000 mg/kg dose level. As discussed in the previous developmental toxicity study with KBR 2738, food consumption is one of the most variable parameters in a developmental toxicity study. In the present study, food consumption fluctuated throughout gestation, in all dose levels, including those exhibiting statistical significance. Based on the absence of a dose-response relationship, the effects on food consumption are not considered compound-related, but within the normal range of variation expected for this endpoint in this test system.

Table 5.6.10-3: Mean maternal food consumption during gestation (g/kg bw/day)

Dose (mg/kg bw/day) **Gestation day**	**0**	**300**	**1000**	**2000**
0 – 6	79.6	78.7	79.5	80.6
6 – 11	78.3	65.0	71.8	64.9
11 – 16	76.1	77.2	77.5	77.3
16 - 20	72.1	77.1*	75.9	77.8*

Significantly different from control: *: $p < 0.05$

Gross pathology

There were no compound-related maternal necropsy findings in animals sacrificed at the termination of the study.

Organ weight

There were no statistically significant effects on mean final body weight, liver, or thyroid weights, expressed as either the absolute weight or as a percent of final body weight. There were no statistically significant effects on mean gravid uterine weight, netbody weight, or net body weight change.

B. Reproductive Parameter

The fertility index for the control, 300, 1000, and 2000 mg/kg groups was 80.0, 86.7, 90.0, and 90.0%, respectively. All pregnant dams terminated on day 20 gestation had viable fetuses, therefore, the gestation index was 100% for all groups. The mating index for each dose level was 100%.

B. Cesarean section data

Embryo implantation/resorption

No statistically significant differences were seen in the mean number of corpora lutea, implantation sites, pre- and post-implantation loss, or early and late resorptions.

Litter effects

There were no statistically significant differences in litter size, number of fetuses per number of implantations, mean fetal weight, or mean placental weight. There was a statistically significant difference in the percentage of male fetuses/implant in the 2000 mg/kg dose group, 40.8% compared to 47.4% in the control group. However, no statistically significant effect was noted on the median percent male fetuses, 46% compared to 50% in control. Moreover, the 40.8% male fetuses/implant observed in the 2000 mg/kg dose group was within the historical control range (38.2%-56.5%) for this finding in the Sprague-Dawley rat. Based on the preceding discussion, this observation is not considered compound-related but within normal variation for this test system.

C. Fetal examination

Fetal external malformations/variations

There were no statistically significant effects on the fetal or litter incidences of external malformations or variations in any dose group.

Fetal external observations included one fetus from one litter in the control group which had a constricted tail, one fetus from one litter in the 300 mg/kg group which exhibited gastroschisis, and one fetus from one litter in the 1000 mg/kg group which had multiple malformations (exencephaly, protruding tongue, spina bifida and a curly tail). There were no findings in the 2000 mg/kg group.

Based on the sporadic, dose independent incidences the aforementioned findings are not considered compound-related.

Fetal Visceral Malformations/Variations

One dam from the control group (no. 18) had only one fetus, which was evaluated for skeletal anomalies. Therefore, the number of litters from the control group which underwent a visceral examination was twenty-three. Fetal visceral malformations were observed in 3 fetuses from 3 litters of the control group, 1 fetus from 1 litter of the 300 mg/kg dose group, and 5 fetuses from 4 litters of the 1000 mg/kg dose group. There were no malformations seen in the 2000 mg/kg group. The incidence of malformations was independent of treatment, and included: transposition of great vessels, observed in the control group and 1000 mg/kg dose group; heart, reduced in size, observed in the control and 1000 mg/kg dose groups; and anophthalmia observed in the 300 mg/kg dose group.

Fetal visceral variations were observed in 12 fetuses from 8 litters of the control group, 6 fetuses from 5 litters of the 300 mg/kg dose group, 8 fetuses from 5 litters of the 1000 mg/kg dose group, and 7 fetuses from 6 litters of the 2000 mg/kg dose group. 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 in the 2000 mg/kg dose group).

Fetal skeletal malformations/variations

Fetal skeletal malformations were observed in 3 fetuses from 3 litters of the control group, 4 fetuses from 3 litters of the 300 mg/kg dose group, 1 fetus from 1 litter of the 1000 mg/kg dose group, and 4 fetuses from 4 litters of the 2000 mg/kg dose group. The incidence of malformations were independent of treatment, and included: missing ribs; extra, fused, or missing thoracic arches; and missing lumbar arches or centra. One fetus in the 1000 mg/kg dose group 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.

Total fetal malformations/variations

There were no statistically significant effects on the total fetal and litter incidences of malformations and variations. There was no effect on the fetal or litter incidence of total affected fetuses.

Relationship between gender and malformations

No gender-related differences were observed on the incidences of external, visceral, or skeletal malformations. No statistically significant differences were observed between the mean affected males and females per litter for total malformations or the individual malformation categories.

III. Conclusion

Based on the findings of the present study, which included a dose level two-fold greater than the previous study, neither the food consumption nor the fetal effects observed in the original study were test compound-related.

NOEL maternal toxicity: 300 mg/kg bw/day / NOAEL maternal toxicity: 2000 mg/kg bw/day based on slightly decreased body weight (by up to 4-5 %) at 1000 and 2000 mg/kg bw

NOEL developmental toxicity: 2000 mg/kg bw

IIA 5.6.11 Teratogenicity test by the oral route in the rabbit

Please refer to the Monograph and baseline dossier of fenhexamid.

IIA 5.7 Neurotoxicity

Summary of neurotoxicity studies

As determined by special functional observations, automated activity measurements and neuro-histopathology, no specific neurotoxic effects -were induced in rats following single oral application of high doses of fenhexamid.

IIA 5.7.1 Acute neurotoxicity – rat

Please refer to the Monograph and baseline dossier of fenhexamid.

IIA 5.7.2 Delayed neurotoxicity following acute exposure

As fenhexamid is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, testing is not necessary.

IIA 5.7.3 28-day delayed neurotoxicity

As fenhexamid is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, testing is not necessary.

IIA 5.7.4 Subchronic neurotoxicity - rat - 90-day

A subchronic neurotoxicity study was not considered to be necessary, since no indication for neurotoxicity was observed in the acute neurotoxicity study up to the high dose of 2000 mg/kg bw as well as in the whole toxicological data package on fenhexamid.

IIA 5.7.5 Postnatal developmental neurotoxicity

Not required according to Regulation 1107/2009/EEC or Directive 91/414/EEC.

IIA 5.8 Toxicity studies on metabolites

No studies were performed as plants metabolites are identical to those formed in animals.

IIA 5.9 Medical and clinical data

IIA 5.9.1 Report on medical surveillance on manufacturing plant personnel

Report:	**KIIA 5.9.1/01, [redacted] 2004 (amended: 2011-10-04)**
Title:	Occupational medical experiences with Fenhexamid
Report No.:	
Document No:	**M-128685-02-1**
Dates of work:	not applicable
Guidelines:	not applicable
GLP:	no

I. Materials and methods

Test material:	fenhexamid, trade name: Teldor
No. of workers exposed:	50 per year, 85 over 3 years
Production period:	one batch production per year (400 – 500 tons per year)
Personal safety measures:	work clothing, safety shoes, chemical protection gloves, safety glasses, for charging and filling in addition Tyrex-type protective suit and dust mask
Medical examinations:	Basic: history, full physical examination with orientating neurological status (reflexes, sensibility, coordination) and skin status Based on German regulation, if applicable for work tasks in the plant, not related to fenhexamid: G7(CO), G25 (driving/steering), G26/2 (breathing protection), G37 (VDU work), G 40 (carcinogens)
Commenced on :	1999
Examination intervals:	Basic and G7 annually, all others every 3 years
Laboratory examinations:	ESR (erythrocyte sedimentation rate), full blood count, AST, ALT, γ-GT, glucose, creatinine, cholesterol, urine status
Technical examinations:	Lung function testing, ecg, vision-testing, audiometry, chest-x-ray, sonography as required by the examination schedules

II. Results and discussion

Occupational medical surveillance of workers exposed to fenhexamid, performed annually on a routine basis, not directly related to exposures, did not reveal any unwanted effects in the workers. The examinations included the above laboratory parameters and clinical and technical examinations. During the batch production period(s) since 1999 no accidents with fenhexamid occurred in the workers, and no consultations of the Medical Department due to work or contact with fenhexamid were required.

III. Conclusion

No unusual occurrences were observed.

IIA 5.9.2 Report on clinical cases and poisoning incidents

With regard to fenhexamid no cases of human poisoning have been reported up to mid of January 2012.

IIA 5.9.3 Observations on general population exposure & epidemiological studies

Up to now there is no known exposure of the general population to fenhexamid. No epidemiological studies have been performed on fenhexamid.

IIA 5.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

No cases of human poisoning with fenhexamid are known so far. In most of the acute toxicity studies the limit dose was tolerated by the animals without any clinical signs or symptoms. Only after oral administration of 5000 mg/kg bw fenhexamid to mice unspecific clinical signs , i.e. apathy, piloerection and in females spastic gait, were observed on the day of exposure. Specific clinical signs and symptoms of poisoning are therefore also not expected in men.

For an exact diagnosis of fenhexamid poisoning in men analytical confirmation of the parent compound or its metabolites in blood, urine orgastrointestinal contents is required.

IIA 5.9.5 First aid measures

- ***Remove patient from exposure / terminate exposure***
- ***Thorough skin deciontamination with copious amounts of water and soap, if available with polyethylenglycol 300 followed by water.***
  <u>Note</u>: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polyethylenglycol 300 is not required.
- ***Flushing of the eyes with lukewarm water for 15 minutes.***
- ***Induction of vomiting does not seem to be required in regard of the low toxicity.***
  It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious. Induced vomiting can remove maximum 50% of the ingested substance.
  <u>Note</u>: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested.

IIA 5.9.6 Therapeutic regimes

- ***As there is no antidote available for fenhexamid, treatment has to be symptomatic and supportive.***
- ***Gastric lavage does not seem to be required in regard of the low toxicity of the compound.***
- ***The application of activated charcoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.***

IIA 5.9.7 Expected effects & duration of poisoning as a function of exposure

No cases of human poisoning with fenhexamid are known. In an acute acute oral toxicity study in rats the limit dose was tolerated by the animals without any clinical signs or symptoms. Only in mice unspecific clinical signs , i.e. apathy, piloerection and in females spastic gait, were observed after a single oral dose of 5000 mg/kg bw fenhexamid. Similar unspecific signs of poisoning could be possible also in man after oral ingestion of such a very high dose of fenhexamid, which, however, is rather unlikely.

After dermal application of 5000 mg/kg bw fenhexamid to rats no systemic nor local signs were noted. This fits to the overall very low acute oral toxicity of fenhexamid and its very limited dermal absorption (fenhexamid WP 50: dermal absorption in the rat in vivo: 2% for the concentrate, 18% for the dilution; fenhexamid WG 50: in vitro dermal absorption through dermatomed human skin: 0.15% for the concentrate, 5.83% for the 1:1333 diluted spray dilution). Due to these facts poisoning via dermal exposure with fenhexamid is also not expected in man.

This applies also for inhalative exposure, since acute inhalation of 5057 mg fenhexamid/m³ as dust for 4 hours was tolerated by rats without any clinical signs and symptoms.

IIA 5.9.8 Effects & duration of poisoning as a function of time

No cases of human poisoning with fenhexamid are known and in most of the acute toxicity studies the limit dose was tolerated by the animals without any clinical signs or symptoms. Only in mice after a single oral dose of 5000 mg/kg bw fenhexamid unspecific clinical signs , i.e. apathy, piloerection and in females spastic gait, occurred 50 to 55 minutes after administration and lasted for the day of treatment in males and up to 4 hours after dosing in females. This time effect relationship reflects pharmacokinetics after oral administration of fenhexamid. Due to the rapid and almost complete absorption of fenhexamid maximal plasma concentrations were reached even after oral administration of a high dose in rats within 4 h. Due to the relatively fast elimination of the compound from the body clinical signs were only visible on the day of dosing, but had ceased 24 h after administration. Similar unspecific signs of toxicity commencing shortly after ingestion and lasting for up to 1 day could also occurr in man after ingestion of such a limit dose of fenhexamid. However, ingestion of such a high dose of fenhexamid is not very likely in man.

IIA 5.9.9 Dermal penetration

Summary of dermal penetration:

A dermal penetration study in vivo was conducted in the rat using pure and diluted fenhexamid WP 50. The resulting overall absorption figures of 2% for the concentrate and 18% for the spray dilution (1:99) were used to conduct the operator risk assessment at Annex I inclusion (see Monograph and Annex III dossier on fenhexamid WP 50).

In addition, a new comparative in vitro dermal absorption study using human and rat skin was performed on the current representative formulation fenhexamid WG 50. Dermal penetration of [^{14}C]-fenhexamid through human and rat dermatomed skin was investigated for the neat product (concentrate, 500 g fenhexamid/kg) and two representative spray dilutions (5 and 0.375 g

fenhexamid/L). The mean percentage of [^{14}C]-fenhexamid considered to be potentially absorbable (directly absorbed plus total remaining at dose site) over a period of 24 hours after 8 hours exposure amounted for the concentrate and the spray dilutions of 5 g/L or 0.375 g/L to 0.15, 0.62 and 5.83% for human skin and to 1.13, 1.03 and 12.22% for rat skin.

Report:	**KIIA 5.9.9/02, [redacted] 2009**
Title:	Fenhexamid WG 50: [^{14}C]-fenhexamid: Comparative *in-vitro* dermal absorption study using human and rat skin.
Report N°:	SA 09113, issued on 17th December 2009
Document N°:	**M-360644-01-1**
Dates of experimental work:	Start: 18th August 2009 End: 16th September 2009
Guidelines:	O.E.C.D. guideline for the testing of chemicals; skin absorption: *in vitro* Method 428 (April 2004). O.E.C.D. Environmental health and safety publications series on testing and assessment N°28, Guidance document for the conduct of skin absorption studies (March 2004). European Commission guidance document on dermal absorption-Sanco/222/2000 rev. 7, (March 2004).
Deviations:	None
GLP	Yes

Material and methods

Rat skin:

Species, strain:	Rat, Wistar Rj: WI (IOPS HAN).
Source:	[redacted] (France).
Sex:	Male.
Number:	10.
Anatomical site:	Dorsal.
Rat Skin Preparation:	Each animal was killed by cervical dislocation. After sacrifice the skin was clipped and removed for use in the study. The dorsal skin was dermatomed by use of a mini-dermatome to obtain samples of *ca* 430 to 510 µm in thickness.

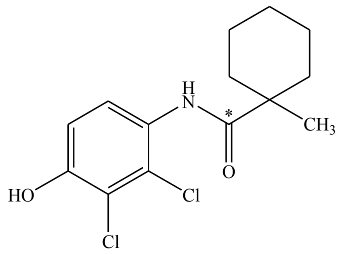
Human skin:

Source: [redacted], France.
Number and sex: 7 donors, female.
Anatomical region: Abdomen.
Thickness: 415 to 550 µm.

Test Material:

Non-radiolabelled:	Batch: KTS10158-1-1. Purity = 98.7%.
Radiolabelled:	[carboxamide-^{14}C] fenhexamid Batch: KATH 6755. Specific activity: 4.18 MBq/mg. Radiopurity of the formulation: >99%.

Structural formula:



* denotes position of radiolabel

Formulation: The formulation used in this experiment was the a fenhexamid WG 50 formulation (specification number 102000007271) used at three nominal concentrations: 500 g a.s./kg, 5 g a.s./L and 0.375 g a.s./L.

Test system: A flow-through diffusion cell system ([redacted] cell modified, [redacted], France) was used to study the absorption of the test substance (exposure area of 1 cm^2 skin). A diffusion cell consisted of a donor chamber and a receptor chamber between which the skin was positioned. The receptor fluid was Eagle's medium supplemented with 5% bovine serum albumin and gentamycin (50 mg/L) at a pH of ca. 7.4. The receptor chamber was warmed by a constant circulation of warm water which maintained the receptor fluid at 32 ± 2°C (close to the normal skin temperature). The receptor fluid was pumped through the receptor chamber at a rate of 1.5 mL/h and stirred continuously whilst in the receptor chamber by means of a magnetic bar.

Skin integrity: Before dose application, the integrity of the skin samples was assessed by measuring the trans-epidermal water loss (TEWL) from the stratum corneum. An evaporimeter probe (Tewameter TM300 system, [redacted]) was placed securely on the top of the donor chamber and the amount of water diffusing through the skin was measured. Human and rat skin with a TEWL of greater than 15 g/hm^2 were considered potentially damaged and were not used. These samples were replaced by new skin fragments which were also tested for integrity before use in the study.

Treatment: The dose preparation was applied to the split-thickness skin sample with a pipette at the rate of approximately 5 mg/cm^2 or10 µL/cm^2 exposed skin. The dose preparations were assayed for radioactivity content (by LSC) by using dose checks (surrogate dose) taken before, during and after the dosing process.

Sampling: The receptor fluid passing through the receptor chamber was collected in glass vials held in a fraction collector. The fraction collector was started after dose application. Samples were then collected hourly for the duration of the experiment (24 hours). At 8 hours post-application, the skin was swabbed with freshly prepared 1% v/v Tween 80 in PBS (phosphate buffer saline) using natural sponge swabs, in order to remove and retain the non-absorbed dose, until no radioactivity was detected with a [redacted] monitor. At the end of the study (24 hours after application), the treated skin and the skin adjacent to the treatment site (surrounding swabs) were swabbed. Each skin sample was tape-stripped to remove the stratum corneum. This involved the application of Monaderm adhesive tape ([redacted], Monaco) for 5 seconds before the tape was carefully removed against the direction of hair growth. This procedure was continued until a ‘shiny’ appearance of the epidermis

was evident, which indicated that the stratum corneum had been removed. The tape-strips were collected into scintillation vials for analysis. The skin surrounding the application site (surrounding skin) was separated from the treated skin. Both surrounding skin and tape-stripped treated skin were retained for analysis.

Radioassay: The amounts of radioactivity in the various samples were determined by liquid scintillation counting (LSC). Samples were counted for 10 minutes or for 2 sigma % in an appropriate scintillation cocktail using a Packard 1900 TR counter with on-line computing facilities. Quenching effects were determined using an external standard and spectral quench parameter (tSIE) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C-n-hexadecane standards. The scintillation counter was recalibrated when a deviation of greater than 2% was observed when counting quality control standards. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.

Findings:

The fenhexamid was demonstrated to be soluble in the receptor fluid up to the maximum amount formulation applied. The solubility in the receptor fluid was deemed to be sufficient to reduce any risk of back diffusion.

Measurements of the homogeneity of the three concentrations of formulation applied indicated that it was acceptable. Good recovery data were obtained, with mean total recoveries of radioactivity in the range of 102.2% to 104.1% of the applied dose.

For the neat formulation, almost all the radioactivity was removed by swabbing (103.7% and 101.0% of dose for human and rat skin, respectively) and by removal of the surface dose (0.06% and 1.45% of dose for human and rat skin, respectively). For the intermediate representative dilution, almost all the radioactivity was also removed by swabbing (102.5% and 100.7% of dose for human and rat skin, respectively) and by removal of the surface dose (0.12% and 0.84% of dose for human and rat skin, respectively). For the low representative dilution, the vast majority of the radioactivity was also removed by swabbing (95.8% and 85.8% of dose for human and rat skin, respectively) and by removal of the surface dose (0.64% and 5.10% of dose for human and rat skin, respectively).

Since the swabbing procedure was intended to reflect a simple washing regimen at the end of the working day, the amount of radioactivity retrieved in this compartment was considered to be non-absorbed. Since the material recovered in the surface tape-strips (first two tape-strips) could be associated with surface residues following incomplete removal of the dose after an 8-hour exposure period and/or material from the superficial stratum corneum, the amount of radioactivity retrieved in this compartment was considered to be non-absorbed.

Based on these results, the mean total amount of radioactivity considered as non-absorbed for the neat formulation was 104.0% and 102.7% dose in the human and rat skin, respectively, the mean total amount of radioactivity considered as non-absorbed for the intermediate representative dilution was 102.7% and 101.6% dose in the human and rat skin, respectively and the mean total amount of

radioactivity considered as non-absorbed for the low representative dilution was 96.4% and 90.9% dose in the human and rat skin, respectively.

The overall amount of [^{14}C]-fenhexamid considered to be directly absorbed was represented by the radioactivity present in the receptor fluid, receptor fluid at termination time and receptor chamber. This accounted for means of 0.04% (human) and 0.12% (rat) of the dose applied for the neat formulation, for means of 0.50% (human) and 0.84% (rat) of the dose applied for the intermediate representative dilution and for means of 4.59% (human) and 5.19% (rat) of the dose applied for the low representative dilution.

The amount of radioactivity recovered in the skin (after tape-stripping and including surrounding skin) in the neat formulation accounted for means of 0.03% (human) and 0.25% (rat) of the applied dose, for means of 0.04% (human) and 0.06% (rat) of the dose applied for the intermediate representative dilution and for means of 0.56% (human) and 2.05% (rat) of the dose applied for the low representative dilution.

The mean quantity of radioactivity recovered in the stratum corneum with the neat formulation accounted for 0.05% (human) and 0.76% (rat) of the applied dose, for 0.08% (human) and 0.14% (rat) of the applied dose for the intermediate representative dilution and for 0.68% (human) and 4.98% (rat) of the applied dose for the low representative dilution.

The radioactivity found in the skin compartment (skin, surrounding skin and stratum corneum) could be considered to be potentially absorbable. Therefore, the mean total amount of radioactivity considered to be potentially absorbable for the neat formulation was 0.15% and 1.13% dose for the human and rat skin respectively. The mean total amount of radioactivity considered to be potentially absorbable for the intermediate representative dilution was 0.62% and 1.03% dose for the human and rat skin respectively. The mean total amount of radioactivity considered as potentially absorbable for the low representative dilution was 5.83% and 12.22% dose for the human and rat skin respectively.

Table 5.9.9-1: Mean distribution of radioactivity at 24 hours after dose application of [14C]-fenhexamid in an WG formulation at the rates of 500 g/kg, 5 g/L and 0.375 g/L to human and rat skin samples. **Results expressed in terms of percentage of applied radioactivity.**

	Distribution of radioactivity (% dose)											
Dose Levels	**Neat formulation: High dose (SYP13458, 500 g/kg)**				**Dilution: Intermediate dose (SYP13461, 5 g/L)**				**Dilution: Low dose (SYP13463, 0.375 g/L)**			
Species	**Human (n=6)**		**Rat (n=6)**		**Human (n=6)**		**Rat (n=6)**		**Human (n=6)**		**Rat (n=6)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
SURFACE COMPARTMENT												
Skin swabs (8h)	**103.68**	**2.02**	**100.95**	**2.86**	**102.4**	**2.12**	**100.6**	**2.34**	**92.07**	**9.27**	**83.72**	**6.55**
Skin swabs (24h)[a]	**0.03**	**0.03**	**0.08**	**0.05**	**0.07**	**0.09**	**0.08**	**0.06**	**3.70**	**4.12**	**2.05**	**1.93**
Surface Dose (tape-strips 1 & 2)	**0.06**	**0.04**	**1.45**	**0.90**	**0.12**	**0.18**	**0.84**	**0.57**	**0.64**	**0.56**	**5.10**	**1.93**
Donor chamber	**0.18**	**0.13**	**0.23**	**0.18**	**0.03**	**0.07**	**0.05**	**0.03**	**n.d.**	**n.a.**	**0.04**	**0.10**
Total % non-absorbed	***103.95***	***2.02***	***102.71***	***3.00***	***102.67***	***2.13***	***101.58***	***2.41***	***96.41***	***10.16***	***90.91***	***7.10***
SKIN COMPARTMENT												
Skin [b]	**0.05**	**0.03**	**0.25**	**0.33**	**0.04**	**0.04**	**0.06**	**0.08**	**0.56**	**0.50**	**2.05**	**3.73**
Stratum corneum [c]	**0.05**	**0.04**	**0.76**	**0.51**	**0.08**	**0.07**	**0.14**	**0.28**	**0.68**	**0.64**	**4.98**	**3.10**
Total % at dose site	***0.10***	***0.05***	***1.01***	***0.61***	***0.12***	***0.09***	***0.19***	***0.29***	***1.24***	***0.81***	***7.02***	***4.85***
RECEPTOR COMPARTMENT												
Receptor fluid (0-24h)	**0.04**	**0.02**	**0.12**	**0.03**	**0.50**	**0.35**	**0.84**	**0.37**	**4.59**	**2.18**	**5.19**	**2.13**
Receptor chamber	**n.d.**	**n.a.**	**n.d.**	**n.a.**	**n.d.**	**n.a.**	**n.d.**	**n.a.**	**n.d.**	**n.a.**	**n.d.**	**n.a.**
Total % directly absorbed [d]	***0.04***	***0.02***	***0.12***	***0.03***	***0.50***	***0.35***	***0.84***	***0.37***	***4.59***	***2.18***	***5.19***	***2.13***
Total % Potentially Absorbable	***0.15***	***0.05***	***1.13***	***0.61***	***0.62***	***0.36***	***1.03***	***0.47***	***5.83***	***2.33***	***12.22***	***5.30***
TOTAL % RECOVERY	**104.1**	**2.03**	**103.8**	**2.38**	**103.3**	**2.17**	**102.6**	**1.91**	**102.2**	**3.65**	**103.1**	**1.81**

[a]: sum of radioactivity found in swabs at termination and in surrounding swabs.
[b]: sum of radioactivity found in skin after tape-stripping procedure and in surrounding skin.
[c]: tape-strips excluding numbers 1 & 2 which are considered to be non-absorbed dose.
[d]: sum of radioactivity found in receptor fluid (0-24h), receptor fluid terminal and receptor chamber.
[e]: total % directly absorbed + total % at dose site
SD: standard deviation
n.d.: not detected (below the limit of detection)
n.a. : not applicable
n: number of skin cells used for calculation
In the above table, the presented means do not always calculate exactly from the presented individual data. This is due to rounding-up differences resulting from the use of the spreadsheet program.

Conclusion:

The dermal penetration of [^{14}C]-fenhexamid through human and rat dermatomed skin from the WG 50 formulation was investigated at three concentrations corresponding to the neat product (500 g /kg) and to two representative dilutions (5 and 0.375 g/L), respectively.

The mean percentage of [^{14}C]-fenhexamid considered to be potentially absorbable (directly absorbed plus total remaining at dose site) over a period of 24 hours for the neat fenhexamid WG 50 formulation was 0.15% and 1.13% for the human and rat skin, respectively, yielding a factor difference of 7.5 between the two species for the neat product.

The mean percentage of [^{14}C]-fenhexamid considered to be potentially absorbable (directly absorbed plus total remaining at dose site) over a period of 24 hours for the intermediate representative dilution of the fenhexamid WG 50 formulation was 0.62% and 1.03% for the human and rat skin respectively, yielding a factor difference of 1.7 between the two species for the intermediate dose formulation.

The mean percentage of [^{14}C]-fenhexamid considered to be potentially absorbable (directly absorbed plus total remaining at dose site) over a period of 24 hours for the representative low representative dilution of the fenhexamid WG 50 formulation was 5.83% and 12.22% for the human and rat skin respectively, yielding a factor difference of 2.1 between the two species for the low dose formulation.

IIA 5.10 Other/special studies

Summary of other studies

The chapter other/special studies comprises new studies on two impurities of fenhexamid and a new study investigating the pharmacological properties of fenhexamid, as well as an acute study in rats with intraperitoneal administration and a subchronic feeding study in rats for the determination of fenhexamid concentrations in plasma and urine.

The impurities 1,2-DCMP-KBR 2738 and 1,3-DCMP-KBR 2738 were investigated for genotoxicity as well as for acute oral toxicity. Like the active ingredient fenhexamid, both impurities were negative for point mutation in Ames test exhibiting an acute oral rat toxicity > 2000 mg/kg bw.

A study investigating the pharmacological effects of fenhexamid showed no marked effects of the compound on the general condition, behaviour, the central and autonomic nervous system, the respiratory and circulating system, the somatic nervous system, the digestive system, renal function and on blood or blood cells. Additionally, the results indicate that at doses below 5000 mg/kg bw fenhexamid causes no symptoms of acute toxicity.

Fenhexamid shows no significant acute toxicity after intraperitoneal administration.

Table 5.10-1: Acute intraperitoneal toxicity

Species	*Vehicle*	*Sex*	*NSD*# *[mg/kg bw]*	*LLD*§ *[mg/kg bw]*	*LD50* *[mg/kg bw]*	*Reference*
rat	*water, demineralized*	*male* *female*	*50* *50*	*> 1000* *1000*	*> 1000* *> 1000*	[redacted] *1991* *Report 20642* *(M-010377-01-1)*

NSD: no-symptoms dose;
§ LLD: lowest lethal dose

A subchronic feeding study in rats for the determination of fenhexamid concentrations in plasma and urine showed that the compound was well absorbed from the gastrointestinal tract. In females there was no saturation of absorption up to a concentration of 20000 ppm. For males saturation can be assumed slightly below this limit concentration.

Studies on impurities

The impurities 1,2-DCMP-KBR 2738 and 1,3-DCMP-KBR 2738 were not detected in the first analysis of batch 4258/76, which was used to conduct the main part of the toxicological studies on fenhexamid. As new impurities they were therefore investigated for genotoxicity in an Ames test as well as for acute oral toxicity in rats.

1,2-DMCP-KBR 2738

Report:	**KIIA 5.10/01,** [redacted]**., 2003**
Title:	1,2-DMCP-KBR 2738 - Acute toxicity in the rat after oral administration
Report No.: Document No.:	AT00510 **M-111009-01-1**
Dates of work:	2003-04-03 to 2003-04-22
Guidelines:	OECD 423; Directive 67/548/EEC; Annex IV B, Part B, B. 1 tris; US-EPA 712-C-98-190, OPPTS 870.1100; Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: 1,2-DMCP-KBR 2738
- Description: white powder
- Lot/Batch no: KTS 9977-2-1
- Purity: 99.1%
- Stability of test compound: guaranteed for study duration; expiry date: 2003-08-27

2. Vehicle: demineralised water with 2% Cremophor EL

3. Test animals:
- Species: Wistar rat
- Strain: HsdCpb:Wu
- Age: approx. 9 – 10 weeks

Weight at dosing:	145 – 161 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	standard diet "[redacted]3883.0.15 Maus/Ratte [redacted], [redacted]Switzerland", ad libitum except for the fasting time before and after administration indicated below
Water:	tap water, ad libitum
Housing:	group caged conventionally in polycarbonate cages, bedding: low dust wood granulate ([redacted] Germany)

B. Study design and methods

1. Animal assignment and treatment:

Dose:	2000 mg/kg bw
Application route:	oral
Application volume:	10 mL/kg bw
Fasting time:	before administration: approx. 16–24 h after administration: approx. 2–4 h
Group size:	3 females/group
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, body weight, gross necropsy

II. Results and discussion

A. Mortality

Table 5.10-2: Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Female rats						
(1st) 2000	0	0	3	--	--	0
(2nd) 2000	0	3	3	5 h – 2 d	--	0
LD_{50}: >2000 mg/kg bw						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd number = number of animals used

h: hours d: days

B. Clinical observations

Diarrhea was observed in 3 of 6 rats from 5 h after administration to 2 days after administration.

C. Body weight

There were no toxicological effects on body weights or on body weight gain.

D. Necropsy

The necropsies performed at the end of the study revealed no particular findings.

III. Conclusion

1,2-DMCP-KBR 2738 is non-toxic after acute oral administration to rats.

Classification/labelling according to Commission Directive 1999/45/EEC as amended: none

Report:	**KIIA 5.10/02 [redacted] 2003**
Title:	1,2-DMCP-KBR 2738 - Salmonella/microsome test - Plate incorporation and preincubation method
Report No.: Document No.:	AT00474 **M-109845-01-1**
Dates of work:	2003-04-09 to 2003-04-25
Guidelines:	OECD 471; Commission Directive 2000/32/EC; B.13/14; US-EPA712-C-98-247, OPPTS 870.5100; Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: 1,2-DMCP-KBR2738

Development no.:	300024188 4
Description:	fine brown powder
Lot/Batch no:	KTS9977-2-1
Purity:	99.1%
Stability of test compound:	guaranteed for study duration, expiry date: 2003-08-27

2. Vehicle and/or positive control:

Vehicle: dimethyl sulfoxide (DMSO, used for solvent control, test substance and positive controls except for Mitomycin C),
deionized water (used for Mitomycin C)

Positive controls:
Sodium azide (Na-azide),
Nitrofurantoin (NF)
4-Nitro-1,2-phenylene diamine (4-NPDA)
Mitomycin C (MMC)
Cumene hydroperoxide (Cumene),
2-Aminoanthracene (2-AA)

3. Test system:

Salmonella typhimurium strains TA1535, TA1537, TA100, TA98, TA102

metabolic activation: S9 mix from Arochlor 1254 induced rat livers

B. Study design and methods

1. Treatment:

Dose:	0-16-50-158-500-1581-5000 µg/plate +/-S9 mix
	0-1.6-5-16-50-158-500 µg/tube +/-S9 mix
	positive controls
	Na-azide 10 µg/plate (TA1535, -S9 mix)
	NF 0.2 µg/plate (TA100, -S9 mix)
	4-NPDA 0.5-10 µg/plate (TA98, TA1537, both -S9 mix)
	MMC 0.2 µg/plate (TA102, plate incorporation trials, -S9 mix)
	Cumene 50 µg/plate (TA102, preincubation trials, -S9 mix)
	2-AA 3 µg/plate (all strains, +S9 mix)
	For each test solution or control n = 3 plates were used.
Application volume:	0.1 mL/plate
Incubation time / temperature:	48 h, 37°C

II. Results and discussion

Doses up to and including 50 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses, the substance had a strong, strain-specific bacteriotoxic effect, so that this range could only be used to a limited extent up to and including 500 µg per plate for assessment purposes.
Evidence of mutagenic activity of 1,2-DMCP-KBR 2738 was not seen. No biologically relevant increase in the mutant count was observed in comparison to the negative controls.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, Mitomycin C, cumene hydroperoxide and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

Table 5.10-3: Summary of mean values without S9 mix

	Strain				
Concentration [µg/plate]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	9	137	5	55	440
16	12	144	7	59	419
50	15	130	6	60	387
158	17	145	5	65	322
500	17	138	5	44	259
1581	-	33	0	0	32
5000	0	-	0	0	--
Na-azide	798				
NF		364			
4-NPDA			83	184	
MMC					801
Concentration [µg/tube]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	14	127	7	19	255
1.6	17	149	9	25	251
5	17	148	6	17	242
16	15	146	7	23	253
50	18	136	5	20	260
158	20	138	5	24	268
500	14	72	0	-	54
Na-azide	688				
NF		545			
4-NPDA			130	174	
Cumene					487

Table 5.10-4: Summary of mean values with S9 mix

	Strain				
Concentration [µg/plate]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	10	157	12	71	349
16	8	140	11	62	312
50	9	164	7	70	298
158	9	144	13	79	263
500	7	130	7	68	242
1581	-	36	0	-	80
5000	-	-	0	-	-
2-AA	149	1789	264	1666	578
Concentration [µg/tube]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	10	149	11	36	378
1.6	9	138	6	31	380
5	12	121	7	33	385
16	11	145	7	33	379
50	9	137	10	28	359
158	9	131	10	24	355
500	3	81	1	16	204
2-AA	159	1791	321	1755	732

III. Conclusion

1,2-DMCP-KBR 2738 is non-mutagenic without and with S9 mix in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

1,3-DMCP-KBR 2738

Report:	**KIIA 5.10/03, [redacted] 2003**
Title:	1,3-DMCP-KBR 2738 - Acute toxicity in the rat after oral administration
Report No.: Document No.:	AT00511 **M-111019-01-1**
Dates of work:	2003-04-03 to 2003-04-22
Guidelines:	OECD 423; Directive 67/548/EEC; Annex IV B, Part B, B. 1 tris; US-EPA 712-C-98-190, OPPTS 870.1100; Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: 1,3-DMCP-KBR 2738

Description: beige crystals

Lot/Batch no.: KTS 9415-4-3

Purity: 99.1%

Stability of test compound: guaranteed for study duration; expiry date: 2003-08-14

2. Vehicle: demineralised water with 2% Cremophor EL

3. Test animals:

Species:	Wistar rat
Strain:	HsdCpb:Wu
Age:	approximately 9 – 10 weeks
Weight at dosing:	149– 164 g
Source:	[redacted], Germany
Acclimatisation period:	at least 5 days
Diet:	standard diet "[redacted] 3883.0.15 Maus/Ratte Haltung, [redacted] Switzerland", ad libitum except for the fasting time before and after administration indicated below
Water:	tap water, ad libitum
Housing:	group caged conventionally in polycarbonate cages; bedding: lowdust wood granulate ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose:	2000 mg/kg bw
Application route:	oral (gavage)
Application volume:	10 mL/kg bw
Fasting time:	before administration: approx. 16– 24 h after administration: approx. 2 – 4 h
Group size:	3 females/group
Post-treatment observation period:	14 days
Observations:	mortality, clinical signs, body weight, gross necropsy

II. Results and discussion

A. Mortality

Table 5.10-5: Doses, mortality / animals treated

Dose (mg/kg bw)	Toxicological result*			Onset and duration of signs	Onset of death after	Mortality (%)
Female rats						
(1st) 2000	0	0	3	--	--	0
(2nd) 2000	0	0	3	--	--	0
LD$_{50}$: >2000 mg/kg bw						

* 1st number = number of dead animals, 2nd number = number of animals with toxic signs
3rd number = number of animals used

B. Clinical observations

No clinical signs were observed.

C. Body weight

There were no toxicological effects on body weights or on body weight gain.

D. Necropsy

The necropsies performed at the end of the study revealed no particular findings.

III. Conclusion

1,3-DMCP-KBR 2738 is non-toxic after acute oral administration to rats.

Classification/labelling according to Commission Directive 1999/45/EEC as amended: none

Report:	**KIIA 5.10/04, [redacted] 2003**
Title:	1,3-DMCP-KBR 2738 - Salmonella microsome test - Plate incorporation and preincubation method
Report No.: Document No.:	AT00466 **M-108538-01-1**
Dates of work:	2003-04-29 to 2003-05-09
Guidelines:	OECD 471, Commission Directive 2000/32/EC, B.13/14; US-EPA 712-C-98-247, OPPTS 870.5100; Deviation(s): none
GLP:	yes

I. Materials and methods

A. Materials

1. Test material: 1,3-DMCP-KBR2738

Development no.:	3000241892
Description:	fine light-brown crystalline powder
Lot/Batch no:	KTS9415.4-3
Purity:	99.1%
Stability of test compound:	guaranteed for study duration; expiry date: 2003-08-14

2. Vehicle and/or positive control: Vehicle: dimethyl sulfoxide (DMSO, used for solvent control, test substance and positive controls except for Mitomycin C),
deionized water (used for Mitomycin C)
Positive controls:
Sodium azide (Na-azide),
Nitrofurantoin (NF)
4-Nitro-1,2-phenylene diamine (4-NPDA)
Mitomycin C (MMC)
Cumene hydroperoxide (Cumene),
2-Aminoanthracene (2-AA)

3. Test system: Salmonella typhimurium strains TA1535, TA1537, TA100, TA98, TA 102

metabolic activation: S9 mix from Arochlor 1254 induced rat livers

B. Study design and methods

1. Treatment:

Dose:	0-16-50-158-500-1581-5000 µg/plate +/-S9 mix 0-1.6-5-16-50-158-500 µg/tube +/-S9 mix positive controls Na-azide 10 µg/plate (TA1535, -S9 mix) NF 0.2 µg/plate (TA100, -S9 mix) 4-NPDA 0.5-10 µg/plate (TA98, TA1537, both -S9 mix) MMC 0.2 µg/plate (TA102, plate incorporation trials, -S9 mix) Cumene 50 µg/plate (TA102, preincubation trials, -S9 mix) 2-AA 3 µg/plate (all strains, +S9 mix) For each test solution or control n = 3 plates were used.
Application volume:	0.1 mL/plate
Incubation time / temperature:	48 h, 37°C

II. Results and discussion

Doses up to and including 50 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses, the substance had a strong, strain-specific bacteriotoxic effect, so that this range could only be used to a limited extent up to and including 500 µg per plate for assessment purposes.
Evidence of mutagenic activity of 1,3-DMCP-KBR 2738 was not seen. No biologically relevant increase in the mutant count was observed in comparison to the negative controls.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, Mitomycin C, cumene hydroperoxide and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

Table 5.10-6: Summary of mean values without S9 mix

	Strain				
Concentration [µg/plate]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	10	153	5	25	217
16	12	165	6	23	233
50	14	173	7	23	255
158	14	159	8	25	229
500	14	112	2	17	185
1581	0	0	0	0	61
5000	0	0	0	0	-
Na-azide	669				
NF		443			
4-NPDA			89	210	
MMC					608

	Strain				
Concentration	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
[µg/tube]					
0	11	122	13	22	493
1.6	10	136	14	18	564
5	9	147	14	18	575
16	8	156	13	20	524
50	10	134	8	17	446
158	10	134	15	17	393
500	3	64	0	0	167
Na-azide	664				
NF		463			
4-NPDA			128	735	
Cumene					726

Table 5.10-7: Summary of mean values with S9 mix

	Strain				
Concentration [µg/plate]	**TA1535**	**TA100**	**TA1537**	**TA98**	**TA102**
0	10	210	13	38	336
16	11	227	12	37	330
50	10	221	13	33	286
158	11	211	11	38	324
500	9	165	7	29	260
1581	8	9	0	0	97
5000	0	0	0	0	0
2-AA	182	1803	286	1589	787
[µg/tube]					
0	10	171	15	33	509
1.6	8	162	14	28	516
5	8	180	14	28	482
16	11	183	12	26	250
50	14	158	12	26	455
158	8	145	16	24	366
500	6	80	2	14	148
2-AA	194	1368	340	1408	737

III. Conclusion

1,3-DMCP-KBR 2738 is non-mutagenic without and with S9 mix in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

Study on pharmacological properties

This study was conducted in order to investigate the pharmacological effects of fenhexamid for the registration in Japan.

Report:	**KIIA 5.10/05, [redacted] 1996**
Title:	A study of the effects of KBR 2738 on physiological functions
Report No.:	MO-99-004880
Document No.:	**M-010427-01-1**

Dates of work:	1996-03-19 to 1996-10-01
Guidelines:	not applicable
GLP:	no

I. Materials and methods

A. Materials

1. Test material: KBR 2738

Lot/Batch no: 17003/94

Purity: 95.8%

Stability of test compound: guaranteed for study duration, expiry date:

2. Vehicle: saline with 2% cremophor EL

3. Test animals:

Species: mouse / rat / rabbit

Strain: mouse: ICR, Crj:CD-1
rat: Sprague-Dawley, Crj:CD
rabbit: Japanese native strain, Kbl:JW

Age: mouse: 8 weeks
rat: 8 weeks
rabbit: 9 weeks

Weight at dosing: mouse: 27.8 - 32.9 g
rat: 258.8 - 322.6 g
rabbit: 1.5 – 2.0 kg

Source: mouse, rat: [redacted], Japan
rabbit: [redacted], Japan

Acclimatisation period: at least 1 week

Diet: mouse, rat: pellet food for laboratory animals, CE-2 ([redacted] Japan), ad libitum - fasting time indicated below
rabbit: pellet food for laboratory animals, RC-4 (Oriental Yeast), ad libitum except for fasting time indicated below

Water: tap water, ad libitum

Housing: mouse, rat: individually in plastic and metal suspended cages; bedding (mice): White Flake® ([redacted] Japan)
rabbit: individually in metal cages

B. Study design and methods

1. Animal assignment and treatment:

Dose: 0-2500-5000 mg/kg bw

Application route: oral (gavage)

Application volume: mouse, rat: 20 mL/kg bw
rabbit: 10 mL/kg bw

Fasting time:	before administration: approx. 18 – 24 h
Group size:	mouse, rat: 5 males/group
	rabbit: 3 males/group

Experimental procedures:

I. Test for the influence on general condition and behavior

1. Gross observation in mice: The gross observation based on Irwin's method was done 30 min, 1, 2, 4, 6 and 24 h after oral administration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738.
2. Gross observation in rabbits: The gross observation of rabbits in their home cage was done 30 min, 1, 2, 4, 6 and 24 h after oral administration of the vehicle, 2500 or 5000 mg/kg bw KBR2738.

II. Test for the influence on the central nervous system

1. Test for the influence on spontaneous movement in mice: The animals were placed in the counting rooms at 14:00. At 16:00 they received the vehicle, 2500 or 5000 mg/kg bw KBR 2738 by gavage. The spontaneous movements of the animals in vertical and horizontal dimension were measured for 15 h up to 7:00 in the following morning. The counts were accumulated every hour.
2. Test for the influence on rectal temperature of rabbits: In preliminary examinations each animal was placed in an animal-holder for 3 h and rectal temperature was measured using thermometers for the pyrogen test. Only animals in which the rectal temperature varied within ±0.6 °C were used for the study. On the next day, the stability of rectal temperature was confirmed for 2 h. Subsequently, the vehicle, 2500 or 5000 mg/kg bw KBR 2738 was administered the rectal temperature was measured for additional 5 h.

III. Test for the influence on the autonomic nervous system

1. Test for the influence on pupillary size in rabbits: The rabbits were placed in an animal-holder and eyelashes were trimmed off. Pupillary size was measured with a Mita pupil size scale before as well as 30 minutes, 1, 2, 4, 6 and 24 h after oral administration of the vehicle, 2500 or 5000mg/kg bw KBR 2738.

IV. Test for the influence on the respiratory and circulatory system

1. Test for the influence on respiration, blood pressure, heart rate and electrocardiogram in rabbits: The animals were anesthetized and a polyethylene catheter filled with heparin containing saline was inserted into the right femoral artery. The catheter was led subcutaneously towards the shoulder, exposed through the skin and fixed at the back of the animal. On the following day, each animal was placed in an animal-holder, and blood pressure was measured through the catheter using a pressure transducer. Heart rate was determined with a tachometer which was triggered by the pulses of the blood pressure. Respiratory rate was measured with a thermo-sensor which was placed close to the nasal cavity. These parameters were recorded on an oscillograph. Electrodes were fixed on the limbs of the animal and an electrocardiogram (ECG) was recorded with an electrocardiometer. These parameters were recorded before as well as 30 min, 1, 2, 4 and 6 hr after administration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738.

V. Test for the influence on the somatic nervous system

1. Test for the influence on the motor coordination in mice:

   (1) Test for the influence on motor coordination in mice using rotating rod: Preliminary examination was performed using a rotating rod apparatus for all animals. The animals which remained on the rotating cylinder of the rod for longer than 1 minute were selected for the study. On the day of examination, the rotating rod performance in individual animals was confirmed before administration. At 30 minutes, 1, 2, 4, 6 and 24 h after p.o. administration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738, the performance was repeatedly examined.

   (2) Test for the influence on muscle force using suspension-wire in mice: The same animals used in (1) were also subjected to the suspension-wire test. The animals were forced to hang on a horizontal wire of 1.5 mm in diameter by their forelimbs and were examined whether they could secure themselves by holding their hind limbs on the wire within 5 sec. Treatments and time schedule for this test were the same as described in test (1).

VI. Test for the influence on the digestive system

1. Test for the influence on charcoal transit in the gastro-intestinal tract in mice: Thirty minutes after p.o. administration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738, 0.2 mL/kg bw of 5% charcoal, suspended in 5% Gum Arabic was administered orally. Thirty minutes after the charcoal administration the animals were killed by cervical dislocation and the gastrointestinal tract was removed immediately. Total length of the small intestine and length of charcoal transit were measured. The transit rate was calculated by dividing the length of the small intestine from the pylorus to the transported charcoal by the length from the pylorus to the ileocaecal valve.

VII. Test for the influence on the renal function

1. Test for the influence on the renal function in rats: Male rats were housed individually in metal metabolic cages for 3 days to be acclimatized and to get control urine. The animals were administered with the vehicle, 2500 or 5000 mg/kg bw KBR 2738 and urine was collected for 6 hr under fasting and suspending of water supply. The urine was examined regarding its volume, concentrations of electrolytes (Na+, K+ and Cl-) and pH by an automatic electrolyte analyzer and a pH-meter.

VIII. Test for the influence on blood coagulation and hemolysis

1. Test for the influence on blood coagulation in rats: Male rats were treated with the vehicle, 2500 or 5000 mg/kg bw KBR 2738. One hour after administration the animals were anesthetized and after performing a median abdominal incision, blood was taken from the inferior caval vein using a sodium citrate solution as an anticoagulant. Plasma was prepared by centrifugation of the samples. Subsequently, prothrombin time (PT) and partially activated thromboplastin time (APTT) were measured by an automatic blood coagulation analyzer.

2. Test for the influence on hemolysis in rats:

(1) in vivo experiment: Male rats received the vehicle, 2500 or 5000 mg/kg bw KBR 2738. One hour after administration, the animals were anesthetized. After performing a median abdominal incision, blood was taken from the inferior caval vein using a sodium citrate solution as an anticoagulant. The blood was centrifuged at 4°C to obtain plasma. The absorbency of the plasma at 540 nm was measured with a spectrophotometer. In the erythrocyte fragility test, 100 µL of the remaining whole blood was added to 5 mL of physiological saline solution and was centrifuged at 4°C. The absorbency of the supernatants at 576 nm was measured in order to determine whether hemolysis occurs or not.

(2) invitro experiment: Male rats were anesthetized. After performing a median abdominal incision, blood was taken from the inferior caval vein using a sodium citrate solution as an anticoagulant and was centrifuged at 4°C. The pellets were washed 3 times by resuspension in physiological saline followed by centrifugation, and were suspended in saline at a concentration of 5%. KBR 2738 was suspended at a concentration of 250 mg/mL in the vehicle, and was diluted to 7*, 0.7 and 0.07 % (weight/volume) with saline. To 1.25 mL of the diluted KBR 2738 suspensions each 62.5 µL of the erythrocyte suspension was added and kept at 38°C for 2 h. After centrifugation of the suspensions absorbency of the supernatants was measured at 540 nm. The assay was performed in duplicate for each sample. Each concentration of KBR 2738 solutions was adjusted to isotonic and the effects of physiological saline solution containing 2% cremophor (corresponding to the vehicle used for the solution containing 7% KBR 2738) was examined as control. The erythrocyte suspension containing 5% glucose was considered as 0% hemolysis.

If 5000 mg/kg bw KBR 2738 p.o. is thoroughly absorbed, the blood concentration of the test substance may approximately rise to 6.5%.

II. Results and discussion

- The general condition of mice and rabbits did not change after oral administration of the vehicle (saline with 2% cremophor EL), 2500 or 5000 mg/kg bw KBR 2738. Some mice given KBR 2738 showed a transient depression of locomotion, which disappeared within 30 minutes after administration.
- The spontaneous movement of mice decreased for 1 h after oral administration of 2500 or 5000 mg/kg bw KBR 2738 compared to the vehicle controls. Although an effect of KBR 2738 cannot be entirely ruled out, the authors supposed that this finding might have been due to the stress of the gavage administration, since the quite thick suspension of the test material with a large volume of 20 mL/kg needed longer restriction for administration than in the vehicle controls. An increase in spontaneous movement observed in the animals treated with 2500 mg/kg bw KBR 2738 from 5:00-6:00 o'clock in the following morning was due to accidentally increased movements in two animals in the group.
- Rectal temperature in rabbits was not changed by the vehicle, 2500 or 5000 mg/kg bw KBR 2738.
- The pupil size of rabbits was not influenced by admistration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738.
- No marked changes of the respiratory and circulatory system were observed in conscious rabbits after oral administration of the vehicle, 2500 or 5000 mg/kg bw KBR 2738.
- Motor coordination in mice tested using a rotating rod apparatus was not affected by the vehicle nor by 2500 or 5000 mg/kg bw KBR 2738.

- Traction force in the skeletal muscles of mice (tested using suspension-wire) was also not affected by the vehicle nor by 2500 or 5000 mg/kg bw KBR 2738.
- No significant differences in charcoal transit through the gastrointestinal tract were noted in mice treated with the vehicle 2500 or 5000 mg/kg bw KBR 2738.
- Urinary volume, pH, and excretion of sodium and chloride did not differ among rats after administration of the vehicle or 2500 or 5000 mg/kg bw KBR 2738 for 6 h. Slightly increased potassium-excretion was found in the animals given KBR 2738. Although the finding was statistically significant in comparison to the vehicle-treated group, the difference between these values was very slight. Moreover, other parameters of the renal function showed no effects of KBR2738. Therefore, the authors concluded that the increased urinary potassium excretion is not necessarily due to any direct effects of the test material.
- Prothrombin time (PT) and partially activated thromboplastin time (APTT) were not changed in rat blood samples obtained after administration of the vehicle or 2500 or 5000 mg/kg bw KBR 2738.
- Oral administration of 2500 or 5000 mg/kg bw KBR 2738 to rats did not cause hemolysis. Additionally, 0.07 and 0.7% KBR 2738 also had no direct hemolytic effect in vitro. At the highest concentration of 7 %, the supernatant was strongly colored dark brown and it was difficult to detect a hemolytic effect. However, there were no peaks in the absorbency chart over 500-700 nm. Therefore, the authors suggested that KBR2738 at the investigated doses and concentrations hardly causes hemolysis.

III. Conclusion

The study results showed that KBR 2738 has no marked effects on the general condition, behaviour, the central and autonomic nervous system, the respiratory and circulating system, the somatic nervous system, the digestive system, renal function and on blood or blood cells. At doses below 5000 mg/kg bw KBR 2738 causes no symptoms of acute toxicity.

Study on acute intraperitoneal toxicity

Please refer to the Monograph and baseline dossier of fenhexamid.

Subchronic feeding study in rats for the determination of fenhexamid concentrations in plasma and urine

Please refer to the Monograph and baseline dossier of fenhexamid.

IIA 5.11 Summary of mammalian toxicity and overall evaluation

Similar to the whole dossier also this summary on mammalian toxicity and overall evaluation consists of old paragraphs, which were originally submitted for Annex I inclusion, and new parts. For discrimination the new parts are written in bold italic letters. The old paragraphs were adopted unchanged as far as possible. In areas in which the assessment changed in the old text, the changed wording is also printed in bold italic letters.

Absorption, distribution, excretion and metabolism

Due to the well known metabolisation and excretion no additional rat metabolism studies have been conducted. No cleavage products were found in the rat and the total identification rate was high. No further rat metabolism study was considered necessary because all metabolites would be detected unchanged with a cyclohexyl label.
The metabolic pathway was similar in rat and goat, with no accumulation observed in fat, meat, and milk. No species-specific metabolites were found.

Acute toxicity, local tolerance and skin sensitization

Fenhexamid has no significant acute toxicity after oral, dermal and inhalatory application. The compound is neither a skin nor an eye irritant. No skin-sensitising potential was found in ***three*** different test systems.

Short-term toxicity

After repeated oral administration of high doses of ***fenhexamid***, no evidence for cumulative toxicity was seen in rats, mice and dogs. The oral administration of 1000 mg/kg bw/day for 4 weeks by gavage did not induce any toxic effects in rats. In subacute/subchronic feeding studies unspecific signs of toxicity such as reduced body weight development, increased feed and water intake were seen in rats and mice. The liver turned out to be the main toxicological target organ in the three species tested. ***In addition, effects on the kidneys were observed in rats and mice at very high doses (effects on kidney weight, increased incidences of basophilic tubules, dilated tubules, tubular casts, increased plasma creatinine and urea).*** In dogs effects on the red blood system (appearance of Heinz' bodies, reduction of numbers of red blood cells) were additionally seen. The red blood system was ***also*** the main toxicological target in dogs following 12 months administration of high dose levels. When comparing the intensity of the effects with those after 3 months, no enhancement was observed indicating that the dogs' organisms are able to compensate these blood effects adequately.

With regard to operator-relevant exposure routes (repeated dermal and inhalatory administration) ***fenhexamid*** shows favourable toxicological properties: No systemic or local effects occurred in rabbits following daily dermal application of the limit dose of 1000 mg/kg bw/day over a period of 3 weeks. In a subacute inhalation study the highest concentration of 486.7 mg /m³ air did not result in specific toxic effects. 68.7 mg ***fenhexamid*** /m³ air is considered to be the NOAEL.

Genotoxicity

The genotoxic action of ***fenhexamid*** was studied in bacteria and mammalian cells with the aid of various in-vitro test systems and in vivo by means of the micronucleus test. None of the tests revealed any evidence of mutagenic or genotoxic potential of ***fenhexamid***. The compound did not induce point mutation, DNA damage or chromosome aberration.

Long-term toxicity and carcinogenicity

The limit concentration of 20000 ppm (equivalent to ~ 1000 mg/kg bw/day) was tolerated by rats over a period of 24 months without increased mortality or major organ damage. In addition to unspecific signs of toxicity, such as reduced body weight development (although the feed intake was increased) and higher water intake, only slight effects on liver and thyroid were established. Some mucosal hyperplasia in the caecum of the rats might be linked to the continuous stimulation by the mild irritant effect of ***fenhexamid***. Following long-term administration of high dose levels to mice, ***again*** the kidneys ***were the*** target organ in this species. The main effects were a decrease of kidney weights and an increased incidence of common morphological findings such as basophilic tubuli and chronic renal disease.

No evidence of an oncogenic potential of ***fenhexamid*** was found in either the rat or mouse long-term feeding studies.

Reproductive toxicity

The limit concentration of 20000 ppm was tolerated by rats in a 2-generation study without adverse effects on reproductive behaviour and efficiency. Reduced pup weights which occured during the first three weeks post partum at high dose levels are attributed to a deficiency of the neonate rat for glucuronidation and, as a consequence, for the excretion of ***fenhexamid***. The reduced pup weights coincided with general toxic effects on the parent animals such as reduced body and organ weights.

In the first developmental toxicity study in rats the limit concentration of 1000 mg/kg bw/day did not induce any adverse effects on intrauterine development; no signs of toxicity were established in the dams. ***In an additionally conducted study employing fenhexamid doses of 300, 1000 and 2000 mg/kg bw/day slightly decreased body weights were observed in dams at 1000 and 2000 mg/kg fenhexamid. No developmental effects occurred up to the high dose of 2000 mg/kg bw.***

In rabbits there was a correlation between slight developmental effects (decreased gestation rate, decreased placental and fetal weights, delayed ossification) and distinct maternal toxicity.

No malformations were observed, neither in rats nor in rabbits.

Overall, it can be concluded that ***fenhexamid*** has no primary reproductive toxicity.

Neurotoxicity

There was no indication for neurotoxic effects, neither in an acute neurotoxicity screening study up to the highest tested dose of 2000 mg/kg bw fenhexamid, nor in the other toxicological studies on

this compound. Tests on delayed neurotoxicity were not conducted, since fenhexamid is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances.

Toxicity of metabolites

No studies were performed as plant metabolites are identical to those formed in animals.

Medical/clinical data

Medical surveillance on manufacturing plant personnel was performed anually between 1999 and October 2011 including clinical examination with orientating neurological status and skin status, laboratory investigations of blood and urine as well as technical examinations of particular organs or organ systems. It did not reveal any unwanted effects. Since 1999 no accidents with fenhexamid occurred in workers and no consultations of the site Medical Department due to work or contact with fenhexamid were required.

No cases of human poisoning have been reported up to January 2012. Up to now there is no exposure of the general population to ***fenhexamid***.

Compound-specific poisoning signs in men after oral ingestion are not expected ***due to the low toxicity of fenhexamid***. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning.

First aid measures comprise the termination of exposure, decontamination of the skin with water and soap and flushing of the eyes with lukewarm water. Induction of vomiting does not seem to be required due to the low toxicity of fenhexamid, in case of the ingestion of formulations containing organic solvents it is forbidden. Treatment has to be symptomatic and supportive, since no specific antidotal therapy is available for ***fenhexamid***. Poisoning signs in men following oral***, dermal or inhalative*** uptake of ***fenhexamid*** are not expected because of the favourable toxicological profile.

Dermal penetration

Dermal absorption figures of 2% for the concentrate and 18% for the spray dilution (1:99) were derived from a dermal penetration study in vivo in rats on fenhexamid WP 50. These values were used to conduct the operator risk assessment at Annex I inclusion.

In addition, a new comparative in vitro dermal absorption study using human and rat skin was performed on the current representative formulation fenhexamid WG 50. After 8 hours exposure 0.15, 0.62 and 5.83% fenhexamid were potentially absorbable in 24 hours through human skin for the concentrate and the spray dilutions of 5 g/L or 0.375 g/L. For rat skin the respective absorption values were 1.13, 1.03 and 12.22%.

Other/special studies

This chapter comprises new studies on two impurities of fenhexamid and a new study on pharmacological properties. In addition it contains previously submitted studies, i.e. an acute study

in rats with intraperitoneal administration and a subchronic feeding study in rats for the determination of fenhexamid concentrations in plasma and urine.

Like the active ingredient fenhexamid, the impurities 1,2-DCMP-KBR 2738 and 1,3-DCMP-KBR 2738 were negative for point mutation in the Ames test and exhibited an acute oral toxicity > 2000 mg/kg bw in the rat.

In a pharmacological study fenhexamid displayed no marked effects on general condition, behaviour, the central and autonomic nervous system, the respiratory and circulating system, the somatic nervous system, the digestive system, renal function and on blood or blood cells. Additionally, the results indicate that at doses below 5000 mg/kg bw fenhexamid causes no symptoms of acute toxicity.

Fenhexamid shows no significant acute toxicity after intraperitoneal administration (LD_{50} > 1000 mg/kg bw).

A subchronic feeding study in rats for the determination of fenhexamid concentrations in plasma and urine showed that the compound was well absorbed from the gastrointestinal tract. In females there was no saturation of absorption up to a concentration of 20000 ppm, while in males saturation can be assumed slightly below this limit concentration.

Literature review

A literature review on fenhexamid revealed no relevant publications in the area of toxicology. In the publication "Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens" by [redacted] et al. (2011)[1] (Publication no. 69 in the Excel document) fenhexamid was identified as an in vitro anti-androgenic compound in the MDA-kb2 screening assay (IC_{20}: 2.02 µM). However, fenhexamid was tested up to very high doses in comprehensive toxicological studies in vivo. In the whole data package there were no effects on male or female reproductive organs nor on reproduction or the development of the young, which could have been triggered by endocrine disrupting properties of the compound. Without any indication for endocrine mediated effects in these higher tier in vivo studies the positive result in the in vitro MDA-kb2 screening assay by [redacted] et al. (2011) is considered to have no relevance for the in vivo situation and thus also not for human risk assessment.

For details on databases and keywords used for this literature package, please refer to the separate document attached to this AIR2 submission (Annex II dossier).

Calculation of the acceptable daily intake (ADI)

At Annex I inclusion an ADI of 0.2 mg/kg bw was established for fenhexamid. This value was derived from the NOEL of 18.3 mg/kg bw from the 52-week study in dogs as the most sensitive

[1] ***Orton, Frances; [redacted] (2011); Widely used pesticides with previously unknown endocrine activity revealed as in vitro antiandrogens, Environmental health perspectives, (2011 Jun) Vol. 119, No. 6, pp. 794-800. Electronic Publication: 2011-02-10.***

species by using a safety factor of 100 and rounding. The more recently conducted studies, the additional subchronic studies in rat and mouse as well as the additional developmental study in rat, did not reveal lower NOELs or more sensitive endpoints. Since the dog still is the most sensitive species for fenhexamid, the rationale for the establishment of the ADI has not changed.

Table 5.11-1: Summary of NOELs in repeated dose and reproduction toxicity studies

Study / Dose levels	NOEL	NOEL (mg/kg bw/day)	Findings at LOEL
subacute rat (gavage) 0-100-300-1000 mg/kg bw/day		1000	no abnormalities detected
subchronic rat (feed) 0-2500-5000-10000-20000 ppm	5000 ppm	415 / 549 (m/f)	***904/1132 mg/kg bw/day (male/female):*** retardation of body weight gain, increase of food consumption
subchronic rat (feed) 0-500-5000-50000 ppm	***500 ppm***	***38.0/47.4 (m/f)***	***403.9/552.8 mg/kg bw/day (m/f): increased feed & water intake and urine excretion (m/f), slightly reduced reticulocyte count (m)***
chronic combined rat (feed) 0-500-5000-20000 ppm	500 ppm	28 / 40 (m/f)	***292/415 mg/kg bw/day (m/f):*** decreased body weight gain ***from week 60 onwards (f)***; decreased plasma cholesterol ***(m)***, decreased GLDH, increased albumin ***(m)***; hyperplasia of caecal mucosa **(m)**
subchronic mouse (feed) 0-100-1000-10000 ppm	1000 ppm	267 / 454 (m/f)	***3284/5151 mg/kg bw/day (m/f):*** effects on liver and kidney (increased levels of cholesterol, creatinine & bilirubin; liver: increased weight & ***reduced glycogen content;*** kidneys: decreased weight, ***basophilic tubuli, protein casts***)
subchronic mouse (feed) 0-200-2000-20000 ppm	***2000 ppm***	***322.9/573.7 (m/f)***	***3416.8/6145.4 mg/kg bw/day (m/f): increased feed & water intake,kidneys: slightly decreased weight, increased incidence of basophilic tubules, dilated tubules, tubular casts***
oncogenicity mouse (feed) 0-800-2400-7000 ppm	800 ppm	247.4 / 364.8 (m/f)	***807.4/1054.5 mg/kg bw/day (m/f):*** decreased kidney weights; reduced no. of sex-specific vacuoles in renal tubuli (not adverse)
subacute dog (feed) 0-50-400-3000-20000 ppm	20000 ppm	500#	no abnormalities detected
subchronic dog (13 weeks, feed) 0-1000-7000-50000 ppm	1000 ppm	***33.9/37.0 (m/f) 35.5 (m+f combined)***	***239.1/261.0 mg/kg bw/day (m/f):*** increased number of Heinz' bodies
subchronic dog (52 weeks, feed) 0-500-3500-25000 ppm	500 ppm	***17.4/19.2(m/f)*** 18.3 ***(m+f combined)***	***124.3/132.7 mg/kg bw/day (m/f):*** increased no. of Heinz' bodies, activity of alkaline phosphatase increased
subacute dermal rabbit 0-1000 mg/kg bw/day		1000	no abnormalities detected
subacute inhalation rat (5x6 h/4 wks) 0-10.2-68.7-486. 7 mg/m³	68.7 mg/m³	respirable dose: 2#	activation of monooxidases in the liver, particle overload phenomena in the lungs
2-generation rat (feed) 0-100-500-5000-20000 ppm	parenta l: 500	38.2/44.8 (m/f)	***406.0/477.2 mg/kg bw/day (m/f):*** lower body weights ***(f, from day 14 on)***, changes in clin. chem. parameters &

	neonatal: 500 reproduction: 20000	76.6 1814	organ weights ***864.1 mg/kg bw/day (f, lactation):*** decreased weight no effects on reproduction
developmental oral rat (gavage) ***0-300-1000-2000 mg/kg bw/day***		***maternal: 300*** ***developmental: 2000***	***1000 mg/kg bw:*** ***slightly decreased body weights*** ***no abnormalities detected***
developmental rabbit (gavage) 0-100-300-1000 mg/kg bw/day		maternal: 100 developmental: 100	***300 mg/kg bw/day:*** decreased feed intake, reduced body weight gain, ***300 mg/kg bw/day:*** slightly decreased gestation rate, placental & fetal weights, delayed ossification

#: calculated value
m: male
f: female

Acceptable Operator Exposure Level (AOEL)

According to Directive 97/57/EC "... the AOEL is based on the highest level at which no adverse effect is observed in tests in the most sensitive relevant animal species or, if appropriate data are available, in humans.". At Annex I inclusion an AOEL of 0.3 mg/kg bw was established for fenhexamid. It was derived from the lowest short-term NOEL of 33 mg/kg bw from the subchronic dietary study in dogs and a safety factor of 100. This NOEL was based on the sole finding of Heinz bodies in the blood at the next higher dose. The formation of Heinz bodies [2] ***is only adverse, if it is associated with anemia, which was not the case at the LOEL. Therefore, and since the dog is also more sensitive with regard to Heinz body formation than man, this AOEL is already a conservative value. Due to the almost complete absorption of fenhexamid from the gastrointestinal tract a correction for oral bioavailability is not needed. Since no lower NOELs were determined in the more recently conducted studies, the systemic AOEL of 0.3 mg/kg bw/day is still considered to be a valid value for the protection of operators with regard to the exposure to fenhexamid.***

An inhalative or dermal AOEL, respectively, has not been set at Annex I inclusion, since it was considered not to be required/applicable most probably due to the low toxicity of fenhexamid after inhalative and dermal exposure.

[2] : ***Heinz bodies: small round inclusion bodies in erythrocytes, which are associated to the inner surface of the erythrocyte membrane. They develop as a consequence of long lasting oxidative stress to the cell by means of drugs, chemicals or toxins, especially basic amines. Heinz bodies adversely affect the fluidity of the erythrocyte membrane and thus the deformability of the red blood cell, so that its movement through the microcirculation is impaired, thus leading to hemolysis of the affected cells. Due to the increased elimination of affected red blood cells by the spleen long term exposure with oxidizing agents may also result in anemia.***

Acute Reference Dose (ARfD)

At Annex I inclusion no acute reference dose was allocated for fenhexamid this was not considered necessary due to the low toxicity of the compound. In order to reassess if it would make sense to set an ARfD for fenhexamid at Annex I renewal, the existing toxicological data base was evaluated again with respect to mortality and acute effects.

According to the OECD Draft Guidance Document for the Derivation of an acute reference dose (version 08 of 2009-02-19) and the publication "Guidance on setting of acute reference dose (ARfD) for pesticides" by [redacted] et al. (2005)[3] an ARfD does not have to be established if:

- ***No findings indicative of effects elicited by an acute exposure are observed at doses which are relevant for the acute risk assessment (e.g. up to about 500 mg/kg bw/day for residues of pesticides) and/or***
- ***No substance-related mortalities are observed at doses up to 1000 mg/kg bw in single dose oral studies (i.e. limit dose for acute testing).***

In the case of fenhexamid no mortalities occurred in single dose oral studies in rats and mice after fenhexamid doses of up to 5000 mg/kg bw.

Furthermore, there was no indication for any acute effects in acute and repeated dose toxicity studies up to 500 mg/kg bw. This comprises also the formation of Heinz bodies in the erythrocytes of dogs as the most sensitive parameter in the most sensitive species. An increase in Heinz` bodies was seen for the first time after 6 weeks of treatment, up to 2 weeks there was no such finding.

In the developmental study in rabbits one female at 300 mg/kg bw aborted on day 26 p.c., but that happened after the animal had consumed almost no food for more than 14 days. However, food consumption data on doe 1626 show that the food intake decreased during the first 5 days of treatment until the animal stopped feeding. The body weight in this single doe showed only a very slight decrease on the first days of treatment. These data show that there were also no acute adverse effects in this single doe of the 300 mg/kg dose group. Data on food consumption and body weight changes are presented in Tables 5.11-2 and 5.11-5 below.

Table 5.11-2: Rabbit developmental study on fenhexamid: food consumption [g/day] of doe 1626 (300 mg/kg bw fenhexamid)

Food consumption [g/day] of doe 1626 (300 mg/kg bw/day fenhexamid)				
Day 0-6	*Day 6-10*	*Day 10-14*	*Day 14-19*	*Day 19-24*
78.7	*42.5*	*0.8*	*0.4*	*0.0*

[3] : [redacted] (2005); Guidance on setting of acute reference dose (ARfD) for pesticides, Food Chem Toxicol. 2005 Nov; 43(11): 1569-93

Table 5.11-3: Rabbit developmental study on fenhexamid: body weight changes [% change in time interval] of doe 1626 (300 mg/kg bw fenhexamid) in comparison to controls

	Body weight changes [% change in time interval]	
Time period	*Day 6-7*	*Day 6-8*
Control group animals	*-1.5% up to +1.4%*	*-2.1% up to +0.8%*
Doe 1626 (300 mg/kg bw)	*-1.4%*	*-1.5%*

Furthermore, for the 300 mg/kg bw dose group a marked effect on body weight gain was reported in this study. However, analysis of the mean body weights of the 300 mg/kg does in comparison to the control does shows only minimal differences. The mean body weights of the 300 mg/kg dose group were slightly exceeding that of the control group during the whole study period (by 2.4-4.1%, for details see Table 5.11-4 below). The margin of 4.1% before start of treatment was slightly decreasing on the first days of treatment, but only down to 2.4% on day 9. Subsequently it varies between 2.4 and 3.6%. These small differences also do not present an acute adverse effect on body weight.

Table 5.11-4: Rabbit developmental study on fenhexamid: mean body weight [g] in the control and 300 mg/kg bw fenhexamid groups

Day	***Mean body weight [g] Control group***	***Mean body weight [g] 300 mg/kg bw fenhexamid***	***Difference to control mean [%]***
0	*2498.1*	*2600.4*	*104.1*
6	*2482.6*	*2574.8*	*103.7*
7	*2482.1*	*2550.3*	*102.7*
8	*2468.3*	*2540.6*	*102.9*
9	*2467.3*	*2525.6*	*102.4*
10	*2464.2*	*2528.4*	*102.6*
11	*2463.6*	*2535.6*	*102.9*
12	*2453.7*	*2540.7*	*103.5*
13	*2462.3*	*2543.4*	*103.3*
14	*2471.9*	*2560.1*	*103.6*
15	*2492.1*	*2568.9*	*103.1*
16	*2503.1*	*2573.7*	*102.8*
17	*2510.5*	*2571.9*	*102.4*
18	*2504.9*	*2574.8*	*102.8*
29	*2655.9*	*2727.6*	*102.7*

In conclusion, without any substance-related mortalities up to 5000 mg/kg bw in acute oral toxicity studies as well as any acute adverse effects at doses up to 500 mg/kg bw the establishment of an ARfD is still not considered to be necessary for fenhexamid.