DIMETHENAMID-P/RACEMIC DIMETHENAMID

First draft prepared by
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Explanation

Dimethenamid-P is the International Standardization Organization (ISO) approved common name for *S*-2-chloro-*N*-(2,4-dimethyl-3-thienyl)-*N*-(2-methoxy-1-methylethyl)-acetamide. This compound belongs to the chemical family of chloroacetamides and is used as a pre-emergent or early post-emergent herbicide with a broad spectrum of activity against most annual grasses and some important broad leaf weeds. It is taken up through the coleoptiles (grass seedlings) or the roots and emerging shoots (dicotyledonous seedlings) and reduces cell division and plant growth.

Dimethenamid is a racemic mixture of the M (or *R*) and P (or *S*) stereoisomers. When this compound was originally registered in various countries, all studies of toxicity were conducted with the racemic mixture. Later, it was discovered that only the P (or *S*) enantiomer has useful

herbicidal activity. Dimethenamid-P and racemic dimethenamid have not been evaluated previously by the JMPR.

All critical studies complied with good laboratory practice (GLP).

Evaluation for acceptable daily intake

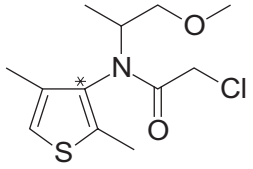
1. Biochemical aspects

1.1 Absorption, distribution and excretion

The studies of rat metabolism were conducted using racemic [3-thienyl-^{14}C]dimethenamid (batch No., RA 683-1, radiochemical purity, > 99%, specific activity, 157 µCi/mg (or 5.8 MBq). See Figure 1 for label position.

The test compound was administered either by intravenous injection or orally by gavage. The study design is summarized in Table 1. There were six males and six females in each of groups 1 to 4 and three males and three females in group 5. Group 1 was given an oral dose at 10 mg/kg bw. Group 2 received an intravenous dose at 10 mg/kg bw. Group 3 was given an oral dose at 1000 mg/kg bw. Group 4 was given unlabelled racemic dimethenamid for 14 days as an oral dose at 10 mg/kg bw per day, followed by an oral dose of radiolabelled dimethenamid at 10 mg/kg bw. The bile ducts of rats in group 5 were cannulated, after which the rats were dosed

Figure 1. Radiolabelled racemic dimethenamid: structure and position of the radiolabel



*Position of the ^{14}C label

Table 1. Summary of design of a study of metabolism of racemic dimethenamid in rats

Group	Sex	No. of rats	Route	Dose (mg/kg bw)	Sample collection time (h)		Sample analysed
					Excreta	Bile	
1	Male	6	PO	10	7, 24, 48, 72, 168	NP	Urine, faeces
	Female	6	PO	10	7, 24, 48, 72, 168	NP	Urine, faeces
2	Male	6	IV	10	7, 24, 48, 72, 168	NP	Urine, faeces
	Female	6	IV	10	7, 24, 48, 72, 168	NP	Urine, faeces
3	Male	6	PO	1000	7, 24, 48, 72, 168	NP	Urine, faeces
	Female	6	PO	1000	7, 24, 48, 72, 168	NP	Urine, faeces
4	Male[a]	6	PO	10	7, 24, 48, 72, 168	NP	Urine, faeces
	Female[a]	6	PO	10	7, 24, 48, 72, 168	NP	Urine, faeces
5	Male[b]	3	PO	10	7, 24, 48, 72, 168	7, 24, 48, 72, 168	Bile, urine, faeces
	Female[b]	3	PO	10	7, 24, 48, 72, 168	7, 24, 48, 72, 168	Bile, urine, faeces

From Vollmin (1992)

IV, intravenous; NP, not performed; PO, oral gavage.

[a] Rats received unlabelled racemic dimethenamid at a dose of 10 mg/kg bw per day for 14 days, then ^{14}C-labelled dimethenamid as a single dose of 10 mg/kg bw.

[b] Rats were bile-duct cannulated, and analysis of excreta was limited .

orally with racemic dimethenamid at 10 mg/kg bw. Excreta from each group and bile from group 5 were collected periodically until sacrifice at 168 h. Additional groups of three males and three females per time-point were dosed at either 10 or 1000 mg/kg bw for the collection of blood and tissue samples at 1, 4, 24, 72 and 168 h. The rats were killed 168 h after the first treatment.

Pooled urine (0–72 h) was desalted using XAD-2 columns. The methanol eluate collected was concentrated, dissolved in water and extracted with dichloromethane. Faecal samples collected at 0–72 h were extracted with methanol and dichloromethane. The non-extractable portion of the urine sample was treated with β-glucuronidase/arylsulfatase and base hydrolysis. The non-extractable portion of the faecal sample was subjected to base hydrolysis to release additional radioactivity. Bile was extracted with dichloromethane. The non-extractable bile sample was treated with β-glucuronidase/arylsulfatase and base hydrolysis. The soluble extracts were analysed using one-dimensional and two-dimensional thin-layer chromatography (TLC). Isolated metabolites were identified by mass spectrometry (MS) and/or nuclear magnetic resonance (NMR).

A summary of the excretion of the radiolabel in the urine, faeces and bile is presented in Table 2. Racemic dimethenamid was well absorbed after oral administration in rats, as demonstrated by addition of the amount of radioactivity excreted in the urine, via the bile duct and that remaining in the organs and carcass. Biliary excretion accounted for 75–82% of the radiolabelled carbon, with an additional 2–4% being found in the faeces and 8–12% in the urine. The total excretion by bile and urine for males was 89.8% and for females was 87.5%. After adding the amount of radioactivity found in the carcass, the total absorption after oral administration was 94.5% in males and 92.8% in females; therefore, essentially 100%.

Excretion was very rapid primarily in the bile. Within 7 h, 45–64% of the orally administered dose was excreted in bile of the cannulated rats. By 168 h after treatment, an average of 90% of the administered dose was eliminated by all routes. There were some dose-dependent differences in the pattern of excretion. At the lower dose (10 mg/kg bw), urinary radiocarbon accounted for 35–47% of the administered dose compared with 62–63% at the higher dose (1000 mg/kg bw). Radioactivity in faeces was 48–58% for groups at the lower dose compared with 26–30% at the higher dose. These data indicated that biliary excretion might be saturated for the group at the higher dose, resulting in more radioactivity being eliminated via the kidney.

Table 2. Summary of material balance for normal and bile-duct cannulated rats

Group	Sex	Route	Dose (mg/kg bw)	Percentage of administered dose recovered by 168 h after treatment				
				Urine	Faeces	Bile	Carcass	Total
1	Male	PO	10	35.3	57.7	NA	6.7	99.7
	Female	PO	10	46.9	47.6	NA	8.0	102.5
2	Male	IV	10	31.2	56.4	NA	11.1	98.7
	Female	IV	10	49.4	36.6	NA	9.9	95.9
3	Male	PO	1000	61.6	30.1	NA	3.4	95.1
	Female	PO	1000	63.1	26.1	NA	3.7	92.9
4	Male [a]	PO	10/day × 14	34.9	61.6	NA	4.4	100.9
	Female [a]	PO	10/day × 14	53.3	39.9	NA	3.6	96.8
5	Male [b]	PO	10	7.6	2.2	82.2	4.7	96.7
	Female [b]	PO	10	12.4	3.7	75.1	5.3	96.5

From Vollmin (1992)

IV, intravenous; NA, not applicable; PO, oral gavage

[a] Rats received unlabelled racemic dimethenamid at 10 mg/kg bw per day for 14 days, then a single dose of ^{14}C-labelled racemic dimethenamid at 10 mg/kg bw.

[b] Bile-duct cannulated.

The concentration of radioactivity in the blood decreased slowly over the experimental period of 168 h. Half-lives of elimination from blood were 255 ± 79 h and 334 ± 192 h for male and female rats, respectively. The radioactivity was mainly associated with erythrocytes (the concentration of radioactivity in the plasma being much lower). A similar binding phenomenon was not observed in human blood; this can be explained by differences between rat and human haemoglobins. After a single oral lower dose (10 mg/kg bw) the maximum concentration of blood radioactivity was reached at about 72 h after administration (0.05 µg of test material/g blood in males and 0.1 µg of test material/g blood in females). Afterwards, radioactivity decreased slowly. For the oral high dose, the maximum blood radioactivity was also reached at 72 h, but did not significantly decrease between 72 and 168 h.

In general, tissue concentrations of radioactivity were similar in both sexes, and the pattern of absorption, distribution and elimination after oral administration was similar. Radioactivity concentrations were higher at 1–4 h in adrenals, pancreas, kidney, spleen, liver and blood. Residue concentrations decreased steadily over time, with the exception of blood. Overall, tissue concentrations were low by 168 h after treatment. For the rats treated at the lower dose, the concentration was < 0.5 ppm in all organs and tissues.

After oral administration, it appeared that there was no significant difference in the absorption, distribution, and elimination of racemic dimethenamid in males and females. There was only a slight difference in the rate of elimination of radiocarbon between single and multiple doses. Residue concentrations in tissues were similar for groups given single or multiple doses, indicating that racemic dimethenamid and its metabolites had no tendency to accumulate in rat tissues (Vollmin, 1992).

The absorption, distribution and excretion of radioactivity was studied in male Wistar rats given a single dermal administration of ^{14}C-labelled racemic dimethenamid or dimethenamid-P. Racemic dimethenamid was dissolved in the undiluted solvent of a commercial formulation (Frontier 6.0 herbicide) at nominal doses of 0.004, 0.04 and 0.4 mg/cm² applied as a patch. The doses were selected on the basis of model calculations giving a range of expected field exposures. Rats were exposed for either 4 or 8 h, after which the patches with test substance were removed and the skin was washed with a mild soap solution. Rats were killed at 4 h for the 4 h exposure and at 8, 24 or 72 h after the 8 h exposure.

Four animals were used per dose and sampling time. In this study of balance/excretion, rats were placed in metabolism cages in order to collect excreta for up to 72 h. At the end of the various collection periods, the rats were killed and the following specimens were collected for measurement of remaining radioactivity: excreta (urine and faeces), blood cells, plasma, kidney, liver, carcass, treated skin (application site) and non-treated skin areas (skin surrounding application site). The cage wash and skin wash as well as the application material (protective cover) were also checked for radioactivity.

To compare rates of dermal penetration for racemic dimethenamid and dimethenamid-P, a second experiment was performed using ^{14}C-labelled dimethenamid-P dissolved in the neat solvent of the formulation BAS 656 07 H, at the same nominal doses of 0.004, 0.04 and 0.4 mg/cm^2. The animals were exposed for 8 h and sacrificed at 72 h after the end of treatment. These exposure and sacrifice times were chosen because they produced the greatest penetration in the study with racemic dimethenamid.

To assess the effect of the formulation on dermal penetration, a third experiment was performed using [^{14}C]dimethenamid in the BAS 656 07 H formulation. The exposure time was 8 h and the rats were killed at 72 h. These times were chosen because they gave the highest penetration in the second experiment using dimethenamid-P and the same solvent. Recoveries of radioactivity from rats at all doses were acceptable.

The results for dermal absorption of ^{14}C-labelled racemic dimethenamid dissolved in the undiluted solvent of a commercial formulation are given in Table 3.

Table 3. Percentage dermal absorption of ^{14}C-labelled racemic dimethenamid dissolved in Frontier 6.0 formulation in rats

Exposure time (h)	Sampling time (h)	Dose (mg/cm²)		
		0.004	0.04	0.4
4	4	10.97	0.97	4.74
8	8	11.36	2.48	4.91
8	24	14.35	6.25	7.38
8	72	18.18	8.42	9.10

From Leibold (1999)

Table 4 Percentage dermal absorption of ^{14}C-labelled dimethenamid-P in BAS 656 07 H formulation in rats

Exposure time (h)	Sample time (h)	Dose (mg/cm²)		
		0.004	0.04	0.4
8	72	15.17	27.32	23.29

From Leibold (1999)

The maximum absorption was 18% at 72 h after an 8 h exposure to the lowest dose, 0.004 mg/cm². With the exception of a low recovery at 4 h after the 0.04 mg/cm² dose, the percentage absorption was essentially the same at the two higher doses and was about half that observed at the lowest dose.

In another experiment, after an 8 h exposure to 0.4 mg/cm^2 of ^{14}C-labelled racemic dimethenamid in BAS 656 07 H, 25.8% was absorbed in 72 h. For comparison, the results of the dermal absorption with [^{14}C]dimethenamid-P in the same proposed BAS 656 07 H formulation are shown in Table 4.

Thus, the dermal absorption of dimethenamid-P and racemic dimethenamid in vivo is very similar, the percentages measured at a dose of 0.4 mg/cm^2 being approximately 23% and 26%, respectively (Leibold, 1999).

The rates of dermal penetration of racemic [^{14}C]dimethenamid through human and rat skin were compared in vitro. Human skin was obtained post-mortem and dermatomed to a thickness of 300 µm. Wistar rats were sacrificed, the abdominal region was clipped free of hair and samples of skin were excised and cut free of fatty tissue; the resulting samples were approximately 2000 µm thick. The excised human and rat skin epidermis and upper dermis were mounted in Franz-type static dermal penetration cells. Each skin sample was checked for integrity by measuring transepithelial electrical resistance. Three different dose solutions were prepared using ^{14}C-labelled racemic dimethenamid, unlabelled racemic dimethenamid and an ethanol/water solvent. The doses used were 2.55, 10.2 and 40.8 mg/cm^2. The test solutions were applied as a single dermal application at a dose volume of approximately 500 µl/cm^2 for 24 h. Three replicates were used for each dose and skin type, and each experiment was repeated twice, giving a total of nine cells tested per dose and skin type. Samples of the receptor fluid (ethanol and water) were taken at 0, 2, 4, 6, 8, 20, 22 and 24 h and analysed for radioactivity. At the end of exposure, the skin samples were removed from the cells, and washed with ethanol and water. The skin samples and the skin wash were analysed for radioactivity. Skin penetration rates found at 24 h are given in the Table 5.

These results do not account for the fact that the samples of rat skin used were 6.67 times thicker than the samples of human skin; the data presented in Table 6 have been corrected accordingly.

Table 5 Penetration rates (µg/cm^2 per h) for ^{14}C-labelled racemic dimethenamid in human and rat skin

Dose (mg/cm^2)	Species	
	Rat	Human
2.55	3.3	3.2
10.2	13.2	11.8
40.8	30.8	67

From Sommer & Müller (1993)

Table 6. Penetration rates (µg/cm^2 per h) for ^{14}C-labelled racemic dimethenamid in human and rat skin, corrected for skin thickness

Dose (mg/cm^2)	Species		Relative penetration
	Rat	Human	(rat/human)
2.55	22	3.2	7
10.2	88	11.8	7
40.8	205	67	3

From Sommer & Müller (1993)

After correcting for the difference in skin thickness, the rate of penetration through human skin is three to seven times less than that through rat skin. However, saturation has occurred at the highest dose in the rat skin, as the penetration rate no longer continues to increase linearly with dose. The predicted exposure of workers involved in the mixing, loading and application of racemic dimethenamid is at most 0.4 mg/cm^2. Therefore, the comparative values for rat/human skin at the lower doses is considered to be more appropriate for use in predicting human exposure (Sommer & Müller, 1993).

Overall the rate of dermal penetration of racemic dimethenamid in humans is predicted to be approximately 4%.

1.2 Metabolism

The proposed metabolic pathways of racemic dimethenamid in rats are shown in Figure 2.

Racemic dimethenamid is rapidly and extensively metabolized (Tables 7–11). Only 1–2%of unchanged racemic dimethenamid was detected in excreta. About 40 metabolites were found in organic extracts that were analysed by TLC. About 20 metabolites were identified. Metabolism occurred primarily via the glutathione conjugation pathways. Racemic dimethenamid was rapidly conjugated with glutathione and then passed through several steps to form cysteine conjugate (M25) and mercapturate (M17). M25 was further oxidized to form additional metabolites (M1, M2, M10, M13, M14, M16, M18, M19, M21, M22, M26, M27, M30, and M31). Although the glutathione adduct was not found in the study in rats, it was identified in the study in vitro. Other metabolites qualitatively identified in the study in vitro included the cysteine conjugate (M25), the mercapturate (M17), the sulfonate (M27), the sulfoxide of thiolactic acid (M30), the sulfoxide of thioglycolic acid (M31), and the thioglycolic acid (M30) (Dorobek & Müller, 1993).

Racemic dimethenamid was also metabolized by reductive dechlorination (M3), oxidation (M4, M23), hydroxylation (M5, M11, M15), *O*-demethylation (M7, M12) and cyclization (M6, M8, M9, M15, M20).

In another supplementary study, metabolites found on maize, the sulfonate (M27) (0.025–0.030%) and sulfoxide of thioglycolic acid (M31) (0.002–0.007%), were identified in rat urine (Yu et al., 1992). These metabolites have also been identified in mouse urine (Ekdawi & Yu, 1992a, 1992b).

Figure 2. Proposed metabolic pathway of racemic dimethenamid in rats

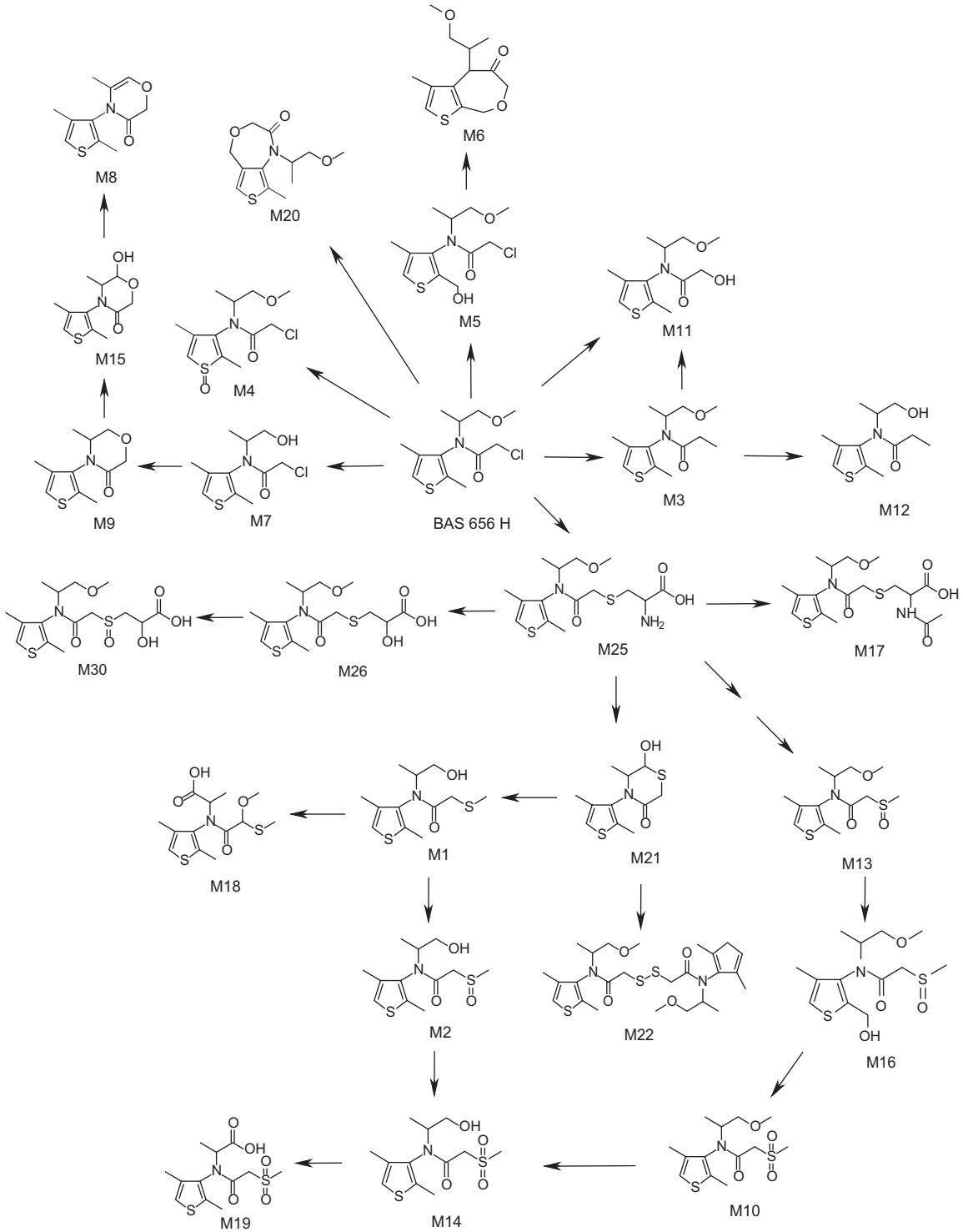


Table 7. Summary of material balance for normal and bile-duct cannulated rats

Group	Sex	Route	Dose (mg/kg bw)	Percentage of administered dose recovered by 168 h after treatment				
				Urine	Faeces	Bile	Carcass	Total
1	Male	PO	10	35.3	57.7	NA	6.7	99.7
	Female	PO	10	46.9	47.6	NA	8.0	102.5
2	Male	IV	10	31.2	56.4	NA	11.1	98.7
	Female	IV	10	49.4	36.6	NA	9.9	95.9
3	Male	PO	1000	61.6	30.1	NA	3.4	95.1
	Female	PO	1000	63.1	26.1	NA	3.7	92.9
4	Male[a]	Multiple, PO	10	34.9	61.6	NA	4.4	100.9
	Female[a]	Multiple, PO	10	53.3	39.9	NA	3.6	96.8
5	Male[b]	PO	10	7.6	2.2	82.2	4.7	96.7
	Female[b]	PO	10	12.4	3.7	75.1	5.3	96.5

From Vollmin (1992)

[a] Rats received unlabelled racemic dimethenamid at a dose of 10 mg/kg bw per day for 14 days, then received radiolabelled dimethenamid as a single dose at 10 mg/kg. bw

[b] Bile-duct cannulated.

IV, intravenous; NA, not applicable; PO, oral gavage

Table 8. Radiolabelled racemic dimethenamid metabolites recovered from urine of rats

Identity	Percentage recovered of administered dose for indicated group[a]							
	1		2		3		4	
	Males	Females	Males	Females	Males	Females	Males	Females
Racemic dimethenamid	0.2	0.7	0.2	0.5	ND	0.2	ND	< 0.1
M1/M7	0.4	1.6	0.3	3.9	5.3	5.9	0.4	2.7
M2	3.3	6.4	2.4	3.4	5.0	6.8	3.7	9.9
M3	0.2	0.1	0.1	0.2	0.3	0.2	< 0.1	0.1
M4	0.2	ND	0.5	0.6	ND	1.1	ND	ND
M5	0.3	1.1	0.2	0.6	7.5	5.0	0.2	1.2
M6	0.1	0.3	0.1	0.5	ND	ND	ND	< 0.1
M8	ND	ND	ND	ND	ND	0.3	ND	< 0.1
M9	< 0.1	0.1	< 0.1	0.2	ND	< 0.1	ND	ND
M10	< 0.1	0.2	0.1	0.3	ND	0.2	ND	< 0.1
M11	< 0.1	0.2	0.2	0.2	0.4	0.2	0.1	0.2
M12	0.3	0.2	0.3	0.3	0.4	0.5	0.4	0.7
M13	0.9	2.9	0.9	2.3	0.5	1.5	0.3	2.1
M14	1.0	2.2	1.0	2.5	2.7	3.9	0.9	2.4
M15	ND	ND	ND	ND	ND	ND	ND	0.1
M16	0.9	1.2	1.1	1.0	2.8	1.7	1.4	2.1
M17	0.3	1.7	0.1	1.9	2.5	3.7	0.2	1.2
M18	0.4	0.6	0.4	0.7	0.9	0.9	0.6	1.1
M19	0.5	0.3	0.2	0.4	0.5	0.6	0.3	0.2
M20	ND	ND	ND	ND	ND	ND	ND	ND
M21	ND	0.5	0.5	0.7	0.9	0.3	ND	0.3
M22	ND	ND	ND	ND	ND	ND	ND	ND
M25	< 0.1	0.2	0.2	0.9	0.1	< 0.1	< 0.1	0.1
M26	0.1	< 0.1	< 0.1	0.1	0.40	< 0.1	0.2	0.1
M30	< 0.1	0.1	0.2	0.2	0.2	0.2	< 0.1	< 0.1

From Vollmin (1992)

ND, not detected

[a]Pooled 1–72 h urine. In a supplementary study (Ekdawi & Yu, 1992a, 1992b), maize metabolites, sulfonate (M27, 0.06–0.07%) and sulfoxide of thioglycolic acid (M31, 0.25–0.24%), were identified in mouse urine

Table 9. Radiolabelled racemic dimethenamid metabolites recovered from rat faeces

Metabolite	Recovery (%)[a]							
	Group 1		Group 2		Group 3		Group 4	
	Females	Males	Males	Females	Males	Females	Males	Females
Racemic dimethenamid	0.9	1.2	2.1	0.8	1.2	1.3	1.4	1.1
M1/M7	2.7	1.8	0.1	2.1	0.6	0.4	2.9	4.5
M2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
M3	0.6	0.3	0.5	0.3	0.1	0.2	0.5	0.2
M4	ND	ND	ND	ND	ND	ND	ND	ND
M5	0.4	0.4	0.4	0.2	0.3	0.1	0.5	0.2
M6	0.8	0.3	0.2	0.3	0.4	0.3	0.3	0.6
M8	0.2	ND	ND	ND	ND	< 0.1	ND	< 0.1
M9	ND	ND	0.3	ND	ND	ND	ND	ND
M10	0.7	0.2	0.1	0.1	0.2	0.1	ND	0.2
M11	0.3	0.2	1.5	0.5	< 0.1	0.3	ND	ND
M12	ND	ND	ND	ND	ND	ND	< 0.1	< 0.1
M13	1.5	1.9	ND	ND	0.3	0.1	< 0.1	1.1
M14	1.4	1.8	0.9	0.6	0.3	0.1	2.1	0.9
M15	< 0.1	ND	ND	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
M16	2.9	3.3	2.4	1.3	2.0	1.0	4.7	1.7
M17	< 0.1	< 0.1	0.2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
M18	0.5	0.5	0.7	0.4	0.3	0.1	0.4	0.3
M19	0.5	0.4	0.2	< 0.1	0.5	< 0.1	0.8	0.3
M20	0.5	0.3	ND	< 0.1	< 0.1	< 0.1	ND	ND
M21	0.4	0.3	0.2	0.2	0.3	0.2	ND	< 0.1
M22	0.7	0.1	0.2	0.2	1.0	1.0	ND	ND
M25	< 0.1	< 0.1	0.2	< 0.1	< 0.1	ND	< 0.1	< 0.1
M26	< 0.1	< 0.1	0.1	< 0.1	< 0.1	ND	< 0.1	< 0.1
M30	< 0.1	< 0.1	0.1	< 0.1	< 0.1	ND	< 0.1	< 0.1

From Vollmin (1992)

ND, not detected

[a] Pooled 0–72 h faeces

In a supplementary study (Ekdawi & Yu, 1992a, 1992b), maize metabolites, sulfonate (M27, 0.25%) and sulfoxide of thioglycolic acid (M31, 0.25–0.4%), were identified in mouse faeces.

In another supplementary study (Yu et al., 1992), maize metabolite, sulfonate (M27, 0.016–0.020%) was identified in rat faeces.

Table 10. Radiolabelled racemic dimethenamid metabolites recovered from bile of rats[a]

Metabolite	Percentage recovery[b]	
	Males	Females
Racemic dimethenamid	< 0.1	< 0.1
M1/M7	5.0	4.8
M2	0.3	0.7
M3	ND	ND
M4	1.8	1.3
M5	6.0	2.0
M6	ND	ND
M8	0.5	0.8
M9	< 0.1	ND
M10	0.1	< 0.1
M11	0.3	0.2
M12	ND	ND
M13	ND	ND
M14	0.4	0.1
M15	ND	ND
M16	0.7	0.3
M17	2.6	3.0
M18	0.2	0.1
M19	0.4	0.1
M20	ND	ND
M21	2.0	1.8
M22	ND	ND
M26	0.1	0.1
M30	0.2	0.1

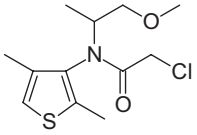
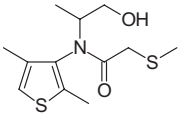
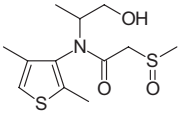
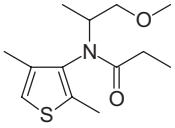
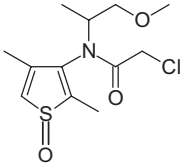
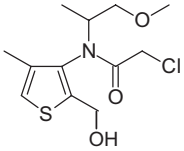
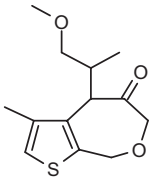
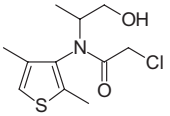
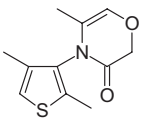
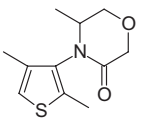
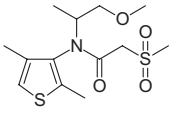
From Vollmin (1992)

ND, not detected

[a]Bile-duct cannulated rats, group 5.

[b]Pooled 1–7 h bile.

Table 11. Structures of identified metabolites in rat urine, faeces and bile

Metabolite	Structure
Racemic dimethenamid	
M1	
M2	
M3	
M4	
M5	
M6	
M7	
M8	
M9	
M10	

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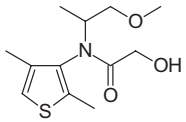
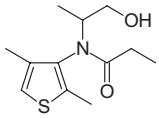
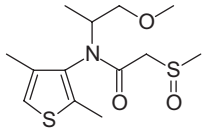
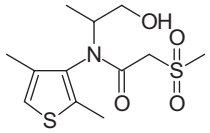
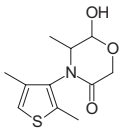
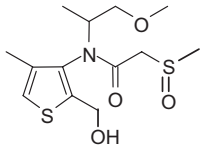
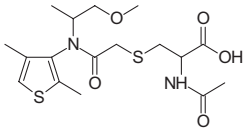
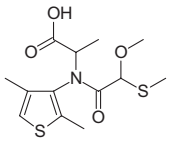
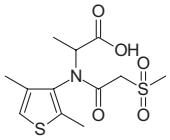
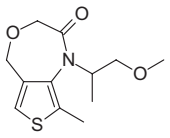
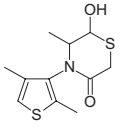
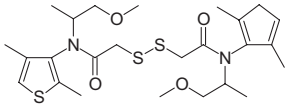
M11	
M12	
M13	
M14	
M15	
M16	
M17	
M18	
M19	
M20	
M21	
M22	

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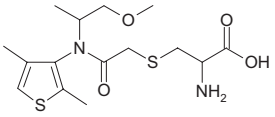
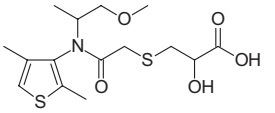
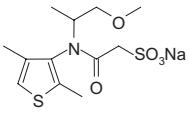
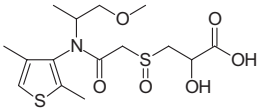
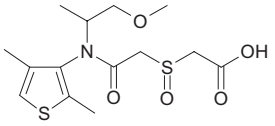
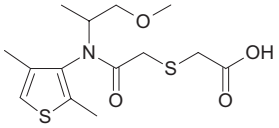
M25	
M26	
M27	
M30	
M31	
M32	

From Vollmin (1992)

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of dimethenamid-P (SAN 1289, batch No. 6663-50-1, purity, 91.1%) was evaluated in groups of five male and five female fasted Sprague-Dawley rats given undiluted herbicide at a dose of 350, 400 or 500 mg/kg bw by gavage. The rats were observed for 14 days after administration. Clinical signs observed in all rats on the day of dosing consisted of lachrymation and excessive salivation. Decreased activity was observed in all rats at 500 mg/kg bw. Signs seen on the day after dosing included: yellow anogenital staining, black or brown staining on the snout, oral area, buccal area and/or extremities, lethargy, decreased food consumption, and decreased faecal volume or no stools at all. All surviving rats were free of clinical signs by day 5 after dosing. Overall, the rats gained body weight as expected for their age. Treatment-related effects on gross pathology were observed only in rats that died and consisted of redness in the region of the thymus, fluid in the thoracic cavity, red lungs, black mucosa or brown fluid in stomach and red testes.

At 350 mg/kg bw, there were no deaths. At 400 mg/kg bw, one male died, but there were no deaths among the females. At 500 mg/kg bw, all five males died and there were two deaths among the five females. The acute oral median lethal dose (LD_{50}) was calculated to be 429 mg/kg bw in male and 531 mg/kg bw in female Sprague-Dawley rats (Blaszcak, 1996a).

The acute oral toxicity of racemic dimethenamid (lot No. 9021; purity, 97.1%) was investigated in groups of five male and five female fasted Sprague-Dawley rats given undiluted herbicide at a dose of 150, 300 or 600 mg/kg bw by gavage. The rats were observed for 14 days after dosing. Clinical observations on the day of dosing included oral and ocular discharges and

hypoactivity at all doses. In addition, at 600 and 300 mg/kg bw, some rats developed nasal discharge, wet rales, faecal staining, soft stools and abdominal griping. Signs seen only at 600 mg/kg bw were irregular gait, coarse and fine tremors, hypopnoea, irregular breathing, urinary staining and prostration. Clinical signs seen after the day of dosing included hypoactivity and decreased food consumption and, in one rat at 150 mg/kg bw, a red ocular discharge. All surviving rats were free of clinical signs by day 4 after dosing. Most surviving rats had gained weight by day 7, and all survivors had gained weight by day 14. Treatment-related effects on gross pathology were observed only in rats that died and consisted of redness in the region of the thymus, fluid in the thoracic cavity, red lungs, black mucosa or brown fluid in the stomach and red testes. Observations made at autopsy on four rats found dead consisted primarily of gastric and intestinal discolouration or thickening of the walls and the presence of red fluid and test material. In addition, at 600 and 300 mg/kg bw some rats that died had discoloured lungs or lungs with red foci.

At 600 mg/kg bw, all male rats and four out of five females died within 1 day of treatment; at 300 mg/kg bw, one out of five males died on day 2. No mortality occurred at 150 mg/kg bw. The LD_{50} was 371 mg/kg bw in male and 427 mg/kg bw in female Sprague-Dawley rats (Blaszcak, 1991a).

The acute percutaneous (dermal) toxicity of dimethenamid-P (SAN 1289, batch No. 6663-50-1; purity, 91.1%) was evaluated in a single group of five male and five female New Zealand White rabbits given undiluted herbicide at a dose of 2000 mg/kg bw. Dimethenamid-P was applied to an area of approximately 12 × 14 cm (at least 10% of the body surface) of the shaved intact skin of the dorsal trunk, which was kept in contact with the skin for 24 h, covered by a gauze patch and impervious plastic. The application site was then washed with 0.9% saline. Observations for mortality and clinical signs were made for up to 14 days. Body weights were measured on days 1, 8 and 15. Autopsies were performed on all rabbits on day 15. No deaths occurred in this study and there were no significant signs of toxicity. Four of the ten rabbits showed slight body-weight losses by day 8, but gained weight thereafter. In the remaining six rabbits there was either body-weight gain or no weight change during the study. There were no internal pathological findings at autopsy. Three of the ten rabbits showed red, subcutaneous discolourations or foci at the application site. The dermal LD_{50} for dimethenamid-P was > 2000 mg/kg bw in male and female rabbits (Blaszcak, 1996b).

The acute percutaneous (dermal) toxicity of racemic-dimethenamid (lot No. 9021; purity, 97.1%) was evaluated in a single group of five male and five female New Zealand White rabbits given undiluted herbicide at a dose of 2000 mg/kg bw. Racemic dimethenamid was applied to an area of at least 10% of the body surface of the shaved intact skin of the dorsal trunk, which was then covered by a gauze patch and kept in contact with the skin for 24 h under an occlusive wrapping. The wrappings were removed after 24 h and the test site wiped free of material with gauze and water. The rabbits were observed for 14 days after the single application. Body weights were measured before the study began and on days 7 and 14. Clinical observations were recorded 1, 2 and 4 h after dosing, and daily thereafter for 14 days. Autopsies were performed on all rabbits. No deaths occurred in this study and there were no significant signs of toxicity. No significant dermal effects were observed. There were no internal pathological findings at autopsy. The dermal LD_{50} for racemic-dimethenamid was > 2000 mg/kg bw in male and female rabbits (Blasczak, 1991b).

The acute toxicity of dimethenamid-P (SAN 1289, batch No. 6663-50-1; purity, 91.1%) administered by inhalation was investigated in a group of five male and five female Sprague-Dawley rats. Exposure was for 4 h to a target atmospheric concentration of ≥ 2000 µg/l. Actual chamber concentrations were determined every hour during the exposure using a gas chromatographic method. Particle size was also determined once each hour and the temperature and relative humidity were recorded every half hour after treatment. The rats were observed for

14 days after the exposure. Body weights were recorded before the test and on days 7 and 14 after exposure. All rats were killed on day 15 and a gross autopsy performed.

The average measured atmospheric concentration of dimethenamid-P was 2200 µg/l. The mass median aerodynamic diameter (MMAD) was 3.4 µm, with a geometric standard deviation of 2.0 µm. Approximately 4% of the aerosol was of MMAD ≤ 1.0 µm, approximately 59% was ≤ 4.0 µm and approximately 94% was ≤ 10.0 µm. All rats survived the observation period. Laboured breathing was noted in the last 2 h of the exposure in two of the ten rats. Clinical signs noted upon removal from the chamber and during the first 2 h after exposure included secretory (lachrymation, chromodacryorrhoea, red and clear nasal discharge and dried red facial material) and respiratory (laboured breathing and moist râles) responses. Similar signs were observed in some rats for up to 2 days after exposure. No clinical signs were observed after day 2. Body weights increased normally and no abnormalities were noted at autopsy. The 4 h median lethal concentration (LC_{50}) of dimethenamid-P was > 2200 µg/l in male and female Sprague-Dawley rats (Hoffman, 1996).

The acute inhalation toxicity of racemic-dimethenamid (SAN 582 H; batch No. 8605; purity, 91.4%) was investigated in a group of five male and five female Wistar rats. Exposure was for 4 h to a target atmospheric concentration of 4990 µg/l. Particle size distribution was determined twice during the exposure and the exposure concentration measured gravimetrically five times during the exposure. The rats were monitored for mortality and clinical signs four times during the first day and daily thereafter. Body weights were recorded before exposure and on days 8 and 15. A gross autopsy was performed on all rats.

Measurements of particle size distribution showed that approximately 6% of the aerosol was of size < 1 µm. All rats survived the exposure and the 14-day observation period. Clinical signs observed were sedation, dyspnoea, curved body position and ruffled fur during the exposure in several rats and during day 4 in one rat. Body weights increased normally and no abnormalities were noted at autopsy. The LC_{50} at 4 h for racemic dimethenamid was > 4990 µg/l in male and female Wistar rats (Ullmann, 1986).

Dimethenamid-P (SAN 1289, batch No. 6663-50-1; purity, 91.1%) was evaluated for acute dermal irritation potential in six male New Zealand White rabbits. Approximately 0.5 ml of the undiluted test substance was applied to the skin beneath a gauze patch (6 cm^2) and secured in position with a semi-occlusive dressing for 4 h, after which the application site was uncovered and washed with 0.9% saline. The skin irritation was scored at 30 min and 24, 48 and 72 h after removal of the test material. Results from the individual animals are shown in Table 12.

Three rabbits developed slight erythema with no oedema and two rabbits developed very slight (barely perceptible) erythema with no oedema. All rabbits were free of dermal irritation by 72 h after removal of the test material. The Meeting concluded that dimethenamid-P produced mild, transient irritation (Blaszcak, 1996c).

Racemic-dimethenamid (SAN 582 H; batch No.8605; purity, 91.4%) was evaluated for acute dermal irritation potential in six male New Zealand White rabbits. Approximately 0.5 ml of the undiluted test subtance was applied to the skin under a gauze patch (6 cm^2) secured in position with transparent tape. The trunk was wrapped in a nonabsorbent binder for 4 h, after which the application site was uncovered and washed with water. The skin irritation was scored at 30–60 min and 24, 48 and 72 h after removal of the test material. Very slight erythema was observed in five of six rabbits after 30–60 min, and very slight oedema occurred in one rabbit during the same period. All rabbits were free of dermal irritation by 24 h after treatment. The Meeting concluded that racemic dimethenamid produced mild transient dermal irritation that cleared within 24 h. The primary irritation index was 0.125 (Lemen, 1988a).

Table 12. Skin irritation (erythema and oedema) in male rabbits

Animal No.	Time after patch removal				Mean scores
	30 min	24 h	48 h	72 h	
1	0/0	1/0	1/0	0/0	0.5
2	1/0	1/0	1/0	0/0	0.8
3	1/0	2/0	2/0	0/0	1.3
4	0/0	0/0	0/0	0/0	0.0
5	1/0	2/0	1/0	0/0	1.0
6	1/0	2/0	1/0	0/0	1.0

From Blaszcak (1996c)

Table 13. Mean eye irritation scores in male rabbits

Rabbit No.	Corneal opacity	Iris	Conjunctival redness	Conjunctival swelling
1	0	0	0	0
2	0	0	0	0
3	0	0	0.33	0
4	0	0	0.33	0
5	0	0	0	0
6	0	0	0	0

From Blaszcak (1996d)

Dimethenamid-P (SAN 1289; batch No. 6663-50-1; purity, 91.1%) was evaluated for acute eye irritation potential in six male young adult New Zealand White rabbits. Approximately 0.1 ml of undiluted dimethenamid-P was administered to one eye of each rabbit. The test substance was not washed from the eyes. Examination of the eyes was carried out 1, 24, 48 and 72 h after the application of the test substance.

The mean eye irritation scores recorded over the 72 h observation period are shown in Table 13.

All six rabbits exhibited slight conjunctival redness and/or chemosis and moderate to severe conjunctival discharge at 1 h after exposure. The discharge and chemosis were not observed at 24 h after treatment. Four animals were free of conjunctival redness by 24 h and the remaining two animals were free by 48 h. There were no corneal or iridial effects observed. The final assessment was that dimethenamid-P is practically non-irritant to the eyes of rabbits (Blaszcak, 1996d).

Racemic-dimethenamid (SAN 582 H; batch No.8605; purity, 91.4%) was evaluated for acute eye irritation potential in six male young adult New Zealand White rabbits. Approximately 0.1 ml of undiluted racemic-dimethenamid was administered to one eye of each rabbit. The test substance was not washed from the eyes. Examination of the eyes was carried out 1, 24, 48 and 72 h after the application of the test substance.

At 1 h after dosing, all six rabbits had conjunctival redness (score 2) and discharge, and four rabbits had chemosis. All rabbits were free of conjunctival irritation by 72 h. No iridial or corneal effects were observed. The final assessment was that racemic-dimethenamid produces mild transient ocular conjunctival irritation in New Zealand White rabbits (Lemen, 1988b).

The potential of dimethenamid-P (SAN 1289; batch No. 6663-50-1; purity, 91.1%) to produce delayed contact hypersensitivitywas assessed using the Buehler test in Dunkin-Hartley guinea-pigs. For induction, 10 male and 10 female guinea-pigs received 0.3 ml of undiluted dimethenamid-P by cutaneous application for 6 h under an occlusive dressing once per week for 3 weeks. Ten untreated guinea-pigs were used as a control group. A cutaneous challenge

application of 0.5 ml of undiluted (100%) test substance was carried out 14 days after the third induction on a non-treated area using the same procedure as that for induction. Control animals were also treated with dimethenamid-P during the challenge phase to differentiate dermal irritation scores from sensitization reactions. Readings for dermal changes were taken 24 and 48 h after patch removal. The sensitivity of the test method in the performing laboratory is tested several times a year with dinitrochlorobenzene.

Irritation increased in incidence and severity during the induction phase. At challenge, dermal responses were observed in 17 out of 20 guinea-pigs previously treated with dimethenamid-P, compared with none out of 10 in the control group. Dimethenamid-P is considered to be a skin sensitizer in the Buehler test (Blaszcak, 1996e).

The potential of racemic dimethenamid (SAN 582 H; batch No. 8502; purity, 91%) to produce delayed contact hypersensitivity was assessed using the Magnusson & Kligman maximization test in Dunkin-Hartley guinea-pigs. Twenty guinea-pigs were used in each of the negative control, test and positive control groups. The first phase of induction was conducted by intracutaneous injections of adjuvant alone, 5% test substance in PEG 400 or 5% test substance in adjuvant. The second phase of induction was a 48 h topical application of undiluted test substance 1 week later. The challenge was performed 2 weeks after the dermal induction, once again using undiluted test substance. Skin reactions were scored at 24 and 48 h after patch removal.

Irritation increased in incidence and severity during the induction phase. At 24 h and 48 h, respectively, after challenge, dermal responses were observed in 9 out of 19 and 15 out of 19 guinea-pigs previously treated with racemic dimethenamid, compared with none out of 10 in the control group. Racemic dimethenamid is considered to be a skin sensitizer according to the Magnusson & Kligman test (Hamburger et al., 1987).

2.2 Short-term studies of toxicity

Mice

Groups of 12 male and 12 female CD-1 mice were given diets containing racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 91.5%) at a concentration of 0, 300, 700, 2000 or 5000 ppm, equal to 0, 46, 105, 301 and 805 mg/kg bw per day in males and 0, 60, 137, 383 and 972 mg/kg bw per day in females, for 13 weeks. Analyses for correct concentration were performed before study start and at monthly intervals thereafter. This was a dose-range finding study conducted according to GLP guidelines, but not regulatory requirements.

Food consumption and body weight were determined once per week. The state of health was checked twice daily. All animals were subjected to complete gross examinations, and weights of selected organs were determined. Histopathological examinations were conducted on the liver and kidney of animals at the lowest and highest dose.

The correct concentrations of the test material in the diet were confirmed by analysis.

Subdued behaviour was observed in mice at 5000 ppm and in one male mouse at 2000 ppm, but behaviour was normal at the lower doses. There were no mortalities during the study. At 5000 ppm, there was a body-weight gain depression of 50% in males, although food consumption was reduced by only 8%. There was no effect on body-weight gain at other doses in males or at any dose in females. Absolute liver weights were increased by 25% in males and 36% in females at 5000 ppm, by 20% in males and 15% in females at 2000 ppm and by 13% in males at 700 ppm. There were parallel increases in relative (to body weight) liver weights of 49% in males and 39% in females at 5000 ppm, 26% in males and 18% in females at 2000 ppm and 17% in males at 700 ppm. In addition, relative kidney weights were increased by 21% in males and 12% in females at 5000 ppm and 11% in males and 14% in females at 2000 ppm. No histopathological lesions were reported for the liver or kidneys at any dose. In the absence of

histological changes, the increased liver weights are most likely to be adaptive, physiological changes induced by the chemical and probably do not represent an adverse effect.

The NOEL was 300 ppm, equal to 46 mg/kg bw per day in males and 60 mg/kg bw in females, on the basis of organ-weight changes at 700 ppm, equal to 105 mg/kg bw per day in males and 137 mg/kg bw in females. However, the NOAEL in mice given diets containing racemic dimethenamid for 90 days was 2000 ppm, equal to 301 mg/kg bw per day in males and 383 mg/kg bw per day in females, on the basis of reduced body weights and body-weight gains and increased absolute and relative liver weights and relative kidney weights in both males and females at 5000 ppm, equal to 805 mg/kg bw per day in males and 972 mg/kg bw per day in females (Warren et al., 1988).

Rats

Groups of five male and five female Sprague-Dawley CD rats were given diets containing dimethenamid-P (SAN 1289 H; batch No. 6663-25-6; purity, 94.7%) at a concentration of 0, 500, 1500 or 3000 ppm, equal to 51, 149 and 298 mg/kg bw per day, for 4 weeks. An additional group received lower doses of 50 ppm for 1 week and 150 ppm, equal to an average of 12 mg/kg bw per day, for 3 weeks. This was a dose-range finding study conducted according to GLP guidelines, but not for a regulatory requirement.

The clinical condition of the rats was monitored throughout the study, while body weights and food consumption were measured weekly. Haematology and examinations of blood and urine chemistry were conducted during the last week of the study. At the end of the feeding period, the rats were killed and subjected to autopsy, and organ weights recorded.

All rats survived for the duration of the study, and there were no clinical signs observed which were considered related to treatment. Also, there were no treatment-related effects on haematological parameters.

In rats at 3000 ppm, body weights were lower, but significantly, by 8% in males and body-weight changes were lower, but not significantly, by 15% in males and 6% in females. There were no effects on food consumption. In this same group, absolute liver weights were 22% higher (not significant) in males and significantly increased by 37% in females, while relative (to body weight) liver weights were increased by 34% in males and 44% in females. Increases in serum γ-glutamyltransferase activity in rats at 3000 ppm are in agreement with the increased liver weights and indicates the possibility of either frank liver toxicity or of an adaptive response to the chemical. There were no gross pathological findings considered to be related to treatment.

The NOAEL for dimethenamid-P was 1500 ppm, equal to 149 mg/kg bw per day, on the basis of reduced body weights and body-weight gain, increased absolute and relative liver weights and increased serum γ-glutamyltransferase activity in both sexes at 3000 ppm, equal to 298 mg/kg bw per day (Randall et al., 1996).

Groups of 10 male and 10 female Sprague-Dawley CD rats were given diets containing dimethenamid-P (SAN 1289 H; batch No. 6663-50-1 purity, 91.1%) at a concentration of 0, 500, 1500 or 3000 ppm, equal to 0, 39, 118 and 239 mg/kg bw per day, for 90 days. Analyses for stability and homogeneity of the test substance in the diet as well as for correct concentration were performed.

Body weight and food consumption were recorded weekly. The state of health of the rats was checked twice daily and comprehensive clinical examinations of the rats were recorded once per week. Haematology and blood and urine chemical analyses were carried out at the end of the administration period. Ophthalmology was carried out in all rats before the start and at the end of the administration period. At the end of the treatment period all rats were killed, subjected to complete gross examinations and selected organs were weighed. Tissues from all rats in the group receiving the highest dose and the control groups were examined histologically and, in addition, the kidneys, liver and lungs were examined from all groups receiving intermediate doses.

Stability analyses demonstrated that dimethenamid-P was stable in the diet for the period of time that it was presented to the rats and that it was homogeneously distributed in the feed. Concentration verification analyses confirmed the target dietary concentrations.

No mortalities occurred during the study, and there were no clinical signs of toxicity or ophthalmoscopy findings that were considered to be treatment-related. Body weights were decreased at 3000 ppm and 1500 ppm by 7% and 5%, respectively, in males and by 5% and 4%, respectively, in females. Body-weight gains were also decreased at 3000 ppm and 1500 ppm by 11% and 8%, respectively, in males and 10% and 9%, respectively, in females. There were no significant body weight changes at 500 ppm. Food consumption was not affected by treatment.

The only haematological change noted was an increase in clotting time as measured by activated partial thromboplastin time in females at 3000 ppm. There were no effects on the urine parameters investigated.

Serum γ-glutamyltransferase activity was increased in both sexes at 3000 ppm and in males at 1500 ppm. Cholesterol concentration was also increased in both sexes at 3000 ppm. In addition, there was a trend towards decreased aspartate aminotransferase activity in males at all doses and decreased alkaline phosphatase activity in males at 3000 ppm and 1500 ppm; however, it is unclear what toxicological significance can be attributed to decreases in activities of these enzymes.

Liver weights were increased in males and females at 3000 ppm by 27% and 18%, respectively, and in males at 1500 ppm. Relative liver weights were increased in males at 3000 ppm, 1500 ppm and 500 ppm by 36%, 21% and 11% respectively. In females, the relative liver weights were increased by 25% at 3000 ppm, but there was no notable change at lower doses. Histologically, the only treatment-related finding was hepatocellular hypertrophy in both sexes at 3000 and 1500 ppm and in females at 500 ppm, where the hypertrophy was described as minimal or slight. Hepatocellular swelling and liver weight increase generally indicates an adaptive, physiological response of the liver to exposure to the chemical rather than frank toxicity. Thus, there was no dose at which an effect was not observed, but the observations recorded at 500 ppm were not considered to be adverse.

The NOAEL in rats given diets containing dimethenamid-P for 90 days was 500 ppm, equal to 39 mg/kg bw per day, on the basis of reduced body weights and body-weight gains and increased serum cholesterol concentration in both males and females and increased serum γ-glutamyltransferase activity in males at 1500 ppm, equal to 118 mg/kg bw per day (Blansett, 1996).

Groups of 10 male and 10 female Sprague-Dawley CD rats were given diets containing racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 91.5%) at a concentration of 0, 50, 150, 500, 1500 or 3000 ppm, equal to 0, 3.5, 10, 33.5, 98 and 204 mg/kg bw per day in males and 0, 3.9, 11.8, 40.1, 119 and 238 mg/kg bw per day in females, for 90 days. An additional 10 rats per sex were used in the control group and at the highest dose to determine recovery from treatment. Analyses for stability and homogeneity of the test substance in the diet as well as for correct concentration were performed.

Body weight and food consumption were recorded weekly. The state of health of the rats was checked twice daily and comprehensive clinical examinations of the rats were recorded once per week. Haematology and blood and urine chemical analyses were carried out at the end of the administration period. Ophthalmology was carried out in all rats before the start and at the end of the administration period. At the end of the treatment period all rats were killed, subjected to complete gross examinations and selected organs were weighed. Tissues from all rats at the highest dose and in the control groups were examined histologically and, in addition, the kidneys, liver and lungs were examined from all groups of rats receiving intermediate doses.

Dietary analyses demonstrated the test material was homogeneously distributed in the feed, that it was stable for the period of time presented to the animals and that the actual concentrations were ± 8% of the target concentrations. No deaths occurred during the study and there were no

clinical signs or ophthalmoscopic effects considered related to treatment. The mean body weights and body-weight gains of the animals treated at 1500 and 3000 ppm were lower than those of thse in the control group. For the 90-day treatment period, the mean body-weight gains of males at the intermediate and highest doses were approximately 17% and 23% below those of controls, respectively, and the females at the intermediate and highest doses were 13% and 24% below those of controls, respectively. For females at 500 ppm and for males and females at 50 and 150 ppm, body-weight gain was comparable with that of the controls. During the recovery period, males and females previously at 3000 ppm showed a higher body-weight gain than did controls. Food consumption was marginally lower in rats at 1500 and 3000 ppm. No treatment-related effects were observed on food consumption at concentrations of 500 ppm and less.

None of the haematological parameters indicated any treatment-related effects. Blood chemistry results showed a decrease for males and females in serum aspartate aminotransferase and alanine aminotransferase activities at all doses, although this did not follow a dose-related trend. Reductions in the activities of these enzymes are of doubtful toxicological significance. Alkaline phosphatase activity was also slightly decreased in both sexes at 1500 and 3000 ppm. Mean γ-glutamyltransferase values were increased in in males and females at 3000 ppm in comparison to those for the controls. Cholesterol concentrations were increased in both sexes at 3000 ppm and in females at 1500 ppm. There were no treatment-related effects on urine analysis parameters. Liver enzyme activities and cholesterol concentrations at 3000 ppm were generally comparable to control after the recovery period.

No macroscopic pathology findings were considered to be treatment-related. Liver weights adjusted with body weight as a covariate were increased in males at 3000 ppm and in females at 1500 and 3000 ppm. Liver weights for animals at 500 ppm and below were similar to controls. Liver weights for animals at 3000 ppm after a 4-week recovery were comparable to controls in males and only slightly increased in females. There were no treatment-related effects on the other weighed organs. Microscopically, minimal to moderate enlargement of centrilobular hepatocytes was observed in females at 1500 and 3000 ppm. The centrilobular enlargement was still present in two out of ten females at 3000 ppm after the 4-week recovery period. There were no other microscopic findings considered related to treatment.

The NOAEL in rats given diets containing racemic dimethenamid for 90 days was 500 ppm, equal to 33.5 mg/kg bw per day, on the basis of reduced body weights and body-weight gains in males and females and increased liver weights and centrilobular hepatocyte hypertrophy and increased serum cholesterol in females at 1500 ppm, equal to 98 mg/kg bw per day (Ruckman et al., 1987).

When comparing the 90-day studies with racemic dimethenamid and dimethenamid-P in rats, the similarity in general toxicity and for liver effects is clear. However, given that these studies were conducted 9 years apart and in different laboratories, some discrepancies would be expected. One discrepancy was in the qualitative and quantitative occurrence of hepatocellular hypertrophy. Consequently, the liver slides were re-evaluated by a single pathologist (Küttler, 1999). The results of the original reviews and the re-evaluations are presented in Tables 14 and 15.

Upon re-evaluation, the occurrence of centrilobular hepatocellular hypertrophy was nearly identical in the female rats treated with racemic dimethenamid or with dimethenamid-P. The incidences were clearly increased at 1500 and 3000 ppm and there were slight increases at 500 ppm. Also, in the re-evaluation, periportal hypertrophy was seen in males after treatment with both racemic dimethenamid and dimethenamid-P at 3000 ppm. In addition, in the case of dimethenamid-P there was a slight increase at 1500 ppm, but the severity was minimal or slight. Overall, there were no qualitative differences, and the only quantitative difference was an increase in incidence of minimal to slight hypertrophy at 1500 ppm in males.

Overall, liver changes were clearly evident with both forms of dimethenamid at 3000 ppm in both sexes and at 1500 ppm for females. This conclusion was based on increased serum

cholesterol and γ-glutamyltransferase activity, increased relative liver weights and hypertophic hepatocytes. With dimethenamid-P, liver changes were also observed at 1500 ppm in males, as indicated by increased serum γ-glutamyltransferase activity, increased liver weight and hepatocyte hypertrophy. Although not observed in males in the 90-day study with racemic dimethenamid at 1500 ppm , liver changes have been seen at similar doses in other studies with racemic dimethenamid. In a 5-week oral feeding study, serum cholesterol and relative liver weights were increased in males treated at 1000 ppm (Carpy, 1987). In a long-term study in rats, males treated with racemic dimethenamid at 1500 ppm had increased serum γ-glutamyltransferase activity, increased liver weight and liver histopathological changes consisting of increased incidences of eosinophilic hepatocytes and ground-glass hepatocytes. Ground-glass appearance in hepatocytes has been shown in the literature to result from an increase in smooth endoplasmic reticulum. This change, like hypertrophy, is usually an adaptation to the handling or detoxification of a chemical.

Table 14. Liver histopathology results as presented in the original reports for 90-day studies in rats

Finding	Dietary concentration (ppm)							
	Racemic dimethenamid				Dimethenamid-P			
	0	500	1500	3000	0	500	1500	3000
Males								
Hypertrophy, periportal	0	0	0	0	0	0	8	10
Minimal	0	0	0	0	0	0	4	1
Slight	0	0	0	0	0	0	4	9
Inclusions	0	0	0	0	0	0	3	7
Minimal	0	0	0	0	0	0	1	3
Slight	0	0	0	0	0	0	2	4
Females								
Hypertrophy, centrilobular	0	1	9	10	0	3	8	8
Minimal	0	1	9	9	0	1	4	3
Slight	0	0	0	0	0	2	4	5
Moderate	0	0	0	1	0	0	0	0

From Küttler (1999)

Table 15. Liver histopathology results from re-evaluation of the slides from 90-day studies in rats

Finding	Dietary concentration (ppm)							
	Racemic dimethenamid				Dimethenamid-P			
	0	500	1500	3000	0	500	1500	3000
Males								
Hypertrophy, periportal	0	0	0	5	0	0	7	10
Minimal	0	0	0	3	0	0	4	5
Slight	0	0	0	2	0	0	3	5
Inclusions	0	0	0	6	0	0	2	6
Minimal	0	0	0	4	0	0	0	2
Slight	0	0	0	2	0	0	2	4
Females								
Hypertrophy, centrilobular	0	3	9	9	0	3	9	8
Minimal	0	3	8	3	0	2	6	4
Slight	0	0	1	6	0	1	3	4
Moderate	0	0	0	0	0	0	0	0

From Küttler (1999)

The lack of pronounced liver changes in males at 1500 ppm in the 90-day study with racemic dimethenamid is considered to be incidental or attributable to normal variation.

Dogs

Groups of four male and four female pure-bred beagle dogs were given diets containing racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 91.4%) at a concentration of 0, 100, 750 or 2000 ppm, equal to 0, 5, 37 and 96 mg/kg bw per day, for 3 months. Analyses for correct concentrations in the diet were performed periodically during the study.

Food consumption of the animals was measured daily and their body weight once per week. The state of health of the dogs was recorded each day, and detailed physical observations were made during weeks 6 and 13 of treatment. Haematology and blood and urine chemistry examinations were carried out once before treatment began and during weeks 6 and 12 of the treatment period. Ophthalmology was conducted before the treatment period and at weeks 6 and 13 of treatment. All dogs were subjected to complete gross and histopathological examinations, and selected organs were weighed.

The correct concentrations of test material were confirmed by analysis for groups at 750 and 2000 ppm. At 100 ppm, analytical values were consistently below target and the actual dose was approximately 91.5 ppm.

No mortality or treatment-related clinical signs were observed at any dose. Body-weight gains were decreased in male and female dogs at 2000 ppm by 70% and 58%, respectively, and in female dogs at 750 ppm by 32%.

Blood chemistry measurements indicated an increase in serum alkaline phosphatase activity in males and females at 2000 ppm. In dogs, this generally correlates with an induction of the microsomal enzyme system of the liver. There was also an increase in serum cholesterol concentrations in males and females at 2000 ppm. No treatment-related changes in blood chemistry were found at 750 ppm and no treatment-related changes in urine chemistry, haematology or ophthalmology at any dose.

Liver weights were increased in males and females at 2000 ppm by 20% and 32%, respectively. Relative liver weights were increased in males and females at 2000 ppm by 49% and 61%, respectively, and in males and females at 750 ppm by 16% and 22%, respectively. There was no notable change in liver weights in the 91.5 ppm dose-group. Histologically, the only treatment-related findings were cytoplasmic vacuolization of the periportal hepatocytes in both sexes at 2000 ppm and 750 ppm and dilatation of the hepatic sinusoids in both sexes at 2000 ppm and females at 750 ppm.

The NOAEL in dogs given diets containing racemic dimethenamid for 90 days was 91.5 ppm, equal to 4.6 mg/kg bw per day, on the basis of reduced body-weight gains, increased absolute and relative liver weights and histological findings in the liver in both males and females at 750 ppm, equal to 37 mg/kg bw per day (Greenough & Goburdhun, 1986; Greenough, 1991).

Groups of four male and four female pure-bred beagle dogs were given diets containing racemic dimethenamid (SAN 582 H; batch No: 8605; purity, 91.3%) at a concentration of 0, 50, 250 or 1500 ppm, equal to 0, 2, 10 and 49 mg/kg bw per day, for 12 months. Analyses to determine stability and homogeneity and correct concentrations of the test substance in the diet were performed.

Food consumption of the animals was measured daily and their body weight once per week. The state of health of the dogs was recorded each day. Haematology and blood and urine chemistry examinations were carried out once before treatment began and during weeks 13, 26 and 51 of the treatment period. Ophthalmology was conducted before the treatment period and at weeks 26 and 51 of treatment. All dogs were subjected to complete gross and histopathological examinations, and selected organs were weighed.

The stability and homogeneity of the test substance in the diet and the correct concentrations were confirmed by analysis.

No mortality or treatment-related clinical signs were observed at any dose. Body-weight gains were decreased in male and female dogs at 1500 ppm by 65% and 32%, respectively. The mean body-weight gain was also decreased at 250 ppm; however, this was caused by a low value for a single dog. The body-weight gains of the other three dogs were generally very similar to those of animals in the control group, except for one dog whose body-weight gain exceeded that of any animal in the control group. It was concluded, therefore, that the mean decrease in body-weight gain at 250 ppm was spurious. There were no treatment related body-weight changes at 250 and 50 ppm and there were no treatment-related effects on food consumption in any group.

Blood chemistry measurements indicated an increase in serum alkaline phosphatase activity in males and females at 1500 ppm. In dogs, this generally correlates with an induction of the microsomal enzyme system of the liver. There was also an increase in serum cholesterol concentrations in males and females at 1500 ppm. No treatment related changes in blood chemistry were found at 250 ppm and 50 ppm and no treatment-related changes in urine chemistry, haematology or ophthalmology at any dose.

Relative liver weights were increased in males and females at 1500 ppm by 31% and 33%, respectively, in females at 250 ppm by 15 % and females at 50 ppm by 19%. There was no notable change in relative liver weights in males at 250 ppm and 50 ppm. The absence of a dose-related response in females at 250 ppm and 50 ppm brings into question whether the differences from the control group were treatment related. Histologically, the only treatment-related findings were cytoplasmic vacuolization of the periportal hepatocytes and dilatation of the hepatic sinusoids in two out of four males and four out of four females at 1500 ppm; no such observations were made at 250 ppm or 50 ppm. Enlargement of mid-zonal hepatocytes was observed in two males and one female at 1500 ppm. Because there were no indications of hepatotoxicity from either blood chemistry or histology at 250 ppm and because the increases in relative liver weights were not dose-related, it was considered that the liver weight increases at 250 ppm were spurious and not toxicologically significant.

The NOAEL in dogs given diets containing racemic dimethenamid for 52 weeks was 250 ppm, equal to 10 mg/kg bw per day, on the basis of reduced body-weight gains, increased relative liver weights in males and histological findings in the liver and increased serum cholesterol concentrations in both males and females at 1500 ppm, equal to 49 mg/kg bw per day (Greenough, 1988a).

Rabbits

Groups of five male and five female New Zealand White rabbits were given racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 91.6%) at doses of 0 (demineralized water) and 1000 mg/kg bw applied to the skin for 6 h per day; 5 days per week, under a semi-occlusive dressing, for 3 weeks. The test material was applied undiluted.

The clinical condition of the rabbits was monitored throughout the study, while body weights and food consumption were measured twice weekly. Skin reactions were recorded daily. Haematology and examination of blood chemistry were conducted on days 9 and 19 of the study. At the end of the feeding period, the rabbits were killed and subjected to autopsy, and weights of major organs were recorded. Histopathological examinations were also performed on all rabbits.

Skin oedema and erythema were observed in rabbits at 1000 mg/kg bw. This effect reached a maximum during the first week of treatment and was followed by a gradual recovery which was nearly complete by the end of the study. Body weights were decreased in the treated group during the first week of treatment, but without any accompanying effect on food consumption. However, the body-weight change was transitory and overall body-weight gains during the treatment period were similar in control and treated rabbits of both sexes. The slight and transient change in body weight is not considered to be toxicologically significant. There were no effects on any haematological or blood chemistry parameters. There were no treatment-related effects on organ

weights or macroscopic findings at autopsy. Histologically, the only change observed was a minimal to slight hyperkeratosis and acanthosis of the skin with inflammatory cell infiltration.

The NOAEL for systemic effects in rabbits given racemic dimethenamid by application to the skin was 1000 mg/kg bw per day, the only dose tested (Sommer et al., 1990).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 52 male and female CD-1 mice were given diets containing racemic dimethenamid (SAN 582 H: batch No. 8605; purity, 91.4%) at a concentration of 0, 30, 300, 1500 or 3000 ppm for 94 weeks. These concentrations provided doses equal to 0, 3.8, 41, 205 and 431 mg/kg bw per day and 0, 4.1, 40, 200 and 411 mg/kg bw per day for males and females respectively. Satellite groups of 16 mice of each sex received diets containing dimethenamid at 0 or 3000 ppm for 65 weeks. Analyses for stability and homogeneity of the test substance in the diet were performed before study start, and analyses to confirm target concentrations were performed periodically during the treatment period.

Food consumption and body weights were measured once per week. At least once per day the mice were examined for mortality and signs of toxicity, and once per week they were subjected to an additional comprehensive clinical examination (including palpation). Blood smears were prepared from all mice killed during the study and from all surviving mice at weeks 52, 78 and at terminal sacrifice (week 66 for mice in the satellite group and week 95 for mice in the main study group). At the end of the treatment period, all surviving mice were killed, subjected to gross pathological assessment, and selected organs were weighed. Histopathology was performed on all organs from animals in the control group and at the highest dose for the satellite group, and on all organs from all animals in the main study.

The stability and homogeneity of the test substance in the diet, and the correct concentrations were confirmed by analysis.

There were no adverse treatment-related effects on survival or clinical observations. Body-weight gains were reduced in males and females at 3000 ppm by 15% and 29%, respectively, and at 1500 ppm by 15% and 16%, respectively. Relative liver weights were increased in males and females at 3000 ppm by 16% and 30%, respectively, and at 1500 ppm by 19% and 26%, respectively. Relative kidney weights were also increased in females at 3000 ppm and 1500 ppm by 21% and 14%, respectively. No other treatment-related organ-weight changes were observed.

Examination of blood smears from all mice in the control group and at 3000 ppm in weeks 52, 65, 78–79 and 95 did not reveal any treatment-related effects on the differential leukocyte counts. The incidence and dose-group distribution of lesions observed at terminal autopsy were within the expected background range for these mice and were therefore considered to be unrelated to treatment.

Histology revealed hepatocytic hypertrophy at 3000 ppm, 1500 ppm and 300 ppm at the end of the study and in the satellite group at 3000 ppm (the only dose group examined) at the interim kill in week 66. At 300 ppm, hypertrophy of the hepatocytes was observed in just one male and two female mice and was of minimal severity. An increased incidence of hyperkeratosis of the gastric limiting ridge was observed in the satellite group at 3000 ppm at 65 weeks, but this was not confirmed in the main study group at 3000 ppm at 95 weeks. In the absence of any other sign of possible toxicity at this dose, the observation in these mice at 300 ppm is not considered to be adverse and is probably a physiological, adaptive response. There were no test substance-related findings at 30 ppm. In all groups, spontaneous changes that were often age-related, were observed in many organs and were within the normal background range for this strain of mouse.

Neoplastic findings were not treatment-related, the incidences showing no significant deviation from the expected tumour profile for this strain of mouse. Lymphoid tumours were the

most commonly recorded neoplasms, mainly among female mice. Liver tumours among male mice and pulmonary tumours among male and female mice showed relatively high incidences.

The NOAEL for racemic dimethenamid in mice was 300 ppm, equal to a mean dose of 40 mg/kg bw per day, on the basis of reduced body-weight gain, increased relative liver weight and increased incidence of hepatocellular hypertrophy at 1500 ppm, equal to 200 mg/kg bw per day (Hooks et al., 1990).

Rats

Groups of 90 male and 90 female Sprague-Dawley rats were given diets containing racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 91.3%) at a concentration of 0, 100, 700 or 1500 ppm. These concentrations provided doses equal to 0, 5, 36 and 80 mg/kg bw per day and 7, 49 and 109 mg/kg bw per day for males and females respectively. Twenty rats of each sex from each group were killed after 52 weeks (this group was referred to as the satellite group), while the remainder continued to be exposed to racemic dimethenamid until they were killed after 24 months. Analyses for stability and homogeneity of the test substance in the diet were performed before the study began. Analyses to verify correct concentrations in the diet were conducted throughout the treatment period.

Food consumption and body weights were measured once per week. Water consumption was measured daily during weeks 12, 25 and 51 for rats in the satellite group. Observations for mortality were made once per day and comprehensive clinical examinations and palpations were made once per week. Ophthalmology was carried out for all rats before the start of the study and again towards the end of the dosing period, and at week 52 on rats in the control group and at the highest dose. Haematology and blood and urine chemistry were measured for 10 rats of each sex per group during weeks 13, 26, 52, 78 and 104 of the dosing period. For these studies, rats in the satellite group were used in weeks 13, 26 and 52, and rats in the main group were used in weeks 78 and 104. All rats were subjected to gross pathological assessment and selected organs were weighed. Full histopathology was performed on all rats in the control group and at the highest dose, any rats that died during the study, lungs liver, kidney and any macroscopically abnormal tissue from animals at the lowest and intermediate doses, and on those tissues from rats at the lowest and intermediate dose for which a treatment-related change was noted at the highest dose.

The stability and homogeneity of the test substance in the diet was demonstrated. Verification of correct concentrations was also confirmed by analysis.

Survival (Table 16) of males was clearly better at 1500 ppm and 70 ppm than in the control group; it was also better among the females of these two groups, although the differences were not as great as those observed among the males.

Body-weight gain through week 80 was reduced in both males and females of the 1500 ppm group by 13% and 21%, respectively, and in females at 700 ppm by 10%. Food consumption was also reduced during the first 10 weeks of the study at 1500 ppm and 700 ppm. Relative liver weight was increased by 16% in females at 1500 ppm and by 15% at 700 ppm, but not in females at 100 ppm or in males at any dose.

There were no haematological changes, but at 1500 ppm there were increases in serum cholesterol in females and γ-glutamyltransferase in males. The activity of this enzyme was slightly higher at week 78 and 104 in males at 700 ppm, but the difference compared with controls was not significant at week 104. Sporadic, higher enzyme activities, that were not statistically significant and generally not dose-related were observed in the males at 700 ppm or 100 ppm in weeks 13, 26 and 52. The increased serum concentrations of cholesterol were statistically significant only in weeks 13 and 104. There were also slightly reduced serum concentrations of calcium in males of all treated groups in weeks 52 and 104; however, the marginal intergroup differences were not dose-related. Urinary ketone bodies were observed in males at 1500 ppm in weeks 26, 52 and 78.

Table 16. Survival in a 2-year study in rats fed diets containing racemic demethenamid

	Dietary concentration (ppm)			
	0	100	700	1500
Males				
No. of survivors	18	20	25	31
% survival	36	40	50	62
Females				
No. of female survivors	25	22	30	31
% survival	50	44	60	62

From Ruckman et al. (1990)

Ophthalmology revealed posterior lenticular opacities in both sexes at 1500 ppm, but not at lower doses. At week 103, the incidences in the control group were 4 out of 20 males and 4 out of 26 females, compared with 13 out of 32 males and 11 out of 34 females at 1500 ppm. Incidences at 700 ppm and 100 ppm were similar to those in the control groups.

Non-neoplastic histological changes that were recorded included increased epithelial hyperplasia at the limiting ridge of the stomach and hyperplasia of the parathyroid, both of which occurred at 1500 ppm. Parathyroid hyperplasia is an age-related, spontaneous phenomenon that is usually associated with progressive chronic nephropathy, although there was no corresponding increased incidence in this study. In this same group, altered eosinophilic hepatocytes were increased in males ($p = 0.044$). The incidence of bile-duct hyperplasia was increased in females, from 3 out of 50 in the control group to 20 out of 50 at 1500 ppm ($p < 0.001$) and 11/50 at 700 ppm ($p = 0.044$). The incidence of dilated bile ducts was also higher in females at 1500 ppm, but did not reach statistical significance ($p = 0.072$).

Neoplastic findings mentioned in the original report indicated a slight increase in ovarian tubular adenomas that was statistically significant by the Peto trend test ($p = 0.046$), but this was not significant in pairwise comparisons (Fisher exact test). All the ovarian tumours in all groups were observed at study termination, except for one tumour at 1500 ppm. Given the inverse dose–response relationship for mortality in this study, the better survival at 1500 ppm may have played some role in the higher incidence of ovarian tumours in this group. Tubular adenomas are a spontaneous neoplasm with variable incidence in Sprague-Dawley rats, although the incidence at 1500 ppm was marginally higher than had been recorded previously in this laboratory (Table 17). A pathology review was conducted by one of the original veterinary pathologists and an independent consultant veterinary pathologist, after the issue of the final report (Alison & Gopinath, 1993). The original and peer review analyses of the ovarian tumours and hyperplasia are shown in Table 18.

Between the original review and the peer review, there had been advances in diagnostic criteria for rodent ovarian tumours. They were originally believed to be of epithelial origin, but had become grouped at the time of the review with other sex cord-stromal neoplasms (Peluso & Gordon, 1992). This change in understanding led to a change in terminology, such that tubular adenomas (and hyperplasias) had become known as Sertoliform tubular adenomas (and hyperplasias). The peer review found one additional tumour in the control group, two additional tumours at 100 ppm, two additional tumours at 700 ppm and one fewer at 1500 ppm. As a result of the relatively small number of diagnostic changes made during the review, the reported increase in incidence of Sertoliform tubular adenomas was no longer significant by the trend test ($p = 0.35$). Hyperplasia was diagnosed more frequently in the re-analysis, particularly in the control group. Differentiation of Sertoliform tubular hyperplasia and adenoma is difficult and subjective, because of the diffuse nature of the lesion. There is a biological continuum from hyperplasia to adenoma, but even when these lesions were combined for statistical analysis, the

Table 17. Incidence of tubular adenoma of the ovaries in Sprague-Dawley rats in historical control groups at the performing laboratory

	Study code											
	86A	86B	85A	85B	84A	84B	84C	84D	84E	83A	83B	83C
Incidence of tubular adenoma	0/50	1/50	0/49	0/50	4/100	0/50	0/50	0/50	0/50	0/50	1/50	5/55

From Ruckman et al. (1990)

Table 18. Incidence of tumours and hyperplasia of the ovary in rats given diets containing racemic demethenamid

Finding	Dietary concentration (ppm)			
	0	100	700	1500
No. of rats examined	50	50	50	50
Original analysis				
Granulosa cell tumour	0	1	1	0
Tubular adenoma	2	1	2	6
Tubular hyperplasia	12	7	14	22
Review analysis				
Granulosa cell tumour	0	0	1	0
Sertoliform tubular adenoma	3	3	4	5
Sertoliform tubular hyperplasia	18	12	12	23
Sertoliform tubular hyperplasia + adenoma	21	12	14	24

From Ruckman et al. (1990)

incidence at 1500 ppm was not different from that in the control group. Sertoliform tubular hyperplasia and adenoma can be induced by hypophysectomy, are considered to be related to gonadotropin deficiency and ovarian senescence (Engle, 1946; Arias & Aschheim, 1974; Arias et al., 1976), and are mainly found in Sprague-Dawley rats. They are rarely found in other strains of rat and are not found in humans, where most ovarian cancers are thought to arise from the surface epithelium (Scully, 1995). The peer-review analysis dispelled doubts regarding the toxicological significance of the ovarian tumours, which do not metastasize. Their potential for malignancy is questionable and they are of very limited relevance for humans.

A marginal increase was observed in the incidence of liver tumours in male rats (Table 19). The incidence of carcinomas at 1500 ppm was not statistically different from that in the control group and it was within the range for historical controls for the testing laboratory (0–6%). The incidence of adenomas at 1500 ppm (6%) was slightly outside the historical range (0–4%), but the difference did not reach statistically significance ($p = 0.13$, Fisher exact test, one-sided because of zero incidence in the control group). The combined incidence of malignant and benign tumours was also slightly outside the range for historical controls (Table 20).

The increase in the incidence of benign liver tumours in male rats at 1500 ppm may be partly attributable to much better survival at this dose (see Table 16); survival was 72% greater than that for males in the control group. This increased survival allowed considerably more animals to reach an older age and develop liver adenomas, which are spontaneously occurring tumours that increase in incidence with age. All the liver tumours were found in rats killed at the end of the 2-year dosing period. In support of this position, females at the highest dose had a much more modest increase in survival and showed no increase in the incidence of liver tumours.

Table 19. Incidence of liver tumours in male rats given diets containing racemic demethenamid

Finding	Dietary concentration (ppm)			
	0	100	700	1500
No. of rats examined	50	50	50	50
Hepatocellular adenomas	0	0	1	3
Hepatocellular carcinomas	0	0	0	2
Combined (adenoma or carcinoma)	0	0	1	4

From Ruckman et al. (1990)

Table 20. Incidence of liver tumours in historical control groups of Sprague-Dawley rats at the performing laboratory

Liver tumour	Study code											
	86A	86B	85A	85B	84A	84B	84C	84D	84E	83A	83B	83C
Benign liver cell tumours	0/50	1/50	0/49	0/50	1/100	0/50	1/50	2/50	0/50	1/50	1/50	0/55
Malignant liver cell tumours	1/50	0/50	1/50	3/50	1/100	0/50	0/50	1/50	0/50	1/50	1/50	1/55

From Ruckman et al. (1990)

The NOAEL for racemic dimethenamid in rats was 100 ppm, equal to a mean dose of 5 mg/kg bw per day, on the basis of reduced body-weight gain, increased relative liver weight and increased incidence of bile-duct hyperplasia at 700 ppm, equal to 49 mg/kg bw per day (Ruckman et al., 1990).

Overall, the Meeting concluded that racemic dimethenamid and dimethenamid-P are unlikely to pose a carcinogenic risk to man.

2.4 Genotoxicity

Dimethenamid-P was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 21). There was no reproducible evidence for genotoxicity in vitro in tests for mutation in four studies in bacteria (*S. typhimurium* and *E. coli*) (Wagner & Coffman, 1996; Engelhardt & Hoffman, 1997a, 1997b; Wagner & Klug, 1997). In the earliest of these studies, there was an increase in the number of *S. typhimurium* TA100 mutants per plate in the standard plate assay, when tested in the absence of an exogenous metabolic activation system. Because the numbers of mutants increased with rising dose above that at which precipitation occurred, it was suspected that the effect could have been caused by the presence of an impurity. When the same batch was re-tested in a different laboratory, using both the standard plate protocol and a preincubation protocol, the earlier observation of a mutagenic effect was not reproduced (Engelhardt & Hoffman, 1997a). A batch of highly purified dimethenamid-P was also tested in this second laboratory using both protocols. Although toxicity was observed at doses of ≥ 2500 µg/plate using the standard protocol and ≥ 500 µg/plate using the preincubation protocol, no increase in the numbers of mutant colonies were observed in either the presence or absence of an exogenous activation system Engelhardt & Hoffman, 1997b). Finally, the original batch was re-tested against *S. typhimurium* TA100 in the laboratory that previously had reported a significant result. No mutagenic response was observed (Wagner & Klug, 1997). No significant response was observed in a single study for mutations at the *hprt* locus in Chinese hamster ovary (CHO) cells in vitro, after exposure for 5 h to dimethenamid-P concentrations up to 400 µg/ml or 450 µg/ml in the absence or presence of an exogenous metabolic activation system, respectively (San & Clark, 1996). This finding gives support to the conclusion that dimethenamid-P does not induce gene mutations in bacteria.

In a study for the induction of chromosomal aberrations in cultures of CHO cells, no significant increases in the proportion of abnormal cells were observed after exposure to dimethenamid-P at concentrations of up to 120 µg/ml for 4 h in the presence of an exogenous activation mixture, or at concentrations of up to 120 or 500 µg/ml for 20 h in the absence of metabolic activation (Curry & Schadly, 1996).

No genotoxic activity was observed in an assay for the induction of unscheduled DNA synthesis in primary cultures of rat hepatocytes exposed for 18–20 h to dimethenamid-P at concentrations of up to 125 µg/ml. A concentration of 250 µg/ml was excessively toxic in a preliminary study (San & Sly, 1996).

In a test for micronucleus formation in bone-marrow cells, groups of 15 male and 15 female ICR mice were treated by intraperitoneal injection with dimethenamid-P as a single dose at 0, 103, 205 or 410 mg/kg bw, these doses being chosen on the basis of a preliminary study in which mortality was observed at 600 mg/kg bw. A group of five mice of each sex was dosed orally with the positive control substance, cyclophosphamide. Subgroups of five males and five females were killed at approximately 24, 48 and 72 h after treatment (except in the positive control group, in which mice were killed at 24 h). Target concentrations of dimethenamid-P were verified analytically. No increases in the incidence of micronucleated polychromatic erythrocytes were observed in either sex in any group dosed with dimethenamid-P. Large increases in the incidence of micronuclei were observed in the positive control group (Putman et al., 1996).

The Meeting concluded that dimethenamid-P is unlikely to be genotoxic.

Tests for genotoxicity have also been conducted with racemic dimethenamid. These have included the following: assay for mutagenicity in *S. typhimurium* (Haworth & Lawlor, 1989); an assay for mutagenicity in Chinese hamster V79 cells (*hprt* locus) Debets & Enninga, 1986); an assay for chromosomal aberration in Chinese hamster ovary (CHO) cells (Taalman, 1985); assays for unscheduled DNA synthesis in primary cultures of rat hepatocytes (Müller, 1986; Cifone, 1989; Dean, 1990); assays for micronucleus formation in bone marrow cells of mice dosed in vivo (Voelkner, 1986; Marshall, 1993).

In these tests, apart from an equivocal result in one of the three assays for unscheduled DNA synthesis in vitro, none of the assays gave any indication that racemic dimethenamid might be genotoxic. Given the non-reproducibility of the equivocal result, the Meeting concluded that racemic dimethenamid also is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of reproductive toxicity, groups of 25 male and 25 female sexually immature Wistar rats (F_0 generation) were fed diets containing racemic dimethenamid (SAN 582 H; batch No. 8710; purity, 92.6%) at a concentration of 0, 100, 500 or 2000 ppm, equal to 0, 9, 45 or 175 mg/kg bw per day averaged over the pre-mating period of exposure of the F_0 and F_1 parental female rats. After 10 weeks, the rats were mated and allowed to rear the ensuing F_1 litters to weaning. The breeding programme was repeated with the F_1 parents (selected from the F_1 offspring) after a 101-day exposure period. The ensuing F_2 litters were reared to weaning. The

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Table 21. Results of studies of genotoxicity with dimethenamid-P

End-point	Test object	Dose (LED/HID)	Ref No.; Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2*uvrA*	333 µg/plate –S9 5000 µg/plate + S9 [a]	6663-50-1; 91.1	Positive –S9 Negative +S9	Wagner & Coffman (1996)
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2*uvrA*	5000 µg/plate ± S9 [a]	6663-50-1; 91.1	Negative	Engelhardt & Hoffman (1997a))
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2*uvrA*	5000 µg/plate ± S9 [a]	RS-1289-111596; 99.4	Negative	Engelhardt & Hoffman (1997b)
Gene mutation	*S. typhimurium* strains TA100	5000 µg/plate –S9 [b]	6663-50-1; 91.1	Negative	Wagner & Klug (1997)
Gene mutation	Chinese hamster ovary cells, *hprt* locus	400 µg/ml –S9 450 µg/ml +S9	6663-50-1; 91.1%	Negative	San & Clark (1996)
Chromosomal aberration *in vitro*	Chinese hamster ovary cells	120 µg/ml –S9 500 µg/ml +S9	6663-50-1; 91.1%	Negative	Curry & Schadly (1996)
Unscheduled DNA synthesis	Male Sprague-Dawley rat liver cells	125 µg/ml, 18–20 h	6663-50-1; 91.1%	Negative	San & Sly (1996)
In vivo					
Micronucleus formation	Male and female ICR mouse bone-marrow cells 24, 48 & 72 h after dosing	410 mg/kg bw, single intraperitoneal dose	6663-50-1; 91.1	Negative	Putman et al. (1996)

LED, lowest effective dose; HID, highest ineffective dose

[a] Standard plate and preincubation protocols

[b] Standard plate protocol

diets containing racemic dimethenamid were fed continuously throughout the study. The stability and homogeneous distribution of racemic dimethenamid in the diet were evaluated before the start of the study. Analyses to confirm correct concentrations were performed periodically during the study. Body weights of F_0 and F_1 parents were measured once per week of the pre-mating period, after which F_0 and F_1 females were weighed on days 0, 7, 14 and 21 of gestationand on postnatal days 1, 4, 7, 14 and 21. The F_1 and F_2 pups were weighed on the day of or day after birth, and on postnatal days 4, 7, 14 and 21. The state of health of the parents and the pups was checked at least once each day, and parental animals were examined for their mating and reproductive performances. Pups were sexed and evaluated as to health, and pup viability was recorded. A gross pathological examination was carried out on all parents and offspring. Histopathology was conducted on all F_0 and F_1 parents, special attention being paid to the reproductive organs.

The stability and homogeneity of the test substance in the diet and target dietary concentrations were confirmed by analysis.

In the parental groups of the F_0 and the F_1 generation there were no adverse effects of racemic dimethenamid on survival and there was no evidence for treatment-related clinical changes. Administration of diet containing racemic dimethenamid at 2000 ppm was associated with reduced food consumption, body weights and body-weight gains. Mean body weights of male rats at the end of the pre-mating period were lower than control values by 6% and 8% for F_0 and F_1 males, respectively, and body-weight gains of male rats were decreased by 12% and 8%

for F_0 and F_1 males, respectively. No such effects were observed at 500 ppm. There was no effect on body-weight gain in F_0 and F_1 female rats.

There was no evidence of an adverse effect of racemic dimethenamid on mean coital time, fertility index, conception-rate, duration of gestation or gestation index at any of the doses tested. There was no effect on litter size at birth that could be attributed to racemic dimethenamid. There were slightly reduced mean numbers of live pups per F_0 pregnancy at 200 ppm and 500 ppm, but this finding was not confirmed in the F_1 generation litters and so it was considered to be incidental. Pup survival was not affected by treatment. In both the F_1 and F_2 pups, mean pup birth weight, per sex, was slightly lower at 2000 ppm compared with controls. In F_1 the reduction was observed from postnatal day 4 until the end of lactation and attained statistical significance on postnatal days 14 and 21. In F_2 the reduction was observed from postnatal day 7 until the end of the lactation period. There was no effect on offspring body weights at 500 or 100 ppm.

At the autopsies at 2000 ppm, it was found that both absolute and relative (to body weight) liver weights were increased in both males and females of the F_0 and the F_1 generations. Absolute liver weights increased by 11% and 19% in males and females, respectively, of the F_0 generation and by 10% and 21% in the F_1 generation. Relative liver weights increased by 17% and 23% in males and females, respectively, of the F_0 generation and by 20% and 21% in the F_1 generation. At 500 ppm there were smaller increases in liver weight (F_0 males, 4%; F_0 females 10%; F_1 males, 3%; F_1 females, 4%). An increase in liver weight without accompanying histopathological changes is more likely to be an adaptive, physiological response of the liver to exposure to the chemical, rather than an expression of toxicity. Thus, the effect on the liver at 500 ppm is not considered to be adverse. There were no effects on pup survival. At 2000 ppm, pup body-weight gains were reduced during lactation in both the F_1 and F_2 generations. There was no effect on pup body weights at 500 or 100 ppm.

The NOAEL for toxicity in adult rats was 500 ppm, equal to 45 mg/kg bw per day, on the basis of reduced body-weight gain at 2000 ppm, equal to 175 mg dimethenamid/kg bw per day. The NOAEL for offspring toxicity was 500 ppm, equal to 45 mg racemic dimethenamid/kg bw per day, on the basis of reduced pup weight gain at 2000 ppm, equal to 175 mg racemic dimethenamid/kg bw per day. There were no effects on reproductive indices at doses up to and including 2000 ppm, so this dose was the NOAEL for reproductive toxicity, equal to 175 mg racemic dimethenamid/kg bw per day, the highest dose tested (Sutter et al., 1989).

The Meeting concluded that racemic dimethenamid is not a reproductive toxicant in rats.

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 25 time-mated, female Sprague-Dawley rats were given dimethenamid-P (SAN 1289 H; batch No. 6663-50-1; purity, 91.1%) at a dose of 0, 25, 150 or 300 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose, after first adhering the test substance to HiSil 233 as the carrier, on day 6 of gestation to day 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The control group of animals received vehicle alone. A standard dose volume of 10 ml/kg bw was used. On day 20 of gestation, the females were killed and assessed by gross pathology (including weight determination of the liver). The number of corpora lutea was determined, and the number and distribution of implantation sites were classified. The uteri were examined for live fetuses and intra-uterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and approximately one half were stained for skeletal examination, while the others were examined for soft tissue alterations.

Analytical verification of stability and homogeneity of dimethenamid-P in 0.5% carboxymethylcellulose were determined before the start of the study. Verification of test concentrations was performed twice during the current study. Analytical determinations demonstrated that dimethenamid-P concentrations were acceptably close to target level.

No mortalities, abortions or premature deliveries occurred during the study. The group at 300 mg/kg bw per day had increased incidences of excess lachrymation, piloerection, excess salivation, decreased motor activity, orange substance on fur, swollen ocular membrane, ptosis, dark pink skin, urine-stained abdominal fur and coldness to touch. These effects were first observed in some rats on days 6–7, and more commonly on days 8–9. There were no observations of significantly increased incidence of clinical toxicity at 150 or 25 mg/kg bw per day. Body-weight gains over days 6–16 of gestation were reduced by 14%, 18% and 25% at 25, 150 and 300 mg/kg bw per day, respectively. The reduced body-weight gain noted at 25 mg/kg bw per day was considered to be of no toxicological significance for several reasons. Body-weight gain decreases were noted at 25 (13%) and 300 mg/kg bw per day (18%) on post coital days 0–6, i.e. before treatment began. These differences from the controls were clearly unrelated to treatment. The reduced body-weight gain observed at 25 mg/kg bw per day during post coital days 6 to 9 (20%) was very similar to the difference noted during the period before treatment. Furthermore, on treatment days 9–12 and 12–16, there was clearly no treatment-related effect on body-weight gain because the weight gains at 300 and 150 mg/kg bw per day were comparable with those of animals in the control group. Over these same periods, weight gain at 25 mg/kg bw per day was slightly less than that of the control. These variations unrelated to treatment led to an overall, statistically significantly reduced body-weight gain at 25 mg/kg bw per day of 14%. An additional factor that may have lead to the difference at 25 mg/kg bw per day was the lower number of implantations in this group than in either the control group or at the higher doses.

A statistically significant reduction in feed consumption occurred at all doses during the treatment period. These were first observed as statistically significant at 150 and 300 mg/kg bw on days 6–9. The reductions over the whole treatment period in comparison with the control group were 9%, 23% and 31% at 25, 150 and 300 mg/kg bw per, respectively. As discussed above, the decreased maternal body-weight gain at 25 mg/kg bw per day was apparently exaggerated by random variability in that group. To appropriately consider any treatment-related effect on food consumption in this group, relative food consumption relative to body weight was calculated. The relative food consumption was statistically significantly reduced over the entire dosing period at 300 and 150 mg/kg bw per day as well as over the individual protocol defined periods, except for days 12–16 of gestation, when there was a rebound, significant increase. Relative food consumption was significantly reduced by 7% at 25 mg/kg bw on days 6–9 of gestation and not significantly different from that of the control group on days 9–12 and 12–16 of gestation. This slight and apparently transient effect on food consumption was not considered to be toxicologically relevant at this dose.

No autopsy observations were considered to be related to treatment. Changes that were observed at this stage were of a type and incidence commonly seen in the strain of rat (Sprague-Dawley) used in this study. Relative liver weights were increased by 8% at 300 mg/kg bw per day, but not at lower doses.

Pregnancies with live litters varied from 83.3% at 150 mg/kg bw per day to 100% at 300 mg/kg bw per day. There were no treatment-related effects on pre- or postimplantation losses, the number of early deaths, or on the sex distribution of fetuses. There were no dead fetuses in any group. Mean fetal weights (g/litter) in the four groups (0, 25, 150 and 300 mg/kg bw per day) were 3.36 ± 0.23, 3.34 ± 0.21, 3.29 ± 0.40 and 3.25 ± 0.23, there being no statistically significant reduction in the treated groups compared with the controls. The values from one litter at 150 mg/kg bw per day with exceptionally low fetal body weights were excluded from this analysis.

No malformations considered to be treatment-related were observed upon external, soft tissue or skeletal examination. Micrognathia and a small tongue occurred in one fetus from the control group and microphthalmia occurred in two fetuses from the group at 300 mg/kg bw per day. In this same group, one fetus had an umbilical hernia and anasarca (oedema of the head, neck and thoracic regions) with malformation of the long bones in both fore- and hindlimbs that were probably secondary to compression associated with the anasarca.

Variations observed in soft tissues were considered to be unrelated to treatment. Distended ureters were seen in seven fetuses in three litters at 300 mg/kg bw per day compared with similar variation in three fetuses in two litters from the control group. Because the litter incidence did not differ significantly from control, this increase was not considered to be treatment-related. At 300 and 150 mg/kg bw there was an increase in incidence of two retarded ossifications, sternal centra and pelvic pubes. Further evaluation of the delayed ossifications indicated that these differences were spurious, primarily attributable to unusually low control values, and not related to treatment (York, 1996).

The Meeting concluded that dimethenamid-P is not teratogenic in rats.

The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of clinical signs at 300 mg/kg bw per day and reduced food consumption at 150 mg/kg bw per day. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested. This decision was based on the arguments that the incidences of delayed ossification observed at 150 mg/kg bw per day were spurious.

In a study of developmental toxicity, groups of 25 time-mated, female Sprague-Dawley rats were given racemic dimethenamid (SAN 582 H; batch No. and purity not specified) in 0.5% aqueous carboxymethylcellulose, after first adhering the test substance to HiSil 233 as the carrier, by gavage at doses of 0, 50, 215 or 425 mg/kg bw per day on days 6 of gestation to day 15 post coitum. The day of confirmation of mating (when spermatozoa were detected) was designated day 0 of gestation. The control group of rats received vehicle alone. A standard dose volume of 10 ml/kg bw was used. On day 20 of gestation, the females were killed and assessed by gross pathology (including weight determination of the liver). Corpora lutea were counted, and the number and distribution of implantation sites were classified. Uteri were examined for live fetuses and intra-uterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and approximately one half were stained for skeletal examination, while the others were examined for soft tissue alterations.

Analytical verification of stability and homogeneity of racemic dimethenamid in 0.5% carboxymethylcellulose were determined before the start of the study. Verification of test concentrations was performed during the study. Analytical determinations demonstrated that concentrations of racemic dimethenamid at the target concentrations of 1.87 mg/ml and 26.1 mg/ml varied over the experiment by +17.8% and –12.9%, respectively.

Two dams in the control group died during the study. No other deaths or abortions occurred. One dam at 50 mg/kg bw per day delivered prematurely. Clinical signs that were observed included excess salivation at 425 and 215 mg/kg bw per day and urine-stained abdominal fur at 425 mg/kg bw per day. Weight loss occurred in dams at 425 mg/kg bw per day and reduced body-weight gain occurred in dams at 215 mg/kg bw per day during the first 3 days of treatment. The weight losses at 215 and 425 mg/kg bw per day continued during days 9–12 of gestation, but were not observed during days 12–16. Resulting from these early effects on body weight, significant decreases in body-weight gain were observed for the overall treatment period (16% at 215 mg/kg bw per day and –35% at 425 mg/kg bw per day). A slight decrease in body-weight gain (11%) was noted in dams at 50 mg/kg bw per day during the first 3 days of treatment. This was transient in that no differences in body weight were noted on subsequent days of treatment. The overall effect, however, was a nonsignificant 9% decrease in body-weight gain during the treatment period. Because the effect was slight and transient, this difference was not considered to be of toxicological significance. Relative feed consumption was significantly reduced at the intermediate dose during the first 3 days of treatment, and at the highest dose from days 6–12. A slight (5%) and transient (days 6–9 only) decrease in food consumption at 50 mg/kg bw per day was not considered to be toxicologically significant. Relative liver weight was statistically increased in dams at 50, 215 and 425 mg/kg bw per day by 6, 8 and 19%, respectively, and absolute liver weights were significantly increased at 215 and 425 mg/kg bw per day by 7% and 15%, respectively.

Table 22. Caesarean delivery and litter data in a study of developmental toxicity in rats given racemic dimethenamid by gavage

Observation	Dose (mg/kg bw per day)			
	0	50	215	425
Early deaths/pregnancy:				
Total (mean ± SD)	14/25 (0.6 ± 1.0)	21/25 (0.9 ± 0.8)	32/25 (1.4 ± 1.3)	47/25 (2.0 ± 2.8)
Historical controls, mean (range)[a]	0.8 (0.3–1.4)	—	—	—
Late deaths/pregnancy:				
Total (mean ± SD)	0 (0.0)	2 (0.1 ± 0.3)	0 (0.0)	2 (0.1 ± 0.3)
% dead implantations/litter ± SD	3.8 ± 6.1	6.2 ± 6.0	9.0* ± 9.1	10.7** ± 10.4
Historical controls, mean (range)[b]	5.9 (2.1–9.4)	—	—	—

From Lochry (1987)

SD, standard deviation

[a] Studies conducted in 1985–1986, 810 litters, 34 groups. An incidence of early death of 1.4 was reached in 1 out of 34 historical control groups only. The next highest incidence reported was 1.3 (one group), followed by 1.2 (two groups) and 1.1 (one group).

[b] Studies conducted in 1985–1986, 497 litters, 36 groups.

* Significantly different from the control value ($p < 0.05$); ** Significantly different from the control value ($p < 0.01$)

Fetal body weights were very slightly, but not significantly lower at 215 (–1%) and 425 (–2%) mg/kg bw per day. There was a dose-dependent increase in the incidence of early deaths at 415 and 215 mg/kg bw per day (Table 22). These increases were not statistically significant, although the incidence in both groups slightly exceeded the range for historical controls and resulted in minimal, statistically nonsignificant reductions in the live litter size. The incidence of early deaths at 50 mg/kg bw per day was not affected by treatment. Fetal sex ratio and body weight were unaffected by treatment. At the highest dose, two fetuses in two litters had incompletely ossified manubria. This low incidence was not considered to be related to treatment. There were no other increased incidences of fetal gross, soft tissue or skeletal variations or malformations.

The Meeting concluded that racemic dimethenamid is not teratogenic in rats.

The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of reduced maternal body-weight gain and reduced food consumption at 215 mg/kg bw per day. The NOAEL for developmental toxicity was 50 mg/kg bw per day on the basis of increased incidence of early deaths and reduced fetal body weight at 215 mg/kg bw per day (Lochry, 1987). A discrepancy between racemic dimethenamid and dimethenamid-P with regard to fetal toxicity was the observation of retarded ossification after treatment with dimethenamid-P. This difference was evaluated in the conducting laboratory (York et al., 1999).

Delayed ossifications in sternebrae and pelvic pubes occur at highly variable incidence and are the most common skeletal variations noted at the performing laboratory. A review of individual studies performed between 1983 and 1998 shows that the incidence of delayed ossifications of sternebrae in the control group varied from 0% to 44.4% in litters and 0% to 7.6% in fetuses. For delayed ossification of pelvic pubes, the litter incidence varied from 0% to 44.2% and the fetal incidence from 0% to 14.8%. The low values for incidence observed in the control group of the study with dimethenamid-P are relatively rare.

A comparison of incidence of the skeletal variations with racemic-dimethenamid and with dimethenamid-P, with ranges for historical controls are given in Table 23.

The litter-based incidence of incompletely ossified sternebrae observed with dimethenamid-P at the highest dose was 12%, which is less than the incidence of 18.2% in the control group (observed in the experiment with racemic dimethenamid) and the corresponding fetal incidence (1.9%) with dimethenamid-P at the highest dose is also less than the incidence of 3.4% observed

Table 23. Incidence of delayed ossifications (%) observed in studies of prenatal toxicity with racemic dimethenamid and dimethenamid-P

Observation	Dose (mg/kg bw)								Historical control range
	Dimethenamid-P				Racemic-dimethenamid				
	0	25	150	300	0	50	215	425	
Sternebrae incompletely ossified									
Litter	0	0	0	12.0	18.2	8.3	8.7	13.6	0–44.4
Fetus	0	0	0	1.9	3.4	2.2	1.1	1.8	0–7.6
Pelvic pubes incompletely ossified									
Litter	4.3	0	25.0	24.0	9.1	4.2	0	0	0–44.2
Fetus	1.0	0	3.6	5.8	1.1	0.5	0	0	0–14.8

From York et al. (1999)

in the control group in the experiment with racemic dimethenamid. In addition, both litter and fetal incidences are well within the range for historical controls. For the pelvic pubes, the incidence in the experiment with racemic dimethenamid is inversely related to treatment. For dimethenamid-P, the incidence of incompletely ossified pelvic pubes at the intermediate and highest doses is well within the range for historical controls.

Delayed ossifications are considered to be developmental delays often seen in smaller fetuses and not caused by any direct toxic effect of the chemical. Although average fetal body weights were only slightly decreased with dimethenamid or with dimethenamid-P, there is a good correlation between reduced body weight and skeletal variations in the pups affected in the studystudy with dimethenamid-P. Fetuses 2095-5 and 2095-3 at the highest dose had incomplete ossification of the pubis, ischium and second sternal centrum and, therefore, appear in all three variation categories. Their fetal body weights were 2.64 and 2.61 g, respectively, which were well below the mean for this group of 3.25 g. Fetuses 2076-17, 2081-16 and 2083-1, 2095-1 and 2095-3, which were also at 300 mg/kg bw per day, had incomplete ossification of the pubis and fetal body weights of 2.99, 3.01, 1.34, 2.73 and 2.77 g, respectively, again well below the average for this dose. At 150 mg/kg bw, fetus 2054-15 had incomplete ossification of the pubis and ischium and a fetal body weight of 2.97 g compared with 3.29 g as an average weight for this group. Fetuses 2057-5, 2058-11 and 2071-14, all with incomplete ossification of the pubis, had fetal body weights of 2.81, 2.47 and 2.97 g, respectively, once again below the group average of 3.29 g. Overall, it can be seen that the incomplete ossifications observed are closely related to reduced fetal weight and most probably are not independent toxicological results. Delayed ossification is considered to be a reversible delay in studies of developmental toxicity when associated with reduced fetal weights, and generally resolves with continued growth (York et al., 1999).

Rabbits

In a study of developmental toxicity, groups of 20 artificially inseminated rabbits were given racemic dimethenamid (SAN 582 H; batch No. 8605; purity, 92%) at a dose of 0, 37.5, 75 or 150 mg/kg bw per day by gavage in 0.5% aqueous carboxymethylcellulose, after first adhering the test substance to HiSil 233 as the carrier, on day 6 of gestation to 18 after insemination. The control group of animals received vehicle alone. A standard dose volume of 10 ml/kg bw was used. The doses were selected on the basis of a preliminary study in which dimethenamid was administered at doses of 0, 37.5, 75, 150, 300 or 425 mg/kg bw per day on days 6 to 18 to groups of four rabbits presumed to be pregnant. Deaths occurred at 300 and 425 mg/kg bw per day and both body-weight gain and food consumption were reduced at 150 mg/kg bw per day. This preliminary study was followed by another experiment in which a high dose of 250 mg/kg bw per day was used, which resulted in 13 deaths and seven abortions and so was rendered invalid.

On day 29 of gestation, the females were killed and assessed by gross pathology. The number of corpora lutea was determined, and the number and distribution of implantation sites were classified. The uteri were examined for live fetuses and intra-uterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and approximately one half were stained for skeletal examination, while the others were examined for soft tissue alterations.

Analytical verification of stability and homogeneity of racemic dimethenamid in 0.5% carboxymethylcellulose were determined before the start of the study. Verification of test concentrations was performed three times during the current study. Analytical determinations demonstrated that concentrations of racemic dimethenamid were acceptably close to target level.

No deaths occurred during the study. At 150 mg/kg bw per day, two rabbits aborted late in the study (days 26 and 26, i.e. well after dosing had ceased), but no abortion or premature delivery occurred at any other dose. Because of the high maternal mortality and incidence of abortion observed at 250 mg/kg bw per day in an earlier study, it was reasonable to assume that these abortions also were dose-related. Clinical signs considered unambiguously related to treatment were localized alopecia at 150 mg/kg bw per day. Dry faeces frequently occurred at all doses, but the incidences were not significantly increased at any dose.

Food consumption was significantly reduced in rabbits at the highest dose. During the treatment period, reduced body-weight gain occurred at 150 and 75 mg/kg bw per day and actually became a body-weight loss at 150 mg/kg bw per day during days 12–15 of gestation.

There were no gross pathological findings that were related to treatment.

There were no treatment-related effects on implantation, live litter size, fetal sex ratio or fetal body weight. Also, there were no effects on external, soft tissue or skeletal variations or malformations that were related to treatment. The Meeting concluded that racemic dimethenamid is not teratogenic.

The NOAEL for maternal toxicity for racemic dimethenamid was 37.5 mg/kg bw per day on the basis of reductions in body-weight gain at 150 mg/kg bw and reduced food consumption at 75 mg/kg bw per day. The NOAEL for developmental toxicity was 75 mg/kg bw per day on the basis of abortion or premature delivery at 150 mg/kg bw per day, which is higher than the dose at which maternal toxicity was first observed (Hoberman, 1988).

2.6 Special studies

(a) *Neurotoxic potential*

Studies of acute toxicity with racemic dimethenamid and dimethenamid-P gave no evidence of a neurotoxic effect.

Racemic dimethenamid and dimethenamid-P were investigated in several short- and long-term studies in three species. The parameters investigated included daily observations of the animals for behavioural effects and a complete histopathological investigation of the nervous system. Since there was no evidence of an effect on the nervous system in any of these studies and the substance is not structurally related to a neurotoxin, it was considered unnecessary to conduct special studies, such as studies of delayed neurotoxicity in hens.

(b) Induction of liver enzymes

Groups of six male Sprague-Dawley rats were given racemic dimethenamid (batch No. 9024; purity, 97.06%) at a dose of 0, 25, 100, 200 or 400 mg/kg bw per day by gavage in corn oil for 4 consecutive days. Another two groups of six male rats were treated with racemic dimethenamid at a dose of 0 or 400 mg/kg bw per day for 4 days and then allowed a 4-day

recovery period. The rats were examined daily for clinical signs and mortality. Body weights were recorded daily and food consumption was determined for the total treatment period and recovery period. At the end of the treatment period, urine was collected for standard analyses and the rats were then killed, blood samples drawn and plasma separated. All rats were subjected to a gross pathological examination and the liver, brain and kidneys were weighed. Samples of the liver were frozen for enzyme analysis.

Plasma concentrations of the following components were determined: total protein, bilirubin, total cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyltransferase, lactate dehydrogenase (LDH), fasting glucose, urea, creatinine, sodium, potassium, calcium, chloride and phosphate.

The following measurements were made on the liver samples: total cytochrome P450 and total glutathione content, and activities of ethoxyresorufin-*O*-de-ethylase (EROD), pentoxyresorufin-*O*-dependentylase (PROD), NADPH-cytochrome P450 reductase (NCPR), glutathione-*S*-transferase and UDP-glucuronyltransferase.

One rat died due to a gavage error and was replaced. No other mortality and no clinical signs were observed. Food consumption was higher for all treatment groups compared with the controls by 10–18%. No significant differences in absolute body weights were observed and body-weight gains were similar day 3 of the 4-day dosing period, when there was a 10% reduction at 400 mg/kg bw per day. The day 4 values were strongly affected by the enforced fasting, the control group losing a mean of 23.6 g and the group at 400 mg/kg bw per day losing 23.0 g during the 1-day period. In the recovery groups, body-weight gains were similar throughout at 0 and 400 mg/kg bw per day.

Absolute and relative (to both body and brain weights) liver weights were increased at doses of 100 to 400 mg/kg bw. At the end of the 4-day dosing period, the liver/body weight ratio was 51% higher at 400 mg/kg bw per day than in the control group, but this difference had fallen to 12% by the end of the recovery period. No dose-dependent changes in kidney and brain weights were observed, although the kidney:body weight ratios were statistically significantly increased at 400 mg/kg bw per day group by 10%. The only statistically significant liver-related plasma enzyme change was a 42% increase in alanine aminotransferase activity at 400 mg/kg bw per day. In the recovery group, there were a number of parameters that were statistically significant from the control at the end of the recovery period, but since these parameters were not affected at the end of the treatment period, they were considered not to be of toxicological relevance. At the highest dose, urine volume was increased, but without any change in specific gravity. Significant decreases were observed in urinary protein (36%) and creatinine (43%), while urinary urea was increased (60%).

The liver enzyme analysis demonstrated significant changes after treatment with racemic dimethenamid. Dose-dependent increases were found in total cytochrome P450 and especially in the cytochrome enzymes PROD (mainly CYP2B) and EROD (mainly CYP2A) as well as in glutathione-*S*-transferase and NADPH reductase activities, although the increases in these last two enzymes were slight at 25 mg/kg bw per day. Increases at 400 and 200 mg/kg bw per day were also observed in UDP-glucuronyl transferase activity, but the increase was no longer significant after the recovery period. Induction of these enzymes represents a physiological adaptation in the liver for the removal of the administered compound and is not considered to be an adverse effect.

Glutathione in hepatocellular cytosol was decreased to 67% of the control value at 400 mg/kg bw per day. This depletion is a likely result of conjugation with xenobiotics, but it is well above the value of 20–30% that is predicted to impair cellular defences against toxic compounds and may lead to cell injury and death (Reed, 1990).

All parameters investigated returned to control or near control levels after the four-day recovery period. In conclusion, oral administration of racemic dimethenamid to rats for 4 days induced several liver enzyme systems. It was demonstrated that the metabolism of racemic dimethenamid involves conjugation with glutathione and glucuronide, and oxidation steps mainly

by cytochrome P450-dependent enzymes. Upon removal from treatment, there is a recovery from the liver changes (Dorobek & Müller, 1994).

(c) Haemoglobin binding potential

The kinetic studies suggested that racemic dimethenamid might bind to blood components in rats. This conclusion was based on the finding that 3% of the radiolabelled material administered remained in the blood fraction. The following study was conducted to investigate the nature of the interaction between racemic dimethenamid and rat blood and to elucidate its relevance for humans.

Male Sprague-Dawley rats were given unlabelled racemic dimethenamid at a dose of 0, 25, 100, 200 or 400 mg/kg bw by gavage in corn oil for 4 days. At the end of the treatment period, blood samples were collected. Methaemoglobin concentrations were determined and reported as part of this study.

Blood samples were collected from human volunteers and Wistar rats. The packed erythrocyte component was obtained and haemolysed chemically. The haemolysed blood components were incubated with 1 µl (0.2 µCi, or 7.4 kBq) racemic [3-thienyl-^{14}C] dimethenamid (batch No. RA 683-2, radiochemical purity, 98.4%) for 15 min at 37 ^{o}C in a shaking water bath. Processing allowed separation of the haemoglobin portion and liquid scintillation counting was used to determine the amount of binding to the haemoglobin.

Analysis of blood samples from Sprague-Dawley rats treated for 4 consecutive days with various concentrations of racemic dimethenamid did not indicate an increase in methaemoglobin. Nevertheless, racemic dimethenamid does bind strongly to rat haemoglobin, while no binding of racemic dimethenamid to human haemoglobin was detected. Further investigation demonstrated that in rats the binding was almost exclusively to the globin moiety, very little radioactivity being associated with haem. The species difference in haemoglobin binding may be explicable in terms of differences in the three-dimensional structure of the molecule in the two species. Human haemoglobin contains two α-globin and two β-globin chains that contain either one cysteine (Cys α-104) or two cysteines (Cys β-93 and Cys β-112) (Dayhoff, 1972). None of these cysteine residues is exposed for chemical substitution. Rat haemoglobin also consists of α- and β-chains. The α-chain contains three cysteine residues, one each at positions 13, 104 and 111, while the β-chain contains two cysteine residues, one each at position 93 and 125 (Dayhoff, 1976). The cysteine residue β-125 is located on the surface of the haemoglobin molecule and is surrounded by hydrophobic residues, thus providing it with a relatively high chemical reactivity (Hughes et al., 1981), a property lacking in human haemoglobin. The strong binding of racemic dimethenamid to rat haemoglobin may be a consequence of reaction of its chemically activated chlorine atom with the sulfhydryl group of rat haemoglobin Cys β-125. If so (and the available evidence would suggest that it is), then the interaction of racemic dimethenamid with haemoglobin has no human consequence. Because dimethenamid-P forms 50% of racemic dimethenamid, the same conclusion can be applied to dimethenamid-P (Villafranca et al.,1992).

(d) Studies on metabolites

(i) Studies of toxicity with metabolites of racemic dimethenamid

Residues of racemic dimethenamid or its metabolites in grain have been shown to be below the currently available limits of quantification. Two metabolites of dimethenamid have been found at low levels in forage and they also occur as soil metabolites. These two metabolites are an oxalamide (M23) and the sulfonate (M27). Both metabolites were also identified in the study of metabolism in rats. These two metabolites were tested in several studies, including an oral LD_{50} test, a test for mutagenicity in bacteria and an assay for chromosomal/genomic aberration in mouse bone-marrow cells.

(ii) Acute toxicity of metabolites

The acute oral toxicity of racemic dimethenamid oxalamide (M23); batch No. RS-582OXA-080194, purity, 99.83%) was evaluated in groups of five male and five female fasted Sprague-Dawley rats given the substance at a dose of 2000 or 5000 mg/kg bw diluted in 0.5% w/v methylcellulose in water by gavage. The dosing volume was 20 ml/kg. The rats were observed for 14 days after dosing. Body weights were determined before dosing and on study days 1, 8 and 15. All rats received a gross autopsy. No deaths occurred at either dose. Clinical signs observed in rats treated at 5000 mg/kg bw were reduced activity, pallor, piloerection, salivation and hunched posture. These signs were seen immediately after exposure, but did not persist to day 2. The only clinical sign observed at 2000 mg/kg bw was piloerection, which did not persist to day 2. Overall, the rats gained body weight as expected for their age. There were no treatment-related effects on gross pathology. The acute oral LD_{50} for racemic dimethenamid oxalamide (M23) was > 5000 mg/kg bw in male and female Sprague-Dawley rats (Cummins, 1995).

The acute oral toxicity of racemic dimethenamid sulfonate (M27; batch No. 4997; purity, 99.45%) was evaluated in a single group of five male and five female fasted Sprague-Dawley rats given the substance at a dose of 5000 mg/kg bw diluted in water by gavage. The rats were observed for 14 days after dosing. Body weights were determined before dosing and on study days 7 and 14. All rats received a gross autopsy. No deaths occurred. Clinical signs observed were yellow anogenital staining and watery or unformed stool. These signs were not observed on day 4 or later. The rats gained body weight as expected for their age. There were no treatment-related effects on gross pathology. The acute LD_{50} for racemic dimethenamid sulfonate (M27) was > 5000 mg/kg bw in male and female Sprague-Dawley rats (Blasczak, 1992).

(iii) Genotoxicity of metabolites

Racemic dimethenamid oxalamide (M23; batch No. RS-582OXA-080194, purity, 99.83%) was tested for its mutagenic potential in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an exogenous metabolic activation system. The initial experiment was a standard plate test using doses ranging from 8 to 5000 µg/plate. Toxicity was observed at a dose of 5000 µg/plate in all strains except for TA100. A second experiment was a preincubation test using doses ranging from 250 to 4000 µg/plate for all strains except TA100, and from 312.5 to 5000 µg/plate for TA100. Toxicity was observed at a dose of 4000 µg/plate in all strains except for TA100. In this strain, toxicity was observed at 5000 µg/plate in the presence of metabolic activation from S9. There were no increases in the frequency of mutation associated with racemic dimethenamid oxalamide (Clare, 1995a).

In a test for micronucleus formation in mouse bone-marrow cells, groups of six male and six female ICR mice were given racemic dimethenamid oxalamide (M23; batch No. L 59-52; purity, 99.83%) as a single dose at 0, 75, 150 or 300 mg/kg bw by intraperitoneal injection , these doses being chosen on the basis of a preliminary study in which clinical signs of toxicity, but no mortalities, were observed at 400 mg/kg bw. A group of six males and six females was dosed orally with the positive control substance cyclophosphamide. These groups were killed at approximately 24 h after treatment. In addition groups of six males and six females were treated in the same way with racemic dimethenamid oxalamide at doses of 0 or 300 mg/kg bw and were killed approximately 48 h later. In the test for micronucleus formation, two male mice died after receiving racemic dimethenamid oxalamide at a dose of 300 mg/kg bw, one each in the groups with a preparation interval of 24 h or 48 h . No increases in micronucleated polychromatic erythrocytes were observed in either sex in any group dosed with racemic dimethenamid oxalamide. Large increases in the incidence of micronucleus formation were observed in the positive control group (Völkner, 1998a).

Racemic dimethenamid sulfonate (M27, batch No. RS-582SSS-071494, purity, 97.2%) was tested for its mutagenic potential in *S. typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an exogenous metabolic activation system. The initial experiment was a standard plate test using doses ranging from 8 to 5000 µg/plate. Toxicity was observed at a dose of 5000 µg/plate in all strains except for TA100. A second experiment was a preincubation test using doses ranging from 312.5 to 5000 µg/plate. No toxicity was observed at any dose and no increases in the frequency of mutation associated with dimethenamid sulfonate were observed (Clare, 1995b).

In a test for micronucleus formation in mouse bone-marrow cells, groups of six male and six female ICR mice were given racemic dimethenamid sulfonate (M27, batch no. RS-582SSS-071494, purity, 97.2%) as a single dose at 500, 1000 or 2000 mg/kg bw by intraperitoneal injection, these doses being chosen on the basis of a preliminary study in which clinical signs of toxicity, but no mortalities, were observed at 2000 mg/kg bw. A group of six males and six females was dosed orally with the positive control substance, cyclophosphamide. These groups were killed at approximately 24 h after treatment. In addition, groups of six males and six females per dose were treated in the same way with racemic dimethenamid sulfonate at a dose of 0 or 2000 mg/kg bw and were killed approximately 48 h later. No increases in micronucleated polychromatic erythrocytes were observed in either sex in any group dosed with racemic dimethenamid sulfonate. Large increases in the incidence of micronucleus formation were observed in the positive control group (Völkner, 1998b).

3. Observations in humans

In a survey in humans, three groups of individuals were assessed: those involved in manufacturing of the active ingredient, the research and development formulations and field personnel involved in product development.

The Sandoz Agro manufacturing facility at Beaumont, Texas, USA, produced three batches of Frontier herbicide in 1991 and one batch in 1992. Between five and ten people were involved in the process, which included charging raw materials, process sampling and manually filling containers with the final formulated material. In 1991, approximately 750 containers with a volume of 1 l litre and 475 containers with a volume of 0.5 l were filled, and in 1992 800 containers of 4 l in volume were filled. Employees wore protective gloves and clothing.

Approximately five to seven people in the Research and Development Formulations group handled racemic dimethenamid and its formulations between 1986 and 1992. Activities included material transfer, drying and packaging. Employees wore protective gloves and clothing and were often working under protective hoods.

During product development field trials, 20 people worked with racemic dimethenamid products between 1984 and 1991. Activities including mixing, loading and application of the formulation product for application and spraying of the product diluted in water. Most applications were made with a backpack hand sprayer and some applications were with a tractor-mounted sprayer. These activities were conducted 7–15 times per year. Personnel wore protective gloves and clothing during handling of the product.

In none of the three groups surveyed were there any cases of skin irritation, skin rash or other signs of allergic response. In addition, no general signs of adverse health effects were reported (Rataj, 1992).

In addition, the sponsor states that: (1) no poisoning incidents are known; (2) no observations regarding health effects after exposure of the general public are known; (3) methods for determination of active substance or metabolites in biological fluids are not established; (4) specific signs of poisoning or clinical tests are not known; (5) no specific antidote is known and

(6) expected effects of poisoning (irritation of exposed eyes and skin, dermatitis and eczema) were derived from studies in animals.

Comments

Biochemical aspects

Racemic dimethenamid was slowly but well absorbed after oral administration and was extensively metabolized by rats. In rats given racemic dimethenamid by gavage, there was no significant difference in the degree of absorption (> 90%) at a low dose of 10 mg/kg bw and a high dose of 1000 mg/kg bw, or between single and multiple doses at 10 mg/kg bw per day. Maximum concentrations in blood were not achieved until about 72 h. Excretion was rapid and primarily via bile, between 45% and 64% of the oral dose being excreted within 7 h by this route; however, biliary elimination appeared to be saturated at 1000 mg/kg bw, because elimination in the urine was increased at the higher dose. By 168 h after treatment, an average of 90% of the administered dose was eliminated. In rats, the concentration of radioactivity in blood decreased more slowly than in tissues and was associated with specific binding to globin; however, similar specific binding to blood components did not occur in human blood. Concentrations in other tissues after 168 h were low regardless of the dose or frequency of dosing. Consequently, there was no evidence of bioaccumulation. There was no significant difference in absorption, distribution and elimination between sexes.

Studies of dermal penetration in vivo in rats demonstrated that dermal penetration of racemic dimethenamid and dimethenamid-P at 24 h was approximately 26%. Based on the results of comparisons of penetration in human and rat skin in vitro, it was concluded that the rate of dermal penetration was lower in humans than in rats.

Metabolism was primarily via the glutathione conjugation pathway, but racemic dimethenamid was also metabolized by cytochrome P450 enzymes via reductive dechlorination, oxidation, hydroxylation, *O*-demethylation, and cyclization pathways, as well as conjugation with glucuronic acid. Unchanged dimethenamid in excreta accounted for only 1–2% of the administered dose, more than 40 metabolites having been detected. At least 20 of these metabolites were structurally identified by mass spectrometry and nuclear magnetic resonance, and confirmed by reference to synthesized standards. There was no significant difference in metabolism between the sexes.

Toxicological data

Although many of the critical studies of toxicity were conducted only with the racemic mixture, some studies were performed with both dimethenamid-P and racemic dimethenamid. These include studies of acute oral toxicity (LD_{50}) in rats, dermal toxicity (LD_{50}) in rats, acute toxicity after inhalation (LC_{50}) in rats, dermal irritation in rabbits, eye irritation in rabbits, dermal sensitization in guinea-pigs, 90-day studies of oral toxicity in rats, prenatal developmental toxicity and teratogenicity in rats, mutagenicity in bacteria and Chinese hamster ovary cells in vitro, chromosome aberrations in Chinese hamster ovary cells in vitro, assays for unscheduled DNA synthesis in rat hepatocytes in vitro, and assays for micronucleus induction in bone-marrow cells in mice in vivo.

The acute toxicities of dimethenamid-P and the racemic mixture are characterized as moderate after oral administration and low after dermal or inhalation administration. The oral LD_{50} values in rats were: dimethenamid-P, 429 mg/kg bw (males) and 531 mg/kg bw (females); racemic dimethenamid, 371 mg/kg bw (males) and 427 mg/kg bw (females). Both substances produced only mild reversible skin and eye irritation. Skin sensitization was produced by dimethenamid-P in guinea-pigs in the Buehler test and by racemic dimethenamid in the Magnusson & Kligman test.

Overall, in short-term studies with racemic dimethenamid, the signs of toxicity observed in mice, rats and dogs were similar, with reduced body-weight gain and liver enlargement being

common features. Dimethenamid-P and racemic dimethenamid produced very similar effects in the liver of rats. The Meeting concluded that increased liver weights were indicative of an adaptive response to exposure. Histopathology confirmed the liver as a target organ with observation of hypertrophy of hepatocytes, although this too is indicative of an adaptive response and was accompanied by the induction of several hepatic microsomal enzymes. These hepatic enzyme changes were resolved upon removal from treatment. In addition, however, vacuolization of hepatocytes and dilatation of liver sinusoids occurred in dogs.

The NOAELs for the short-term dietary studies were for dimethenamid-P and racemic dimethenamid, respectively: 90-day study in rats, 500 ppm (equal to 39 mg/kg bw per day) and 500 ppm (equal to 34 mg/kg bw per day); and, for racemic dimethenamid alone: 90-day dietary study in mice, 2000 ppm (equal to 301 mg/kg bw per day); 90-day study in dogs, 92 ppm (equal to 4.6 mg/kg bw per day); 12-month study in dogs, 250 ppm (equal to 10 mg/kg bw per day). In a 3-week study of dermal toxicity with racemic dimethenamid in rabbits, no substance-related systemic findings were detected at 1000 mg/kg bw per day, the highest dose tested.

Long-term feeding studies with racemic dimethenamid in rats and mice demonstrated that the primary target organ was the liver. There was no evidence for a carcinogenic potential in these studies. The NOAELs obtained in long-term studies were: rats, 100 ppm (equal to 7 mg/kg bw per day, on the basis of bile-duct hyperplasia and reduced body-weight gain in females); and mice, 300 ppm (equal to 40 mg/kg bw per day, on the basis of decreased body-weight gain and hepatocellular hypertrophy).

Dimethenamid-P and racemic dimethenamid were tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test with dimethenamid-P. Apart from an equivocal result in one of three assays for unscheduled DNA synthesis in vitro with racemic dimethenamid, none of the assays gave any indication that racemic dimethenamid might be genotoxic. The Meeting concluded that both dimethenamid-P and racemic dimethenamid are unlikely to be genotoxic.

In the absence of genotoxicity and any evidence of carcinogenicity in rodents, the Meeting concluded that dimethenamid-P and racemic dimethenamid are unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of racemic dimethenamid was investigated in a two-generation study of reproduction in rats and in a study of developmental toxicity in rabbits. The developmental toxicity of both dimethenamid-P and racemic dimethenamid was studied in rats.

Reproductive function was not affected in rats in the two-generation study of racemic dimethenamid and the NOAEL for reproductive function was 2000 ppm (equal to 175 mg/kg bw per day), the highest dose tested. The NOAEL for systemic toxicity in the parental animals in the two-generation study was 500 ppm (equal to 45 mg/kg bw per day). The only effect on pups noted was a decreased body-weight gain during lactation at the highest dose. The NOAEL for developmental toxicity in the F_1 and F_2 litters was 500 ppm (equal to 45 mg/kg bw per day).

In a study of developmental toxicity, rats were given dimethenamid-P at doses of up to 300 mg/kg bw per day. Both maternal and developmental toxicity were observed. There was an increased incidence of clinical signs of toxicity in the group receiving the highest dose. The effects on development included increases in delayed ossifications, but further evaluation demonstrated that these were attributable to unusually low control values and were not related to treatment. The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreased body-weight increment, and the NOAEL for developmental toxicity was 300 mg/kg bw, the highest dose tested.

In a study of developmental toxicity, rats were given racemic dimethenamid at doses of up to 425 mg/kg bw per day. Signs of maternal toxicity that were recorded included excess salivation at 215 mg/kg bw per day and 425 mg/kg bw per day, and urine-stained abdominal fur at 425 mg/kg bw per day. Fetal body weights were reduced and the frequency of early deaths was

increased at doses of 215 mg/kg bw per day and 425 mg/kg bw. The NOAELs for both maternal toxicity and developmental toxicity were 50 mg/kg bw per day.

In a study of developmental toxicity in rabbits given racemic dimethenamid at doses of up to 150 mg/kg bw per day, significant maternal toxicity (body-weight loss preceded by reduced food consumption and associated with dry faeces) was observed at the highest dose and less severe effects were noted at 75 mg/kg bw per day. Abortions in two rabbits at 150 mg/kg bw per day were considered to be treatment-related, but secondary to the clear maternal toxicity. The NOAEL for maternal toxicity was 37.5 mg/kg bw per day and the NOAEL for developmental toxicity was 75 mg/kg bw per day.

No evidence of neurotoxicity was noted in any studies.

The plant and soil oxalamide (M23) and sulfonate (M27) metabolites of racemic dimethenamid, which also occur as products of metabolism in rats, were tested in studies of acute oral toxicity, assays for mutagenicity in bacteria and for micronucleus formation in bone-marrow cells of mice. Both compounds had low acute oral toxicity with LD_{50} values of > 5000 mg/kg bw. Neither compound was mutagenic in bacteria or induced micronucleus formation in bone-marrow cells of mice.

Interviews with and written surveys of 50 people handling racemic dimethenamid and its formulated products over 7 years have been conducted. There were no reported cases of skin irritation or other adverse health effects.

Comparison of racemic dimethenamid with dimethenamid-P has been possible for a number of types of study. These have shown that there is little difference in the toxicological profile or, where appropriate, the NOAELs for these materials. Consequently, the Meeting concluded that data derived from assays with the racemic mixture could be used to supplement data from assays with dimethenamid-P. In the following tables, the actual material tested was identified.

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

Toxicological evaluation

The Meeting concluded that the toxicology of the *S* enantiomer (dimethenamid-P) is not significantly different from that of the racemic mixture. For the purpose of dietary risk assessment, the residues of concern were defined as parent dimethenamid (*R* and *S* enantiomers); therefore the derivation of a separate ADI or ARfD for dimethenamid-P is not necessary.

An ADI of 0–0.07 mg/kg bw was established for dimethenamid-P and racemic dimethenamid based on the NOAEL of 7 mg/kg bw per day for bile-duct hyperplasia and reduced body-weight gain observed only in female rats in a 24-month study in rats given diets containing racemic dimethenamid, and a safety factor of 100.

The Meeting established an ARfD of 0.5 mg/kg bw for dimethenamid-P and racemic dimethenamid based on an overall NOAEL of 50 mg/kg bw for maternal clinical signs of toxicity and developmental toxicity (fetal body-weight deficits and increases in early deaths) in studies in rats, and a safety factor of 100.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	94-week study of toxicity and carcinogenicity with the racemic mixture	Toxicity	300 ppm, equal to 40 mg/kg bw per day	1500 ppm, equal to 200 mg/kg bw per day
		Carcinogenicity	300 ppm[a], equal to 411 mg/kg bw per day	—

Rat	104-week study of toxicity and carcinogenicity with the racemic mixture	Toxicity	100 ppm, equal to 7 mg/kg bw per day	700 ppm, equal to 49 mg/kg bw per day
		Carcinogenicity	1500 ppm[a], equal to 80 mg/kg bw per day	—
	Two-generation study of reproductive toxicity with the racemic mixture[b]	Reproductive toxicity	2000 ppm[a] equal to 175 mg/kg bw per day	—
		Parental toxicity	500 ppm, equal to 45 mg/kg bw per day	2000 ppm[a], equal to 175 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 45 mg/kg bw per day	2000 ppm[a], equivalent to 175 mg/kg bw per day
	Developmental toxicity with dimethenamid-P[c]	Maternal toxicity	25 mg/kg bw per day	150 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw[a] per day	—
	Developmental toxicity with the racemic mixture[c]	Maternal toxicity	50 mg/kg bw per day	215 mg/kg bw per day
		Embryo- and fetotoxicity	50 mg/kg bw per day	215 mg/kg bw per day
Rabbit	Developmental toxicity with the racemic mixture[c]	Maternal toxicity	37.5 mg/kg bw per day	75 mg/kg bw per day
		Embryo- and fetotoxicity	75 mg/kg bw per day	150 mg/kg bw per day
Dog	1-year study of toxicity with the racemic mixture	Toxicity	250 ppm, equal to 10 mg/kg bw per day	1500 ppm[a], equal to 49 mg/kg bw per day

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the pre-mating phases for F_0 and F_1 females

[c] Gavage administration

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate of acute reference dose

0.5 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to dimethenamid-P and racemic dimethenamid

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Low, plasma T_{max} 7 h; high, > 90% absorbed in rats
Dermal absorption	> 20% (dimethenamid-P and racemic dimethenamid) in rats
Distribution	Distributed throughout the body; higher concentrations in adrenals, pancreas, kidney, liver, spleen and blood
Potential for accumulation	Very low
Rate and extent of excretion	High (determined by the slow absorption); essentially 100% excretion within 168 h

Metabolism in animals	Extensive, about 40 metabolites, little parent compound remaining
Toxicologically significant compounds (animals, plants and environment)	Parent
Acute toxicity	
Rat LD_{50} oral	429 mg/kg bw (dimethenamid-P); 371 mg/kg bw (racemic mixture)
Rat LC_{50} inhalation	> 2.2 mg/l (4 h) (dimethenamid-P and racemic mixture)
Rabbit LD_{50} dermal	> 2000 mg/kg bw (dimethenamid-P and racemic mixture)
Rabbit, skin irritation	Slightly irritating (dimethenamid-P and racemic mixture)
Rabbit, eye irritation	Not irritating (dimethenamid-P and racemic mixture)
Skin sensitization (test method used)	Sensitizing (Buehler test) (dimethenamid-P) and Magnusson & Kligman (racemic mixture)
Short-term studies of toxicity	
Target/critical effect	Body-weight gain decrement, increased absolute and relative liver weight (dimethenamid-P and racemic mixture)
Lowest relevant oral NOAEL	10 mg/kg bw per day: (12-month study in dogs) (racemic mixture)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day (21-day study in rabbits) (racemic mixture)
Lowest relevant inhalation NOAEC	No data available and not required
Genotoxicity	
	Not genotoxic in vivo or in vitro (dimethenamid-P and racemic mixture)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver, bile-duct hyperplasia (racemic mixture); body weight
Lowest relevant NOAEL	7 mg/kg bw per day (24-month study in rats) (racemic mixture)
Carcinogenicity	Dimethenamid-P and racemic dimethenamid are unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproductive target/critical effect	None
Lowest relevant reproductive NOAEL	175 mg/kg bw[a, b] per day (racemic mixture)
Developmental target/critical effect	Not teratogenic; reduced fetal body weight (dimethenamid-P); not teratogenic; reduced fetal body weight and increased early deaths (racemic dimethenamid)
Lowest relevant developmental NOAEL	300 mg/kg bw[a] per day (rat) (dimethenamid-P) and 50 mg/kg bw per day (rat) (racemic mixture)
Neurotoxicity/delayed neurotoxicity	
	No signs of neurotoxicity
Other toxicological studies	
	Liver xenobiotic metabolizing enzyme induction. Strong binding to haemoglobin in rats, but this has no relevance to humans
Medical data	
	There have been no reports of toxicity in workers exposed during manufacture or use

Summary

	Value	Study	Safety factor
ADI	0–0.07 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity (racemic mixture)	100
ARfD	0.5 mg/kg bw	Rat, study of developmental toxicity (racemic mixture)	100

[a] Highest dose tested

[b] Measurements of intake of the compound are the mean of the pre-mating phases for P and F_1 females

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* Study complies with GLP

† Study does not comply with GLP