ZOXAMIDE

First draft prepared by
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Explanation

Zoxamide is the International Organization for Standards (ISO) approved name for (*RS*)-3,5-dichloro-*N*-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide (Chemical Abstracts Service; CAS No. 156052-68-5). Zoxamide is a chlorinated benzamide fungicide that acts against late blight (*Phytophthera infestans)* and powdery mildew (*Plasmopara viticola)*. The mechanism of fungicidal action involves disruption of microtubule formation by binding to β-tubulin.

Zoxamide has not been evaluated previously by the JMPR and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR).

All the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) or national test guideline and contained certificates of compliance with good laboratory practice (GLP).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

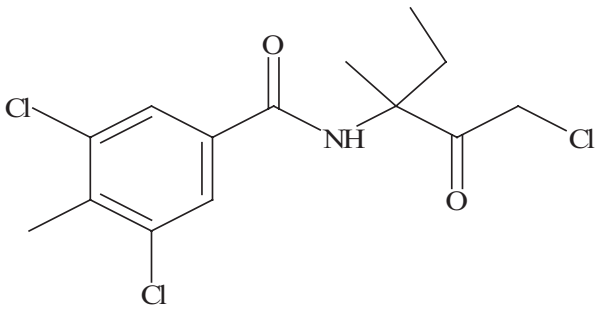
In a GLP-compliant study, the balance and excretion patterns of ^{14}C- and ^{13}C-phenyl ring-labelled zoxamide (Figure 1), and the amount of residual radioactivity in blood, organs and tissues were determined in male and female Sprague-Dawley Crl:CDBR rats (body weight, 220–426 g) treated orally.

Groups of three to six males and three to six females were given [^{14}C]zoxamide (radiochemical purity, 97.6–99.5%; 45.8–90.2 mCi/g or 1.69–3.34 GBq/g) at nominal dose levels of 10 (lower dose) or 1000 (higher dose) mg/kg bw by gavage—details of the main groups are outlined in Table 1. The test materials were prepared as suspensions in corn oil and administered in a volume of 5 ml/kg bw. For all groups, [^{14}C]zoxamide was combined with appropriate amounts of non-radiolabelled zoxamide (purity, 92.9–94.2%). In addition, ^{13}C-labelled zoxamide (purity, 96.8%) was added to the dosing solution for groups A, B, Q and R to assist in metabolite determinations.

The following experiments were conducted:

- Determination of the excretion, distribution and mass balance of radioactivity (up to 120 h after dosing) in groups A and B (lower dose) and C and D (higher dose);
- Evaluation of the pharmacokinetics of radioactivity in blood (to determine the time to C_{max} and ½ C_{max}) (groups 1, 2 (also investigated for expired air) (higher dose) and 3 (lower dose);
- Determination of the tissue distribution of radioactivity at C_{max} and ½ C_{max} (groups I to P; both dose levels),
- Investigation of effects after repeated doses (groups E, F, G and H). Rats in groups E and F received diets containing non-radiolabelled zoxamide technical at a concentration of 200 ppm for 2 weeks before receiving [^{14}C]zoxamide as a single oral dose at 10 mg/kg bw. Rats in groups G and H received [^{14}C]zoxamide as five consecutive daily doses at 10 mg/kg bw/day by gavage.

Figure 1. Structure of zoxamide



The excretion of radioactivity in rats given a single oral dose of [^{14}C]zoxamide followed similar patterns, with a higher (approximately threefold) proportion in the urine at the lower dose (Table 1). More than 85% of the administered radioactivity was excreted during the first 24–48 h after dosing. The major route for the elimination of radiolabel was in the faeces, which contained 74–92% of the administered dose. The remaining radioactivity (4–27%) was excreted in the urine, with females tending to have a higher level of urinary excretion than males. A significant proportion of the absorbed dose (approximately 45%) was excreted in the bile. Biliary excretion of radioactivity in rats given [^{14}C]zoxamide as a single oral dose at 10 mg/kg bw was rapid. Most of the dose was recovered within 12 h after dosing. Based on the recovery of radioactivity from the bile, blood, urine, tissues and carcasses, 59–63% of the administered oral dose of [^{14}C]zoxamide was considered to be systemically absorbed. Very little radioactivity remained in tissues (0.04–0.17% of the administered dose) or carcass (0.34–1.9% of the administered dose) at 5 days after dosing, indicating that zoxamide has a low potential for accumulation. No radioactivity was recovered as either $^{14}CO_2$ or volatile organic compounds. Pre-treatment of animals with diets containing non-radiolabelled zoxamide for 2 weeks or with five daily doses of radiolabelled zoxamide did not significantly alter the absorption

Table 1. Recovery of radiolabel in excreta, blood, tissues and residual carcass of rats treated with radiolabelled zoxamide

Group	Dose (mg/kg bw)	Route[a]	Sex	Time of sacrifice	Recovery (% of administered dose)						
					Urine[d]	Bile	Faeces	Blood	Tissues	Carcass	Total[e]
2	1000	Oral	Male	7 days	6.4	—	94.1	0.01	0.02	0.30	100.9[f]
			Female	7 days	11.2	—	84.4	0.01	0.02	0.33	96.0[f]
C	1000	Oral	Male	5 days	3.5	—	92.4	0.00	0.04	0.34	96.2
D	1000	Oral	Female	5 days	8.1	—	88.8	0.01	0.05	0.55	97.5
A	10	Oral	Male	5 days	10.3	—	87.8	0.01	0.16	1.86	100
B	10	Oral	Female	5 days	26.8	—	73.5	0.02	0.17	1.87	102.4
Q	10	Oral (bile); bile-duct cannulated	Male	72 h	9.5	45.8	32.2	0.01	0.18	2.98	90.8[g]
R	10	Oral (bile) bile-duct cannulated	Female	72 h	12.0	47.8	33.9	0.01	0.14	2.79	96.7[g]
E	10	Oral, pulse[b]	Male	5 days	16.3	—	78.6	0.02	0.58	3.41	98.9
F	10	Oral, pulse[b]	Female	5 days	28.7	—	71.0	0.02	0.19	1.55	101.5
G	10	Oral, repeat[c]	Male	C_{max} after fifth dose	7.9	—	60.3	0.03	2.69	17.77	96.3[g]
H	10	Oral, repeat[c]	Female	C_{max} after fifth dose	19.9	—	51.6	0.04	3.27	12.65	93.0[g]

From Swenson et al. (1998)

[a] All rats except those in groups G and H were administered a single dose by gavage in a constant volume of 5 ml/kg bw.

[b] These rats received diets containing non-radiolabelled zoxamide at a concentration of 200 ppm for 2 weeks before receiving radiolabelled zoxamide as a single oral dose at 10 mg/kg bw.

[c] These animals received five consecutive daily oral doses by gavage and were sacrificed at the C_{max} time-point (8 h after dosing) on day 5 of dosing.

[d] Includes urine, urine funnel wash and urine cage wash.

[e] Mean total percentage of administered dose, reflects the mean and standard deviation for the individual animals.

[f] No radioactivity was recovered as either $^{14}CO_2$ or volatile organic compounds.

[g] Stomach contents, stomach wash, and intestinal tract contents and wash accounted for 7.55% and 5.42% of the administered dose for males and females, respectively.

or distribution of [^{14}C]zoxamide when compared with rats that were not pre-treated and that received a single dose of [^{14}C]zoxamide (Table 1).

In pharmacokinetic studies, [^{14}C]zoxamide was observed to be rapidly absorbed by rats. The maximum concentrations of radioactivity in plasma were observed at 8 h after dosing (C_{max} in plasma, 8 h; ½ C_{max}, 22 h). Peak blood and tissue concentrations were noted to be low. Elimination of radiolabel from plasma followed a biphasic pattern. The overall elimination half-life of the ^{14}C radiolabel in plasma was essentially similar (12–14 h) in male and female rats at the lower and at the higher dose (Table 2).

The concentrations of radioactivity in the tissues were highest in the organs associated with oral absorption—liver, stomach, intestines, and carcass (which included the caecum). The results for tissue distribution were consistent with the pharmacokinetic data and indicated that radioactivity was rapidly cleared from the tissues (Table 3). A comparison of repeated-doses (five) and single-dose C_{max} values indicated slightly higher (approximately twofold) values after repeated administration; these results were within typical variability for a study of this type and were not seen as a clear indication of bioaccumulation (Swenson et al., 1998).

In an investigation of the distribution of zoxamide in bone marrow, [^{14}C]zoxamide as a single dose at 2000 mg/kg bw was administered orally by gavage in corn oil to groups of four male and four female CD-1 mice. Mice were killed at 4, 8, 24 and 48 h after administration of the test material, bone marrow tissue samples were collected and analysed for radiolabel. The study was certified to be compliant with GLP and was conducted to support a study of micronucleus formation in mice that was conducted in accordance with OECD 474 guidelines. At all time-points (i.e., 4, 8, 24 and 48 h), radiolabel derived from [^{14}C]zoxamide was present in the bone marrow of male and female mice. Peak concentrations of ^{14}C, 55 and 39 µg equivalents/g in males and females respectively were seen at 4 h; declining to approximately 9 µg equivalents/g by 24 h (Swenson & Frederick, 1998).

(b) Dermal route

Rats

In a study of dermal absorption, male rats received single dermal applications of two formulations of [^{14}C]zoxamide (each of two different batches):

- [^{14}C]zoxamide 80WP (lot No. TEM-2627, 80% active ingredient (a.i.); radiochemical purity, 96.3%; specific activity, 0.67 mCi/g (24.79 MBq/g); or lot No. TEM-2629; 0.15% a.i., radiochemical purity, 96.3%; specific activity, 0.05 mCi/ml (1.85 MBq/ml);

Table 2 Pharmacokinetic half-lives ($t_{1/2}$) of ^{14}C-radiolabel and elimination and peak plasma concentration for rats given oral doses of radiolabelled zzoxamide[a]

Group	Dose (mg/kg bw)	Sex	Elimination half-life[b] (h)	Alpha-Phase half-life[c] (h)	Beta-Phase half-life[d] (h)	Peak concentration[e] (ppm)	AUC[f] (ppm.h)
1	1000	Male	11.7	5.5	100	32	1360
1	1000	Female	13.8	6.3	107	43	1882
3	10	Male	14.0	5.6	70	0.62	26
3	10	Female	13.1	6.6	164	0.98	45

From Swenson et al. (1998)

AUC, area under the curve of concentration–time.

[a] Elimination rates calculated for a two-compartment pharmacokinetic model (PK Analyst® Model 13) with first-order input and first-order output (elimination).

Table 3. Mean concentration of radiolabel in blood, carcass, plasma and tissues of rats at 8 h or 22 h after oral doses of radiolabelled zzoxamide

Tissue	Dose (mg/kg bw)									
	1000 mg/kg		1000 mg/kg		10 mg/kg		10 mg/kg		5 × 10 mg/kg	
	C_{max}		1/2 C_{max}		C_{max}		1/2 C_{max}		C_{max} at 8 h	
	8 h		22 h		8 h		22 h		Fifth dose[b]	
	M	F	M	F	M	F	M	F	M	F
Adrenals	59	185	16	33	0.51	0.66	0.87	0.93	1.2	1.8
Bone marrow	14	22	6.5	7.5	0.19	0.20	0.13	0.17	0.22	0.39
Brain	2.5	4.2	0.48	1.2	0.04	0.03	0.01	0.02	0.04	0.07
Carcass (residual)	805	727	88	159	5.8	3.9	2.5	4.0	11	8.9
Fat	7.5	14	3.7	9.5	0.11	0.23	0.09	0.14	0.17	0.52
Heart	19	31	4.4	8.5	0.20	0.25	0.10	0.16	0.30	0.53
Intestinal tract	1779	1826	257	375	33	33	6.4	6.1	29	20
Kidneys	120	177	18	31	1.7	2.1	0.41	0.59	1.9	4.2
Liver	879	1131	71	175	15	25	2.5	4.1	15	32
Lungs	29	41	6.4	11	0.29	0.39	0.13	0.19	0.41	0.69
Muscle (thigh)	6.7	9.6	2.0	3.5	0.08	0.07	0.04	0.06	0.12	0.18
Ovaries	NA	34	NA	12	NA	0.65	NA	0.21	NA	0.56
Plasma	50	64	11	17	0.53	0.73	0.20	0.30	0.76	1.0
Spleen	21	28	5.3	8.2	0.19	0.21	0.09	0.16	0.28	0.53
Stomach	4846	1750	23	93	14	20	1.0	0.44	8.5	9.1
Testes	7.7	NA	2.1	NA	0.09	NA	0.06	NA	0.16	NA
Thyroid	30	39	13	23	0.34	0.43	0.24	0.29	0.57	0.81
Whole blood	38	49	12	18	0.49	0.55	0.22	0.31	0.73	1.0

From Swenson et al. (1998)

F, female; M, male; NA, not applicable.

[a] These rats received diets containing non-radiolabelled zoxamide at a concentration of 200 ppm for 2 weeks before receiving a single oral dose (pulse) of radiolabelled zoxamide.

[b] These rats received fiveconsecutive daily oral doses of ^{14}C-labelled zoxamide by gavage and were sacrificed at the C_{max} time-point (8 h after dosing) on day 5 of dosing.

- [^{14}C]zoxamide 2F (lot No. TEM-2616; 24% a.i.; radiochemical purity, 96.3%; specific activity, 0.068 mCi/ml (2.52 MBq/ml); and lot No. TEM-2618; 0.015% a.i.; radiochemical purity, 96.3%; specific activity, 0.005 mCi/ml (0.19 MBq/ml).

The study was certified to be compliant with GLP and conducted in accordance with OECD guideline 417.

The [^{14}C]zoxamide 80WP formulation was given either as a 10 mg aliquot of the undiluted wettable powder (80% a.i.) or as 100 µl aliquots diluted in water. This gave concentrations of 0.15% active substance (a.s.) and 0.015% a.s. respectively. Similarly, the [^{14}C]zoxamide 2F formulation was given at concentrations of 24% and 0.015% a.s. These concentrations were chosen to represent exposure to the concentrated product and a typical in-use dilution.

After administration of undiluted [^{14}C]zoxamide 80WP or [^{14}C]zoxamide 2F formulations to male rats, approximately 1% of the administered dose was absorbed within 24 h. After administration of the 0.015% a.i. dilutions of either formulation, 5–6% was absorbed after 24 h. These studies

indicated that zoxamide is very poorly absorbed after dermal exposure, irrespective of formulation. The low rate of dermal absorption was considered to be attributable to the very low solubility of zoxamide in water (log Kow 3.8; water solubility, < 1 mg/l) (Frederick & Swenson, 1998).

(c) Biotransformation

Metabolites in samples of urine, faeces and bile from the studies described above were identified and quantified. Samples from rats given [^{14}C]zoxamide were pooled by sex and dose to create composite samples.

Faeces were studied by extracting homogenates with methanol, then analysing the methanol fractions by reverse-phase high-performance liquid chromatography (RP-HPLC) and normal-phase thin-layer chromatography (TLC). Samples of urine were filtered and examined directly by HPLC and TLC. Bile was analysed directly by RP-HPLC and liquid chromatography-mass spectroscopy (LC-MS). The amounts of metabolites were quantified using liquid scintillation counting (LSC) of collected HPLC fractions, except for two benzoic acid metabolites RH-141,455 and RH-141,452, which were quantified from samples from rats at the highest dose by gas chromatography-electron capture detection (GC-ECD) using a method not requiring radiolabel. Isolated metabolites were characterized and identified by TLC, GC-MS analyses (either as derivatized or underivatized metabolites), and/or LC-MS with electrospray (ESI) or atmospheric chemical ionization (APCI), and/or by comparison to authentic reference standards.

Zoxamide was found to be extensively metabolized. Including parent compound, a total of 36 metabolites were found in the faeces and urine; 24 of these were identified. In bile, 17 products were detected and 13 were identified. Altogether, 32 structures were determined. No single metabolite other than parent zoxamide accounted for more than 10% of the administered dose. Zoxamide was observed in faeces at 12% and 23% of the administered dose at 10 mg/kg bw and 72% and 74% at 1000 mg/kg bw for females and male, respectively, probably from unabsorbed material. The levels of major metabolites are given in Table 4 and a proposed metabolic pathway is given in Figure 2. The main reactions occurring were hydrolysis and dehalogenation with subsequent oxidation and conjugation.

The primary metabolites in bile were M14A/B (10–12%), a glutathione conjugate; M25 (8%), the glucuronide of M4; and M26 (6%), also a glutathione conjugate. The pattern of biliary metabolites was very similar in males and females.

Overall metabolism was similar irrespective of dose or sex. The metabolic profile in samples of faeces from rats at the highest dose was qualitatively similar to that in rats at the lowest dose, except for the large amount of parent compound that was considered to be suggestive of incomplete absorption for the higher dose. The amount of parent compound found in rats given repeated doses in a dietary 14-day study (5.56–5.84%) were reduced compared with rats at the lower dose (12.10–22.96%) suggesting increased metabolism after repeat dosing (Swenson et al., 1998).

In a supplementary study, bile samples from males and urine samples from females that had been retained from the above study of metabolism were analysed in order to determine whether the benzamide metabolite RH-139432 was present. Quantitative analysis was by two-dimensional TLC with reverse-phase and normal-phase coatings. Radioanalysis was performed before chromatography to allow for material balance calculations. For bile, RH-139432 was found to account for 0.21–0.25% of the radioactivity applied to the TLC plate, depending on the TLC phase used. For urine, RH-139432 accounted for 0.02–0.03% of the applied radioactivity, depending on the TLC phase used. After purification of the material obtained from the bile sample, the identity of RH-139432 was confirmed by GC-MS and HPLC-MS. This study showed there was no significant production of RH-139432 in rats treated with zoxamide (Reibach & Detweiler, 2001).

Table 4. Distribution of metabolites in rats given radiolabelled zoxamide as a low dose at 10 mg/kg bw

Metabolite	Recovery (% of applied dose)											
	Single dose						Repeated doses					
	Male			Female			Male			Female		
	Faeces	Urine	Subtotal	Faeces	Urine	Subtotal	Faeces	Urine	Subtotal	Faeces	Urine	Subtotal
M-1 (zoxamide)	22.96	—	22.96	12.10	—	12.10	5.56	—	5.56	5.84	—	5.84
M-2 (RH-127,450)	2.75	—	2.75	2.43	—	2.43	2.64	—	2.64	2.28	—	2.28
M-3 (RH-141,643)	7.44	—	7.44	4.93	—	4.93	5.30	—	5.30	5.37	—	5.37
M-4 (RH-141,288)	2.84	—	2.84	3.15	—	3.15	4.02	—	4.02	6.43	—	6.43
M-5	2.46	—	2.46	1.54	—	1.54	2.09	—	2.09	1.76	—	1.76
M-7 (RH-141,454)	5.74	—	5.74	3.73	—	3.73	6.19	—	6.19	2.79	—	2.79
M-8A, M-8B, and M-15	3.36	0.70	4.06	2.94	5.05	7.99	2.22	1.38	3.60	3.84	3.08	6.92
M-9	4.14	—	4.14	3.58	—	3.58	5.12		5.12	3.78		3.78
M-10A, M-10B, M-16, M-17 (RH-141,452), and M-18	7.47	4.30	11.77	9.14	4.93	14.07	9.42	5.90	15.32	8.12	3.64	11.76
M-12	—	0.11	0.11	—	1.09	1.09	—	0.75	0.75	—	1.58	1.58
M-13	—	—	—	—	5.06	5.06	—	0.32	0.32	—	9.64	9.64
M-14A and M-14B	—	0.55	0.55	—	1.27	1.27	—	1.67	1.67	—	1.71	1.71
M-19 and M-20	—	1.78	1.78	—	2.65	2.65	—	2.46	2.46	—	2.26	2.26
M-21A and M-21B	—	0.94	0.94	—	1.02	1.02	—	1.34	1.34	—	1.48	1.48
Sum of identified metabolites	59.16	8.38	67.54	43.54	21.07	64.61	42.56	13.82	56.38	40.21	23.39	63.60
% of administered dose submitted for analysis	87.78	8.84	96.62	73.50	23.17	96.67	78.57	15.30	93.87	71.05	26.45	97.50
Total % of administered dose	87.78	10.29[a]	98.07	73.50	26.85[c]	100.35	78.57	16.27[a]	94.84	71.05	28.72[a]	99.77

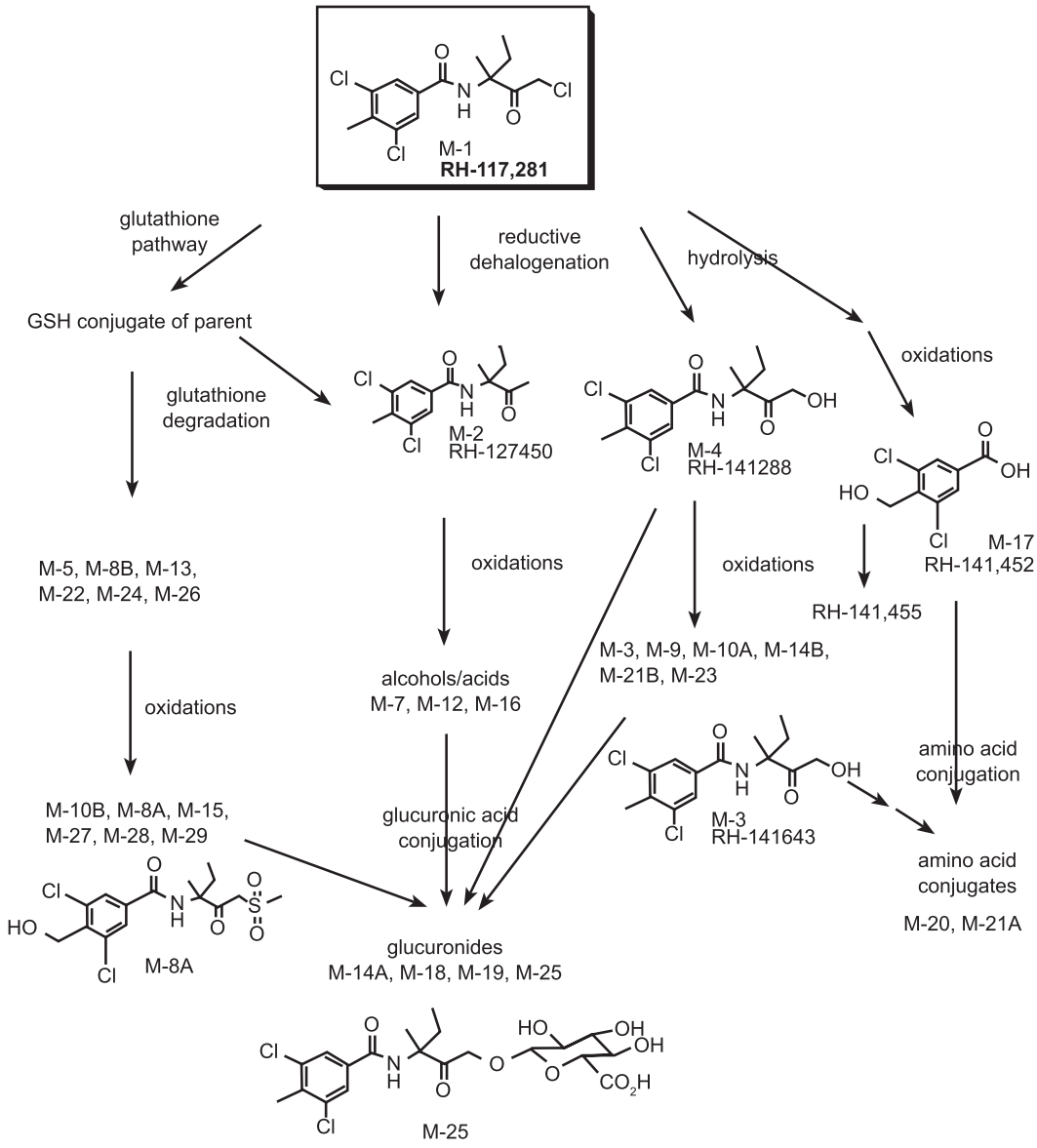
[a] This includes urine, urine funnel wash and urine cage wash. Urine funnel wash and urine cage wash were not analysed for metabolites.

Metabolite RH141,452 (3,5-dichloro-4-hydroxymethyl benzoic acid)

The absorption, distribution, metabolism and elimination of [^{14}C]RH-141,452 (lot No. 955.0005, specific activity, 75.38 mCi/g (2.79 GBq/g) were studied in male rats. Four male rats were each given a single oral dose of [^{14}C]RH-141,452 in pH-adjusted water at a nominal dose of 1000 mg/kg bw. The rats were sacrificed 78 h after dosing and the total recovery of radiolabelled residues was determined.

Most of the radioactivity was eliminated in the urine (approximately 98% in urine and cage rinse), with only a small amount being excreted from faeces (< 2%). Approximately 0.01% of the administered dose of radioactivity was found in the expired air. The excretion of the ^{14}C was rapid, with more than 97% being excreted within 24 h. Studies of metabolism showed that most of the RH-141,452 was eliminated unchanged in the urine, accounting for > 94% of the administered dose. Three minor conjugates, M-2, M-3 (glucuronide conjugates), and M-4 (glycine conjugate)

Figure 2. Summary of the metabolic pathway of zoxamide in the rat



From Swenson et al. (1998)

were also found in the urine, accounting for approximately 3% of the administered dose. An additional 1.6% of the administered radioactivity was excreted in the faeces as the parent chemical (Wu & Gu, 1998a).

Metabolite RH141,455 (3,5-dichloro-terphthalic acid)

The absorption, distribution, metabolism and elimination of [^{14}C]RH-141,455 (lot No. 958.0005; specific activity, 74.46 mCi/g) in male rats were studied. Four male rats were given a single oral dose of [^{14}C]RH-141,455 in pH-adjusted water at a nominal dose of 1000 mg/kg bw. The rats were sacrificed 168 h after dosing and the total recovery of radiolabelled residues was determined. More than 96% of radioactivity excreted from faeces (72%) and urine plus cage wash (20%) was identified as unchanged RH-141,455. Some minor metabolites were also observed in urine samples, but were not identified owing to their low concentrations (Wu & Gu, 1998b).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with zoxamide administered by the oral, dermal and inhalation routes, and two of its metabolites, are presented in Table 5. Zoxamide was of low acute toxicity by all routes. The metabolites RH 141-452 & 141,455 were of low acute oral toxicity.

(b) Dermal and ocular irritation and dermal sensitization

Zoxamide was not irritating to the skin of rabbits (Gingrich & Parno, 1996c) but exhibited slight, transient irritation to the rabbit eye (Gingrich & Parno, 1996d). Zoxamide produced delayed contact hypersensitivity in the guinea-pig in the Magnusson & Kligman maximization study (Glaza, 1997) and in Buehler skin sensitization tests (Robison et al., 1998a). Low concentrations (< 0.25% w/w) were shown to be not sensitizing to the skin of guinea-pigs (Robison et al., 1998b).

Table 5. Acute toxicity with zoxamide and its metabolites

Test substance	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Vehicle	Reference
Zoxamide	Rat	Crl:CDBR	Males & females	Oral	> 5000	—	92.3	Corn oil	Gingrich & Parno (1996a)
Zoxamide	Mouse	Crl:CD-1-(ICR)BR	Males & females	Oral	> 5000	—	94.4	Corn oil	Ferguson & Lutz (1998)
Zoxamide	Rat	Crl:CDBR	Males & females	Dermal	> 2000	—	92.3	Corn oil	Gingrich & Parno (1996b)
Zoxamide	Rat	Crl:CDBR	Males & females	Inhalation (4-h, nose-only)	—	> 5.3 (MMAD 4.3 µm)	92.3	None (dust aerosol)	Bernacki,& Ferguson (1996)
RH-141,452	Mouse	Crl:CD-1-(ICR)BR	Males & females	Oral	> 5000	—	97.7	Corn oil	Ferguson et al. (1998c)
RH-141,452	Mouse	Crl:CD-1-(ICR)BR	Males & females	Oral	> 5000	—	98.7	Corn oil	Ferguson et al. (1998a)

MMAD, mass median aerodynamic diameter.

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female Crl:CD-1(ICR)BR mice were given diets containing zoxamide (purity, 94.2%) at a concentration of 0, 70, 700, 2500 or 7000 ppm (equal to 0, 12, 123, 436 and 1212 mg/kg bw per day respectively in males, and 0, 17, 174, 574, 1666 mg/kg bw per day in females) for 90 days. All mice were observed daily for signs of ill health or reaction to treatment. Physical examinations were performed each week. Body weight and feed consumption were determined each week. At the end of the study period, all surviving mice were bled for haematology and clinical chemistry investigations, killed and necropsied. Selected organ weights were recorded and tissues were collected for histopathological evaluation. The study was certified to be compliant with GLP and satisfied the essential criteria of OECD guideline 408.

No treatment-related deaths or clinical signs of toxicity were observed. There were no treatment-related effects on body weight or body-weight change in males at any dose. After 4 weeks of treatment, there was a persistent and apparently treatment-related but statistically non-significant decrease in body weight and cumulative body-weight gain in females at 7000 ppm compared with concurrent controls (Table 6). In males, a number of statistically significant decreases in mean body weight and cumulative body-weight gain were observed in the groups at 700 ppm and 2500 ppm throughout the treatment period, but these decreases were not considered to be treatment-related

Table 6. Body weights, body-weight gain and liver weight in mice fed diets containing zoxamide for 90 days

Parameter	Dietary concentration (ppm)				
	0	70	700	2500	7000
Males					
Body weight (g):					
Week 0	30.8	30.4	30.2	29.2	29.8
Week 4	34.6	33.7	33.2*	32.0	32.8
Week 8	37.9	36.3	35.8*	34.3*	35.4
Week 13 (mean ± SD)	41.1 ± 4.9	38.5 ± 2.2	38.0 ± 1.9*	36.7 ± 3.5	38.3 ± 3.9
Cumulative body-weight gain, week 13 (g, mean ± SD)	12.3 ± 4.2	9.5 ± 1.9	9.1 ± 1.1*	8.9 ± 2.7	10.0 ± 2.7
Absolute liver weight (g, mean ± SD)	2.1 ± 0.24	2.0 ± 0.20	1.9 ± 0.13	2.1 ± 0.25	2.2 ± 0.21
Relative liver weight (%, mean ± SD)	5.2 ± 0.41	5.2 ± 0.39	5.2 ± 0.25	5.8 ± 0.49*	5.8 ± 0.55*
Females					
Body weight (g):					
Week 0	23.3	23.3	22.2	22.8	20.9
Week 4	26.8	26.8	25.6	25.1	23.6
Week 8	28.1	28.9	27.3	26.8	25.0
Week 13 (mean ± SD)	30.6 ± 4.8	31.1 ± 3.2	29.3 ± 3.8	28.7 ± 3.3	25.8 ± 2.1
Cumulative body-weight gain, week 13 (g, mean ± SD)	7.4 ± 3.1	7.7 ± 2.4	7.0 ± 2.6	5.9 ± 2.0	4.9 ± 1.7*
Absolute liver weight (g, mean ± SD)	1.5 ± 0.16	1.6 ± 0.16	1.7 ± 0.21	1.6 ± 0.24	1.5 ± 0.17
Relative liver weight (%, mean ± SD)	5.2 ± 0.51	5.3 ± 0.26	5.7 ± 0.52	5.7 ± 0.57	6.0 ± 0.51*

From Shuey et al. (1996)
SD, standard deviation.
* Significant difference from controls ($p < 0.05$).

owing to the absence of a dose–response relationship. There was no statistically significant treatment-related effect on feed consumption in either sex at any dose; but mice at the highest dose regularly consumed less food than did other groups. However, in females there was a statistically non-significant but dose-related reduction in body-weight gain (34%) and in body weight in females at 7000 ppm compared with controls. The body-weight effects in females at the highest dose did not appear to be directly related to the lower body weight at the start of the study. Haematology and clinical chemistry parameters did not reveal any significant treatment-related differences. There were no treatment-related effects on absolute or relative organ weights in either sex at any dose, other than a statistically significant increase in relative liver weights in males receiving at_2500 ppm or greater and in females at 7000 ppm; there was evidence of a dose response in the females (Table 6). No treatment-related gross pathological changes or histopathological findings were observed in any tissues. In the absence of clinical chemistry and histopathological correlates, the increase in liver weight per se is not considered to be an adverse effect.

The no-observed-adverse-effect level (NOAEL) was 2500 ppm, equal to 574 mg/kg bw per day, on the basis of reduction in body-weight gain and in overall body weight in female mice at 7000 ppm (1666 mg/kg bw per day) (Shuey et al., 1996)

Rats

Groups of 15 male and 15 female Crl:CD®BR rats were given diets containing zoxamide (purity, 92.9%) at a concentration of 0, 1000, 5000, or 20,000 ppm (equal to 0, 74, 372, and 1509 mg/kg bw per day in males, and 0, 80, 401, and 1622 mg/kg bw per day in females) for 90 days. All rats were observed daily for signs of ill health or reaction to treatment. Physical examinations were performed each week. Body weight and feed consumption were monitored each week. Ten males and ten females per group were randomly selected for in-life testing for neurotoxicity via a standard battery of behavioural observations and motor activity before treatment and at weeks 4, 8, and 13; five of these were randomly selected for whole-body perfusion and special neuropathology evaluation. Haematology, urine analysis and clinical chemistry tests were conducted on samples taken from the remaining rats during week 13. The rats were then killed and necropsied. Selected organ weights were recorded and tissues were collected for routine histopathological evaluation. All animals were given ophthalmological examinations before testing and during week 13. The study was certified to be compliant with GLP and satisfied the essential criteria of OECD guideline 408.

No treatment-related mortalities or clinical signs of toxicity were observed during the study period. Body-weight gain and feed consumption showed no treatment-related inter-group differences. There were no treatment-related effects on haematology, clinical chemistry, or urine-analysis parameters. The functional observation battery (FOB) and motor activity assessments did not show any indications of neurotoxicity. No treatment-related effects on organ weights or ophthalmological changes were observed. There were no observations of treatment-related macroscopic or histopathological changes after routine necropsy or perfusion in males or females selected for neuropathology examinations.

The NOAEL was 20000 ppm, equal to 1509 mg/kg bw per day, the highest dose tested (Morrison & Gillette, 1996)

Groups of 10 male and 10 female Crl:CD®BR rats received zoxamide (purity, 93.83%) as 22 dermal doses at 0, 150, 400 or 1000 mg/kg bw per day. The material was moistened with tap water (1 : 2 w/v) and applied topically under an occlusive dressing to the shaved intact skin (an area approximately of 10% of the total body surface area) for at least 6 h per day, 5 days per week. The study was certified to be compliant with GLP and was conducted in accordance with OECD guideline 410.

The only findings of note were a dose-related and time-related increase in the incidence of reddening and scabbing at the application sites, a dose-related increase in leukocytes and a decrease in albumin : globulin ratio (A : G) in females,; and an altered differential count in males (Table 7). The study investigators suggested that the alteration in A : G were secondary to the skin irritation, but it is of note that reductions in globulin levels were also seen in the dietary studies in dogs (see Table 11) and do not explain the absence of an equivalent effect in males. The albumin and globulin values in treated animals were within ranges for historical controls and would not be considered to be adverse in isolation.

Zoxamide produced significant local effects at doses of 107 mg/kg bw per day and greater. Findings of systemic toxicity were most likely secondary to the local effects and the NOAEL for systemic effects was 714 mg/kg bw per day (corrected for administration on 5 days/week) (Robison et al., 1998d).

Dogs

Groups of two male and two female beagle dogs were given diets containing zoxamide (purity, 92.9%) at a concentration of 0, 500, 5000, 15 000 or 30 000 ppm (equal to 0, 20, 175, 542 and 1045 mg/kg bw per day for males and 0, 20, 191, 579 and 1085 mg/kg bw per day for females) for 28 days. All dogs were observed daily for signs of ill health or reaction to treatment. During pre-test and throughout treatment, feed consumption was monitored daily, physical examinations were performed and body weights were determined weekly. During the first week of pre-test and after 2 and 4 weeks of treatment, blood samples were collected from all dogs (fasted) for haematology and clinical chemistry analysis. After 4 weeks, all dogs were killed and organs and tissues were grossly examined. Selected organ weights were recorded at necropsy. Microscopic examination was conducted on all gross lesions and on all tissues and organs collected from all dogs in the group receiving the highest dose and in the control group.

There were no deaths during the study. The only treatment-related clinical signs were soft faeces present throughout the dosing period in one out of four dogs at 5000 ppm and in all dogs at 15 000 ppm and greater. There were no overt treatment-related effects on any other measured parameter; but the dogs varied markedly in size at the start of treatment.

Table 7. Findings in rats treated dermally with zoxamide

Finding	Dose (nominal mg/kg bw per day)			
	0	150	400	1000
Males				
Application site reaction	0/10	4/10	9/10	10/10
Leukocytes	6.3	5.7	6.7	6.0
Lymphocytes (%)	88	90	83	80*
Segmented neutrophils (%)	11	9	15	18*
Females				
Application site reaction	4/10	9/10	10/10	10/10
Leukocytes	3.6	4.5	5.1	5.8*
Albumin (g/dl)	4.2	3.9	3.7*	3.7*
Globulin (g/dl)	1.7	1.9	2.0*	2.1*
Albumin : globulin ratio	2.5	2.1*	1.9*	1.8*

From Robison et al. (1998d)
* Significant difference from control ($p < 0.05$).

The NOAEL was 30 000 ppm, equivalent to 1045 or 1085 mg/kg bw per day. The only finding was the occurrence of a minimal incidence of soft stools at 5000 ppm (175 or 191 mg/kg bw per day) and an increased incidence at doses of 15 000 ppm and greater; however, soft faeces was not a consistent finding in other studies in dogs with a longer duration and using the same formulated diet (Vandenberghe et al., 1996).

Groups of four male and four female beagle dogs were given diets containing zoxamide (lot No. DSR-9510, purity 92.3%) at a concentration of 0, 1500, 7500 or 30 000 ppm (equal to 0, 55, 281, and 1139 mg/kg bw per day in males, and 0, 62, 322 and 1055 mg/kg bw per day respectively in females) for at least 90 days. One female in the group at 1500 ppm was moved to a concurrent 1-year study and replaced with another dog during the third week of dosing, hence the treatment period was extended to 16 weeks. All dogs were observed daily for signs of ill health or toxicity. Feed consumption was determined daily for all dogs beginning 2 weeks before treatment and throughout the treatment period. Physical examinations were performed and body weights were determined each week. Blood samples were collected from all dogs for haematology and clinical chemistry analyses during pre-test, and after 8 and 16 weeks of treatment. Urine analysis and ophthalmology examinations were performed on all dogs during pre-test and after the treatment period. Selected organs were weighed and tissues were collected for histopathological evaluation. The study complied with GLP and with OECD guideline 409.

The study investigators considered that there were no treatment-related deaths or clinical signs indicative of systemic toxicity during the treatment period. Two dogs were killed humanely during the study, a female at 30 000 ppm and a male at 7500 ppm. The female was diagnosed with

Table 8. Body weight and feed consumption in dogs fed diets containing zoxamide for 90 days

Parameter	Dietary concentration (ppm)			
	0	1500	7500	30 000
Males				
Body weight (g):				
Week 0	7911	7821	8017	7537
Week 1	8339	8091	8118	7515
Week 8	9861	9714	9584	7803*
Body-weight gain (g), week 1–16 (mean ± SD)	3296 ± 477	3290 ± 437	2718 ± 894	1296 ± 813*
Food consumption (g), week 1	290	300	285	226*
Females				
Body weight (g):				
Week 0	6562	6846	6659	6665
Week 1	6885	7116	6753	6529
Week 8	8043	7749	7728	6669
Body-weight gain (g), week 1–16 (mean ± SD)	2314 ± 891	1658 ± 928	1899 ± 398	824 ± 528[a]
Food consumption (g/day), week 1	270	278	280	187*

From Ferguson et al. (1997)
SD, standard deviation.
* Significant difference from controls ($p < 0.05$).
[a] $p = 0.052$ (two-sided t-test).

canine juvenile polyarteritis syndrome (CJPS[1]); the male with pneumonia. Two additional males (at 7500 ppm and 30 000 ppm) exhibited signs consistent with CJPS. Sporadic instances of soft faeces were recorded pre-test and during treatment. There was a treatment-related decrease in mean body weight, cumulative body-weight gain, and feed consumption in males and females at 30 000 ppm (Table 8). These findings were evident in the first weeks of dosing and did not appear to be related to the sick animals in the groups at the intermediate and highest dose.

Haematology investigations did not reveal any treatment-related changes in either sex at doses of 7500 ppm or less (Table 9). Statistically significant changes, including decreased erythrocyte count, increased mean cell haemoglobin, and increased mean cell haemoglobin concentration were seen in females at 30 000 ppm. A similar but statistically non-significant trend was seen in males. A dose-related decrease in lymphocytes and increased segmented neutrophils were also observed in males from week 8 to 16; the total leukocyte count was very variable but did not show any treatment-related differences. The toxicological significance of these haematological changes was unclear, as they were not reproduced in the 1-year study in dogs.

Many clinical chemistry parameters exhibited considerable variation between dogs, but did not show any changes in males or females at doses of 7500 ppm or less. At 30 000 ppm, apparent treatment-related decreases in albumin and in the albumin to globulin ratio were seen in both sexes after 8 and 16 weeks of treatment and an increase in serum gamma glutamyl transferase activity was seen in males (less than twofold). Urine analysis parameters did not show any treatment-related differences. Ophthalmology at 16 weeks showed no treatment related effects. Organ weights showed a treatment-related increase in the absolute and relative liver weights in females at 7500 ppm and greater and in males at 30 000 ppm. The increase in absolute (≥ 23%) and relative liver weight (29%) of the females at 7500 ppm was considered to be treatment-related but not an adverse effect, since no corresponding clinical pathology or histopathological findings were observed at this dose (Table 10).

There were no treatment-related findings on gross examination. Treatment-related microscopic changes were observed in the group at 30 000 ppm and consisted of a diffuse hepatocellular hypertrophy affecting all males and females, and hypertrophy of the thyroid follicular epithelium in one male and one female. Neither of these findings were reported in the data for historical controls; however, hypertrophy of the thyroid was not reported in the 1-year study in dogs.

The NOAEL was 7500 ppm, equal to 281 mg/kg bw per day, on the basis of treatment-related changes including reduction in body weight and in body-weight gain, thyroid hypertrophy, changes in erythrocyte and leukocyte parameters and reduced albumin concentrations. Increases in absolute and relative liver weights were noted at 7500 ppm, but the changes in organ weights were not accompanied by any clinical chemistry histopathological changes and were not considered to be adverse effects (Ferguson et al., 1997).

Groups of four male and four female beagle dogs were given diets containing zoxamide (purity, 92.3%) at a concentration of 0, 1500, 7500 or 30 000 ppm (0, 50, 255, and 1016 mg/kg bw per day respectively in males, and 0, 48, 278 and 994 mg/kg bw per day respectively in females) for 1 year. All dogs were observed daily for signs of ill health or reaction to treatment. Feed consumption was determined daily for all animals beginning 2 weeks before treatment (i.e. pre-test) and continued until the end of week 13 of treatment. Thereafter, feed consumption was measured for 1 week every 4 weeks until the end of the study. Body weights were determined each week for all animals, beginning 2 weeks before treatment (i.e. pre-test) and continued until the end of week 13 of treatment. Thereafter, body weights were measured once every 4 weeks until the end of the study. Physical examinations

[1] CJPS is reported to be a spontaneous disease of uncertain etiology (Ruben et al., 1989; Snyder et al., 1995; Son, 2004).

Table 9. Haematological findings in dogs fed diets containing zoxamide for 90 days (mean)

Finding	Dietary concentration (ppm)			
	0	1500	7500	30 000
Males				
Erythrocytes (10^6/mm^3):				
Week 8	5.92	6.05	6.11	5.72
Week 16	6.61	6.75	6.45	5.88
MCH (pg):				
Week 8	21.4	22.0	21.3	22.1
Week 16	21.5	21.9	21.6	22.6
MCHC (%):				
Week 8	34.0	34.2	34.3	34.7
Week 16	34.2	34.1	34.4	34.9
Segmented neutrophils (%):				
Week 8	64	62	64	69
Week 16	56	71	77	83*
Lymphocytes (%):				
Week 8	31	35	30	30
Week 16	38	26	20	14*
Females				
Erythrocytes (10^6/mm^3):				
Week 8	6.33	6.07	5.89	5.71*
Week 16	7.01	6.57	6.63	5.98
MCH (pg) :				
Week 8	21.7	22.1	22.0	23.5*
Week 16	21.7	22.3	22.2	24.3*
MCHC (%):				
Week 8	34.0	34.3	34.2	35.1
Week 16	33.7	34.6	34.5	35.8*

From Ferguson et al. (1997)
MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration.
* Significant difference from control ($p < 0.05$).

were performed weekly beginning 2 weeks before treatment. Clinical chemistry, haematology, and urinary parameters were evaluated for all dogs during pre-test, after 3, 6, and 9 months of treatment, and for all dogs surviving to necropsy. Ophthalmological examinations were performed on all dogs during pre-test and just before the end of treatment. Selected organs were weighed and tissues were collected for histopathological evaluation. The study was certified to be compliant with GLP and was conducted in accordance with OECD guideline 452.

There were no deaths or clinical signs indicative of systemic toxicity during the study. A female from the group at 1500 ppm was found dead (presumed cause, bilateral haemorrhagic pneumonia) and replaced with an equivalent animal from the 90-day study (see above). A female at the highest dose was humanely killed on week 38; the clinical signs and post-mortem findings were reported to be consistent with CJPS. A male at the lowest dose showed evidence of CJPS at post mortem examination. Soft faeces were seen in all groups and in some animals before testing, but appeared to be more persistent in animals at 30 000 ppm. There was a treatment-related reduction in mean body

Table 10. Organ weight and histopathological findings in dogs receiving zoxamide

Finding	Dietary concentration (ppm)			
	0	1500	7500	30 000
Mean organ weights (mean ± SD)				
Males				
Absolute liver weight (g)	304 ± 14	319 ± 33	310 ± 116	371 ± 45*
Relative liver weight (%)	2.66 ± 0.24	2.80 ± 0.22	2.80 ± 0.87	4.14 ± 0.06*
Females				
Absolute liver weight (g)	244 ± 49	269 ± 39	300 ± 48	329 ± 45*
Relative liver weight (%)	2.68 ± 0.20	3.11 ± 0.40	3.44 ± 0.30*	4.37 ± 0.31*
Histopathology (No. affected/No. examined)				
Males				
Liver hypertrophy	0/4	0/4	0/4	4/4
Thyroid hypertrophy	0/4	0/4	0/4	1/4
Females				
Liver hypertrophy	0/4	0/4	0/4	4/4
Thyroid hypertrophy	0/4	0/4	0/4	1/4

From Ferguson et al. (1997)
* Significant difference from controls ($p < 0.05$).

weight, cumulative body-weight gain, and feed consumption in both sexes at 30 000 ppm; it was not until week 7 that all the females returned to their pre-test body weight. Females at 7500 ppm had reduced body-weight gain in the early stages of the study and this early deficit persisted (Table 11). Haematology and urine analysis parameters did not reveal any treatment-related differences. Clinical chemistry parameters showed treatment-related decreases in albumin and increases in alkaline phosphatase activity in both sexes at 30 000 ppm (Table 11). Ophthalmology at termination did not reveal any treatment-related ocular changes.

Organ weights showed a treatment-related increase in absolute and relative liver weights in both sexes at 30 000 ppm and in females at 7500 ppm (Table 12). A dose-related increase in absolute and relative thyroid weights was also evident (Table 12). No treatment-related gross findings were made at necropsy. Treatment-related microscopic changes were observed in the livers of some animals from the group at 30 000 ppm and consisted of diffuse hepatocellular hypertrophy in two males and one female; another female had multifocal haemorrhage and necrosis; a third female had congestion and mononuclear cell infiltration, a finding also seen in a male without hypertrophy (Table 12). None of the animals in this study had thyroid cell hyperplasia.

The NOAEL was 1500 ppm, equal to 48 mg/kg bw per day, on the basis of reduction in body-weight gain in females at 7500 ppm, equal to 255 mg/kg bw per day (Ferguson et al., 1998b).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a long-term study of toxicity/carcinogenicity, groups of 60 male and 60 female Crl:CD-1 (ICR) BR (VAF/+) mice were given diets containing zoxamide (purity, 92.3%) at a concentration of 0, 350, 1750, or 7000 ppm (equal to 0, 51.1, 251 or 1021 mg/kg bw per day and 0, 60.4, 326 or 1289 mg/kg bw per day in males and females respectively) for 18 months. All mice were observed

Table 11. Body weight, food consumption and clinical chemistry findings in dogs fed diets containing zoxamide for 1 year

Finding (means)	Dietary concentration (ppm)			
	0	1 500	7 500	30 000
Males				
Body weight (g):				
Week 0	7 846	8 036	7 576	7 882
Week 4	8 939	9 094	8 374	8 136
Week 13	10 640	10 773	10 349	9 466
Week 25	11 623	12 063	11 933	10 718
Week 52	11 734	12 318	12 247	11 041
Body-weight gain (g):				
Week 1	169	180	9	36*
Week 4	1 093	1 058	799	254*
Week 13	2 794	2 737	2 773	1 584*
Week 25	3 777	4 027	4 357	2 836
Week 52	3 888	4 283	4 672	3 159
Food consumption (g):				
Week 1	290.1	285.7	241.2	217.1
Week 25	324.4	343.1	330.8	359.8
Albumin (g/dl):				
Pre-test	3.7	3.3	3.4	3.6
Day 93	3.7	3.7	3.5	3.4*
Day 183	3.7	3.5	3.6	3.3*
Day 274	3.7	3.4	3.4	3.2*
Day 365	3.8	3.7	3.7	3.5
Alkaline phosphatase (U/l):				
Pre-test	260	402	498	325
Day 93	190	241	388	367
Day 183	122	168	275	434*
Day 274	117	174	236	412*
Day 365	97	159	200	414*
				Females
Body weight (g):				
Week 0	7 004	6 781	6 755	6 980
Week 4	7 898	7 658	7 137	6 839*
Week 13	9 242	9 008	8 322	8 035
Week 25	10 176	9 511	9 179	8 503*
Week 52	10 171	9 576	9 293	8 912
Body-weight gain (g):				
Week 1	251	415	139	−175*
Week 4	895	877	382*	−141*
Week 13	2 238	2 227	1 567	1 056*
Week 25	3 172	2 730	2 425	1 523

Week 52	3 167	2 795	2 538	2 052
Food consumption (g/day):				
Week 1	259.9	254.4	250.5	196.4*
Week 25	281.6	252.6	313.5	274.2
Albumin (g/dl):				
Pre-test	3.4	3.6	3.5	3.3
Day 93	3.6	3.8	3.6	3.2*
Day 183	3.6	3.8	3.5	3.1
Day 274	3.6	3.8	3.5	3.3
Day 365	3.7	3.6	3.6	3.3*
Alkaline phosphatase (U/l):				
Pre-test	283	314	311	290
Day 93	208	177	258	311*
Day 183	170	116	242	297*
Day 274	176	106	219	304*
Day 365	152	108	183	330*

From Ferguson et al. (1998b)
* Significant difference from controls ($p < 0.05$); ANOVA, Dunnett t-test.
a Mainly due to one dog that lost 397 g in body weight, but consumed typical amounts of food.

Table 12. Organ weights and histopathological findings in dogs fed diets containing zoxamide for 1 year (means)

Finding	Dietary concentration (ppm)			
	0	1500	7500	30 000
Mean organ weights:				
Males				
Absolute liver weight (g)	293	330	353	412*
Relative liver weight (%)	2.5	2.7	2.9	3.7*
Absolute thyroid weight (g)	0.81	0.92	1.07	1.04
Relative thyroid weight (%)	0.007	0.007	0.009	0.010
Females				
Absolute liver weight (g)	278	276	309	341
Relative liver weight (%)	2.7	2.9	3.4*	3.8*
Absolute thyroid weight (g)	0.73	0.75	0.81	0.98*
Relative thyroid weight (%)	0.007	0.008	0.009	0.011*
Histopathology (No. affected/No. examined):				
Males				
Liver hypertrophy	0/4	0/4	0/4	2/4
Hepatocellular necrosis	0/4	0/4	0/4	0/4
Females				
Liver hypertrophy	0/4	0/4	0/4	1/4
Hepatocellular necrosis	0/4	0/4	0/4	1/4

From Ferguson et al. (1998b)
* Significant difference from controls ($p < 0.05$); ANOVA, Dunnett t-test.

daily for signs of moribundity, mortality, ill health or reaction to treatment. Physical examinations were performed each week. Body weights and feed consumption were monitored weekly beginning 1 week before initiation of treatment until week 13 and then every fourth week thereafter for the duration of the study. After 12 and 18 months, all mice were bled for leukocyte differential counts. After 18 months, all mice were killed, and organs and tissues grossly examined at necropsy. Selected organ weights were recorded and histopathological examinations were conducted on tissues. The study was certified to be compliant with GLP and was conducted in accordance with OECD guideline 451.

There were no effects on survival in mice at any dose; absolute survival was more than 50% in all groups. No treatment-related deaths or clinical signs indicative of systemic toxicity were observed in any of the treatment groups. Males at 350 and 7000 ppm had lower body weights than did controls (< 10% reduction) for much of the study, but there was no clear dose–response relationship. There were no other notable effects on body weight, cumulative body-weight gain, or feed consumption. Leukocyte differential counts from all mice at the highest dose were similar to those of controls at 12 or 18 months of treatment. Organ-weight measurements at necropsy did not show any treatment-related intergroup differences. Relative liver weights in all treated groups of males were approximately 10% higher than in controls, but there was no dose–response relationship and no associated histopathological findings, therefore this was not considered to be an adverse effect of treatment. Gross examination at necropsy and microscopic examination of organs and tissues did not reveal any treatment-related changes. There were no treatment-related effects on the type or incidence of any of the neoplasms observed in this study.

The Meeting concluded that zoxamide is not carcinogenic. The NOAEL was 7000 ppm, equal to 1021 mg/kg bw per day, the highest dose tested (Robison et al., 1998c; Gillette & Brown, 1998).

Rats

In a combined long-term study of toxicity and carcinogenicity, groups of 70 male and 70 female Sprague-Dawley Crl:CD®BR rats were given diets containing zoxamide (purity, 92.0%) at a concentration of 0 (control), 1000, 5000, or 20 000 ppm (equal to 0, 51, 260, and 1058 mg/kg bw per day in males and 0, 65, 328, and 1331mg/kg bw per day in females) for 2 years. Ten males and 10 females per group were randomly selected before treatment for interim sacrifice after 52 weeks. Routine observations were performed for survival, clinical signs, body weights and food consumption. During weeks 13, 26, 52, 78, and 104, blood samples were collected for haematology and clinical chemistry tests from 20 males and 10 females per group, and urine samples were collected from 10 males and 10 females per group. The same animals were bled at each interval when possible. After either 52 (10 males and 10 females per group) or 104 (all survivors) weeks of treatment, the animals were weighed, anaesthetized, killed, and necropsied. At necropsy, macroscopic observations were recorded, selected organs were weighed, and selected tissues were collected and preserved. Animals that died during test or were sacrificed at an unscheduled interval were also necropsied; however, organs were not weighed. Microscopic examinations were performed on tissues from each rat in the control group and in the group at the highest dose and from each rat that died or was sacrificed at an unscheduled interval. The lung, liver, kidney, and any gross lesions were also examined microscopically from each rat at the lowest and intermediate dose. The study was certified to be compliant with GLP and satisfied the essential requirements of OECD guideline 453.

There were no test material-related effects on survival or in clinical signs; overall survival was > 44% in all groups. Clinical chemistry and haematology did not reveal any adverse, treatment-related differences. Observed changes in the haematology and serum chemistry parameters were considered incidental to the administration of the test material owing to the lack of dose–response relationship, the low magnitude of the change, the lack of histopathological correlation, or the inconsistent occurrence of the differences at the different sampling times. There were no treatment-related effects in any urine analysis parameter or in ophthalmical findings in either sex at any dose. Liver

weights were increased in a dose-related manner in females at the interim kill (Table 13), but not at termination, nor in males. There were no indications of treatment-related histopathological changes in the liver. Gross and histopathological examinations of organs and tissues did not reveal any treatment-related abnormalities. An apparent increase in thyroid C-cell lesions in males at the highest dose was not statistically significant ($p > 0.05$), did not exhibit a dose–response relationship, was not reproduced in females and was within the range for historical controls (Giknis & Clifford, 2001).

Zoxamide was not carcinogenic and did not produce any evidence of significant systemic toxicity at doses of up to 20 000 ppm (1058 mg/kg bw per day) in this 2-year dietary study in rats. The NOAEL was 20 000 ppm (1058 mg/kg bw per day) on the basis of the absence of toxicity at the highest dose tested (Ivett, 1998a; 1998b)

2.4 Genotoxicity

The genotoxic potential of zoxamide and two of its metabolites has been investigated in a range of studies (Table 14). Zoxamide was not genotoxic in an assay for reverse mutation in *Salmonella typhimurium*; an assay for gene mutation in mammalian cells nor in an assay for micronucleus formation in mice in vivo. In a study of chromosome aberration in Chinese hamster ovary cells in vitro, there was no increase in structural aberrations, but mitotic accumulation was observed at concentrations that inhibited cell growth in tests with and without metabolic activation. The increases observed in all cultures were noted to be predominantly attributable to increases in the frequency of cells with polyploidy. Such a finding is consistent with the mode of pesticidal action of zoxamide, which involves binding to β-tubulin (Young, 1998). To investigate this finding, a study of micronucleus formation in bone marrow with kinetochore staining was performed in rats in vivo; this produced negative results for both micronucleus formation and kinetochore staining.

Table 13. Liver weights and thyroid pathology findings in rats given diets containing zoxamide for 2 years

Finding	Dietary concentration (ppm)			
	0	1000	5000	20,000
Females				
Week 53 ($n = 10$):				
Body weight (g)	398	380	355	383
Absolute liver weight (g)	9.5	10.0	0.1	11.1
Relative liver weight (%)	2.38	2.64	2.86*	2.91*
Week 105 (n = 26–30):				
Body weight (g)	406	389	383	388
Absolute liver weight (g)	11.5	11.7	11.9	11.8
Relative liver weight (%)	2.90	3.02	3.15	3.06
Thyroid C-cell hyperplasia	8/58	2/31	4/33	5/60
Thyroid C-cell carcinoma	4/58	0/31	2/33	2/60
Thyroid C-cell adenoma	4/58	2/31	3/33	5/60
Males				
Thyroid C-cell hyperplasia	6/58	2/33	0/23	8/58
Thyroid C-cell carcinoma	2/58	2/33	0/23	3/58
Thyroid C-cell adenoma	2/58	2/33	2/23	6/58

From Giknis & Clifford (2001)
* Significant difference from controls ($p < 0.05$).

Table 14 Results of studies of genotoxicity with zoxamide and its metabolites

Test substance	End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro						
Zoxamide	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	16–5000 µg/plate (precipitation at ≥ 160 µg/plate)	92.3	Negative +S9 Negative –S9	Sames & Ciaccio (1996)
Zoxamide	Chromosome aberrations	Chinese hamster ovary cells	1–100 µg/ml (1–4 µg/ml –S9 and 1–16 µg/ml +S9 scored) Cytotoxicity at 4–µg/ml – S9; and 11–µg/ml +S9	92.3	Negative +S9 Negative –S9 Positive for polyploidy ± S9	Riley (1998)
Zoxamide	Gene mutation (*HPRT*)	Chinese hamster ovary cells (CHO-K1, BH4)	–65 µg/ml (precipitation at 50 µg/ml)	94.2	Negative +S9 Negative –S9	Pant (1994)
In vivo						
Zoxamide	Micronucleus formation	Bone marrow from CD-1 mice (five males and five females per group per time-point)	200, 1000 or 2000 mg/kg bw by gavage in corn oil (sacrifice at 24 or 48 h)	92.3	Negative	Sames & Vandenberghe (1996)
Zoxamide	Micronucleus formation with kinetochore staining	Bone marrow from Crl:CD-BR rats (five males per group)	Two administrations at 500, 1000 or 2000 mg/kg bw in corn oil. All doses scored for micronucleus formation; highest dose only for kinetochore staining.	97.35	Negative	Gudi & Krsmanovich (2003)
Metabolites						
RH141,452	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	50–5000 µg/plate	97.7	Negative +S9 Negative –S9	Sames & Ciaccio (1998a)
RH141,455	Reverse mutation	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	50–5000 µg/plate	98.7	Negative +S9 Negative –S9	Sames & Ciaccio (1998b)

S9, 9000 × *g* supernatant from livers of rats.

The zoxamide metabolites, RH 141,452 and RH 141,455 gave negative results in an assay for reverse mutation in *S. typhimurium*.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female rats were given diets containing zoxamide (purity, 92.3%) at a concentration of 0, 1000, 5000 or 20 000 ppm (active ingredient) over two generations. Parental rats of the first generation (P_1) were exposed from age approximately 6 weeks and of the second generation (P_2) from weaning (21 days). P_1 and P_2 rats were mated initially (to produce F_{1a} and F_{2a} litters) after at least 10 weeks of exposure to treated diets. Treatment continued throughout gestation, lactation, and until terminal necropsy. P_1 and P_2 rats were each mated a second time to produce a second set of litters (designated F_{1b} and F_{2b}, respectively). Rats were re-mated approximately 1 week after weaning of all litters from the first mating was complete. A second mating of the P_1 rats was conducted to verify apparent findings among the F_{1a} offspring. The P_2 rats were re-mated to ascertain if the apparent findings observed in the F_{1a}, F_{1b} and F2a litters were related to decreased feed intake, direct toxicity of the test substance, and/or from maternally mediated effects. To accomplish this, litters resulting from the second mating of P_2 rats (F_{2b} pups) were randomly divided into two subgroups within each dose group on postnatal day 0. From day 0 to day 14, the treatment regimen and evaluation of the rats remained unchanged. On days 14 to 21, all dams and their litters were removed from their respective diets and placed on untreated feed. All dams were given zoxamide (suspended in corn oil and dosed at a constant volume of approximately 5 ml/kg) at a dose of 0, 50, 250 or 1000 mg/kg bw per day (corresponding to 0, 1000, 5000, or 20 000 ppm, respectively) daily by oral gavage. Pups in litters assigned to one subgroup (at each dose) remained untreated during this period. Pups in litters assigned to the second subgroup (at each dose) were dosed daily by oral gavage at the same dose as their dams. All dams were placed back on treated feed on day 21 and maintained on treated feed for 3–5 weeks until terminal necropsy.

Body weight, feed consumption, and clinical signs were monitored in parental animals throughout treatment. Estrous cycling was evaluated in P_1 and P_2 females for 3 weeks before initial mating. Male parental rats were killed and necropsied after the second mating. Female parental rats were killed and necropsied after weaning of their second litters. Sperm evaluation was performed for all P_1 and P_2 males at the time of necropsy. Selected tissues (including reproductive tissues) were weighed from all P_1 and P_2 rats at necropsy. Histopathological evaluation was performed for all selected tissues for rats in the control group and at the highest dose, and rats found dead or sacrificed during the course of the study. Reproductive tissues were also examined in all rats suspected of reduced fertility.

Survival and growth of offspring were monitored throughout lactation. Litters were culled to eight pups (four males and four females per litter where possible) on day 4 of lactation. Stillborn pups, pups that died during lactation, pups culled at day 4, or sacrificed at weaning were examined grossly. Selected tissues were collected, weighed and preserved from F_{1a}, F_{1b}, F_{2a}, and F_{2b} litters at weaning (one male and one female per litter) for possible histopathology. Microscopic evaluation was performed on spleens of the F_{1a}, F_{2a} and F_{2b} weanlings (both subgroups) and on stomachs of the F_{2a} and F_{2b} weanlings. Sexual maturation (age at preputial separation in males, vaginal patency in females) was evaluated in F_{1a} offspring selected as P_2 parental animals.

The achieved intakes of zoxamide for the P_1 and P_2 parents during the pre-mating period were 71–100, 360–489 and 1474–2091 mg/kg bw per day, in males and 82–108 , 409–534 , and 1624–2239 mg/kg bw per day, in females at 1000, 5000 or 20 000 ppm, respectively. Similar intakes were achieved during gestation; with intakes during lactation being higher than during the pre-mating

phase. The study was certified to be compliant with GLP and satisfied the essential criteria of OECD guideline 416.

There were no treatment-related deaths or clinical signs of systemic toxicity. At 20 000 ppm, a treatment-related decrease in cumulative body-weight gain was noted in P_1 rats during the first week of treatment in both sexes and the decrease was evident throughout the pre-mating period in P_1 females. Female (P_1) feed consumption was decreased at 20 000 ppm during the first week of treatment, but there were no consistent effects on food consumption over the whole study. There were no treatment-related effects on estrous cycling nor on sperm motility, morphology, epididymal sperm count or concentration, or testicular sperm count and concentration of P_1 or P_2 males. Reproductive performance, gestation and birth parameters were similar in all groups. The viability of offspring at birth and the ratio of male to female pups in either generation did not show any treatment-related differences. There was a slight delay in sexual maturation in F_{1a} animals at the highest dose (approximately 1 day), but this was considered to be associated with lower body weights (Table 15).

Gross examination and histopathology of P_1 and P_2 rats did not reveal any treatment-related abnormalities. Statistically significant increases in relative liver weight were seen in males and females at 5000 and 20 000 ppm, but these findings were not considered to be adverse as there were no associated gross or histopathological changes.

There were no treatment-related gross findings in F_{1a}, F_{1b}, F_{2a} or F_{2b} pups. There was an apparent decrease in body-weight gain in the F_{1a}, F_{1b}, and F_{2a} pups at all doses, particularly during the latter stages of lactation (Table 14), which was accompanied by a decrease in spleen weights and histopathological changes in the spleen (decreased extramedullary hematopoiesis) (Table 16). These effects were not evident in the F_{2b} litters that were placed on untreated diets from day 14 to 21 of lactation and dosed by gavage during that time with the equivalent dose of zoxamide. In these litters, no effects on body weight, changes in spleen weight or histopathology of the spleen were seen (Tables 15 and 16). The decrease in body weight seen in the F_{1a}, F_{1b} and F_{2a} litters was judged to be a secondary effect related to the palatability of the treated diets and not a systemic toxic effect of zoxamide. The effects noted in the spleen (F_{1a}, F_{1b}, F_{2a}) were considered to be potentially secondary to the decreased body-weight gain since these effects were not seen in the F_{2b} litters where body-weight gain was not affected although an equivalent dose was given by gavage.

There were no adverse reproductive effects in rats given diets containing zoxamide. Some reductions in pup body-weight gain, spleen weight and spleen pathology were seen, but the evidence indicated that these were potentially related to palatability as they were not present in the F_{2b} litters that received equivalent doses of zoxamide via gavage rather than in the diet. A slight delay in sexual maturation was seen in pups, but this was considered to be secondary to the reduced body weight. An increase in relative liver weight was noted at doses of 5000 ppm and greater in both sexes and in absolute liver weight in males only at 20 000 ppm. These effects were not associated with any histopathological changes and appear to be consistent with the metabolic burden associated with high doses of a xenobiotic rather than direct toxicity of zoxamide.

The NOAEL for reproductive toxicity, parental toxicity and toxicity in pups was 20 000 ppm (equal to 1474–2091 mg/kg bw per day in males and 1624–2239 mg/kg bw per day in females), the highest dose tested. The findings seen in the study were not considered to be adverse effects of treatment with zoxamide (O'Hara et al., 1998).

(b) Developmental toxicity

Rats

Groups of 25 mated female rats were given zoxamide (purity, 94.2%) at a dose of 0 (control), 100, 300, or 1000 mg/kg bw per day in corn oil by gavage on days 6–15 of presumed gestation. All rats were examined daily for signs of ill health or reaction to treatment. Body weight and feed

Table 15 Pup body weights during lactation in a two-generation study of reproductive toxicity in rats exposed to zoxamide

Mean weights (g)	Dietary concentration (ppm)			
	0	1000	5000	20 000
P_1/F_{1a}				
Pup weight:				
Day 0	6.6	6.7	6.7	6.6
Day 7	18.3	17.8	18.5	17.4
Day 14	37.8	35.9	37.3	35.2*
Day 21	60.2	54.5*	54.8*	51.0*
P_1/F_{1b}				
Pup weight:				
Day 0	6.4	6.4	6.5	6.3
Day 7	17.6	17.2	16.5	16.6
Day 14	36.0	34.6	34.6	33.8
Day 21	58.3	54.4	52.8*	51.2*
P_2/F_{2a}				
Pup weight [litter weight]:				
Day 0	6.4 [86]	6.3 [92]	6.2 [91]	6.2 [91]
Day 7	17.3 [137]	16.2 [126]	15.8 [117]	15.6* [123]
Day 14	35.4 [280]	34.1 [252]	33.7 [236]	32.4* [243]
Day 21	55.9 [442]	52.1* [386]	49.0* [343]	47.8* [358]
P_2/F_{2b} [a]				
Pup weight [litter weight]:				
Day 0	6.6 [80]	6.3 [92]	6.5 [92]	6.2 [95]
Day 4 – before culling	11.2 [131]	10.0* [140]	9.6* [126]	9.3* [140]
Day 4 – after culling	11.2 [77]	10.0* [76]	9.6* [74]	9.3* [74]
Day 7	18.1 [125]	16.1* [121]	15.7* [113]	15.3* [119]
Day 14	36.1 [242]	35.0 [220]	34.7 [230]	33.4* [247]
Day 14 – pups treated by gavage	35.0	34.1	34.3	33.1
Day 14 – pups on control diet	37.2	36.0	35.1	33.7
Day 21 – pups treated by gavage	57.3	55.3	56.2	54.1
Day 21 – pups on control diet	60.0	56.9	57.6	55.3

From O'Hara et al. (1998)

* Significant difference from control ($p<0.05$); ANOVA, Dunnett's t-test

[a] Rats were exposed to diets containing zoxamide until day 14 of lactation (postpartum). From day 14 to day 20 of lactation, doses of 0, 50, 250 or 1000 mg/kg bw per day (corresponding to dietary concentrations of 0, 1000, 5000, or 20 000 ppm, respectively) were administered by gavage. All dams received the appropriate dose by gavage. Pups in half of the litters received the dose by gavage; pups in the remaining litters were not treated during this period.

Table 16. Spleen weights and histology for pups in a two-generation study of reproductive toxicity in rats exposed to zoxamide

Generation	Finding	Dietary concentration (ppm)			
		0	1000	5000	20 000
F_{1a}	*Males*				
	Absolute spleen weight (g)	0.283	0.223*	0.236*	0.205*
	Relative spleen weight (%)	0.473	0.415*	0.420*	0.394*
	Reduced extramedullary haematopoiesis (No. affected/No. examined)	0/24	6/26*	7/24*	9/26*
	Females				
	Absolute spleen weight (g)	0.286	0.248*	0.226*	0.208*
	Relative spleen weight (%)	0.489	0.461	0.424*	0.408*
	Reduced extramedullary haematopoiesis (No. affected/No. examined)	1/24	5/25	12/25*	15/27*
F_{1b}	*Males*				
	Absolute spleen weight (g)	0.263	0.242	0.220*	0.203*
	Relative spleen weight (%)	0.442	0.431	0.404	0.385*
	Females				
	Absolute spleen weight (g)	0.267	0.235	0.216*	0.199*
	Relative spleen weight (%)	0.457	0.442	0.419	0.395*
F_{2a}	*Males*				
	Absolute spleen weight (g)	0.253	0.229	0.217*	0.172*
	Relative spleen weight (%)	0.439	0.425	0.425	0.354*
	Reduced extramedullary haematopoiesis	1/23	5/24	6/24	13/22*
	Females				
	Absolute spleen weight (g)	0.267	0.234	0.211*	0.184*
	Relative spleen weight (%)	0.479	0.460	0.429*	0.380*
	Reduced extramedullary haematopoiesis	0/23	5/24	12/23*	12/22*
F_{2b}	*Males (dams and pups dosed by gavage)*				
	Absolute spleen weight (g)	0.269	0.280	0.270	0.252
	Relative spleen weight (%)	0.457	0.534	0.481	0.448
	Reduced extramedullary haematopoiesis	0/10	—	—	0/11
	Females (dams and pups dosed by gavage)				
	Absolute spleen weight (g)	0.251	0.265	0.273	0.258
	Relative spleen weight (%)	0.453	0.455	0.483	0.481
	Reduced extramedullary haematopoiesis (n=)	0/9	—	—	0/10
	Males (dams only dosed by gavage)				
	Absolute spleen weight (g)	0.290	0.295	0.282	0.285
	Relative spleen weight (%)	0.474	0.484	0.468	0.478
	Reduced extramedullary haematopoiesis (n=)	0/10	—	—	1/11
	Females (dams only dosed by gavage)				
	Absolute spleen weight (g)	0.286	0.266	0.283	0.267
	Relative spleen weight (%)	0.483	0.485	0.510	0.476
	Reduced extramedullary haematopoiesis (n=)	0/9	—	—	0/11

From O'Hara et al. (1998)
* Significant difference from controls ($p < 0.05$);

consumption were monitored throughout gestation. Dams were killed on day 20 of presumed gestation, and subjected to a gross necropsy and uterine examination. Each live fetus was removed, weighed individually, and examined for external abnormalities. One half of each litter was examined for soft-tissue and head alterations; skeletal examinations were performed on all fetuses. The study was certified to be compliant with GLP and satisfied the essential criteria of OECD guideline 414.

There were no treatment-related mortalities, or clinical signs of toxicity at any dose. Maternal body weight, body-weight gain, feed consumption or gravid uterine weights showed no treatment-related differences. Pregnancy rates were > 90% in all groups. There were no dams aborting or treatment-related effects on the numbers of early or late resorptions, live fetuses per litter, fetal body weight or sex ratio. There were no notable increases in external, soft-tissue, head or skeletal malformations, variations, or developmental retardations observed at any dose.

The NOAEL was 1000 mg/kg bw per day for maternal and fetotoxicity; on the basis of the absence of effects at 1000 mg/kg bw per day, the highest dose tested. Under the conditions of this study of developmental toxicity in rats, zoxamide was not teratogenic (Kane & Shuey, 1995).

Rabbits

Groups of 16 artificially-inseminated New Zealand White rabbits were given zoxamide (purity, 92.3%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day suspended in 0.5% aqueous methylcellulose solution by gavage on days 7–19 of presumed gestation. All doses were administered in a constant volume of 20 ml/kg. Clinical signs were recorded routinely. The does were weighed on days 0, 7, 9, 11, 14, 17, 20 and 29; feed consumption was recorded daily. On day 29, the does were killed and the thoracic and abdominal cavities were examined for gross changes and a full uterine examination was performed. All live fetuses were sexed, weighed and examined for external and visceral alterations (Staples' technique). After the visceral examinations, fetuses were stained with Alizarin Red S and examined for skeletal alterations. The study was certified to be compliant with GLP and satisfied the essential criteria of OECD guideline 414.

There were no treatment-related deaths or clinical signs of toxicity in does. No treatment-related effects were noted for maternal body weight, body-weight change, feed consumption or gravid uterine weights at any dose. No treatment-related gross lesions were observed in does during post-mortem examinations at any dose. Pregnancy rates were 100% in all groups; no dams aborted. The number of viable litters, mean numbers of resorptions, live or dead fetuses, and sex ratio per litter did not reveal any treatment-related intergroup differences. No treatment-related differences were observed in fetal body weights. External, visceral and skeletal examinations of fetuses did not reveal any treatment-related abnormalities; all findings were typical of the background observations in studies of developmental toxicity in rabbits.

The NOAEL for fetotoxicity and maternal toxicity with zoxamide in rabbits was 1000 mg/kg bw per day, the highest dose tested, on the basis of the absence of treatment-related toxicity in dams or fetuses. Zoxamide was not teratogenic in rabbits (Shuey, 1997).

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity, groups of 10 male and 10 female Crl:CD®BR rats were given zoxamide (purity, 92.9% active ingredient) at a dose of 0 (control), 125, 500 or 2000 mg/kg bw suspended in corn oil by gavage in a volume of 10 ml/kg). All rats were observed daily for signs of ill health or reaction to treatment. Each rat received a pre-test functional observational battery (FOB) and motor activity assessment 7 days before dosing. FOB and motor activity testing was repeated approximately 5 h after dosing (day 0) and on days 7 and 14 after dosing. On the day after the final

FOB/motor activity assessment, rats were anaesthetized, perfused with neutral buffered formalin and given a limited gross necropsy. Twelve randomly selected control rats (six males and six females) and twelve randomly selected rats at the highest dose (six males and six females) received a special neuropathology evaluation that included microscopic examination of the brain, spinal cord, peripheral nerves of the hindlimb and selected ganglia. It was claimed that the study was compliant with GLP and met the basic requirements of EPA guideline 81-8.

Dosing solutions were confirmed to be within 3% of nominal values. There were no mortalities, treatment-related clinical signs of toxicity or body-weight effects observed during the study period. There were no treatment-related effects on motor activity or any of the FOB parameters. Motor activity was lower in males at the highest dose on the day of dosing, but as this did not achieve statistical significance and was not evident in females this was not considered to be an adverse effect of treatment. No treatment-related morphological alterations occurred in any of the examined areas of the central or peripheral nervous systems.

The NOAEL for general toxicity and neurotoxicity was 2000 mg/kg bw, the highest dose tested (Danberry & Gillette, 1997).

A study of neurotoxicity/general toxicity with repeated doses of zoxamide in rats is reported in section 2.3.

(b) Mechanism of action

A range of studies have been performed (Young, 1998) in order to characterize the antifungal mechanism of action of zoxamide and the potential relevance to mammals. These have included investigations in fungi, plant cells, mammalian cells and on isolated tubulin. In some studies, a compound encoded RH-54032 was also investigated: RH-54032 is a dichlorobenzamide compound like zoxamide, but without the 4-methyl group on the aromatic ring.

Investigations on the effects of zoxamide and RH-54032 on morphology and nuclear division in *Phytophthora capsici* showed that the encystment of *P. capsici* zoospores and germination were unaffected by treatment with zoxamide at high concentrations (25 ppm, 74.3 µmol/l) and RH-54032 (25 ppm, 77.5 µmol/l). However, germ-tube elongation was completely arrested shortly after germination even by treatment at very low concentrations (zoxamide at 0.5 ppm, 1.49 µmol/l), and this was accompanied by swelling of the germ tube. Mithramycin staining of treated cells revealed that the first cycle of nuclear division, which occurred in untreated cells at between 1 and 2.5 h, failed to occur in the presence of zoxamide or RH-54032. Treated cells contained a single nucleus located in the cyst whereas untreated cells contained multiple nuclei, which migrated into the growing germ tubes. When the effects of zoxamide and RH-54032 at various concentrations on the first cycle of nuclear division were compared with their inhibition of radial growth in poison-agar assays, it was shown that concentrations that inhibited growth strongly inhibited nuclear division. Inhibition of nuclear division was found to occur very rapidly when zoxamide, RH-54032 or the anti-microtubule agent colchicine were added to germlings at the onset of the first cycle of nuclear division.

In an investigation of the effect of RH-54032 on mitosis in mouse lymphoma cells, RH-54032 inhibited the growth of mouse lymphoma cells, and at concentrations that inhibited growth produced an accumulation of cells in arrested metaphase as reflected by an increase in the mitotic index. Normal mitotic figures were absent in treated cells, and the cells contained scattered chromosome pairs that failed to align at the equatorial metaphase plate. RH-54032 was considerably less potent than colchicine: RH-54032 produced a mitotic index of 27.4 at 2 µmol/l (0.642 ppm), while colchicine produced a mitotic index of 45.6 at 0.125 µmol/l (0.05 ppm).

In investigations on the effects of RH-54032 and zoxamide on the assembly of microtubules in vitro, RH-54032 and zoxamide inhibited the assembly of bovine tubulin into microtubules in vitro in a dose-dependent manner. In these experiments, purified bovine tubulin was incubated with the

anti-tubulin compounds at 37 °C for an appropriate time, then cooled on ice. Microtubule assembly was initiated by addition of guanosine triphosphate (GTP) and incubation at 37 °C. The ability of anti-microtubule agents to inhibit microtubule assembly was characterized by the need for a relatively long pre-incubation period with tubulin before initiation of assembly, indicating a low potency.

A comparison of RH-54032, zoxamide, and other anti-microtubule agents with respect to their ability to inhibit microtubule assembly in vitro and the growth of mouse lymphoma cells is shown in Table 17. EC_{50} values for inhibition of growth of mouse lymphoma cells by RH-54032, zoxamide and carbendazim fell in the range of 1.1 to 9.0 µmol/l), while colchicine and taxol were more than two orders of magnitude more potent, and vinblastine was more than three orders of magnitude more potent. RH-54032, zoxamide and carbendazim were also less potent inhibitors of microtubule assembly in vitro than were colchicine and vinblastine.

Binding of RH-54032 to isolated bovine tubulin was shown to involve the β-subunit of tubulin, in agreement with results from labelling experiments using whole cells. Further information about the anti-tubilin benzamide binding site on tubulin was obtained by testing the effect of other anti-microtubule agents on binding of [^{3}H]RH-54032 to bovine tubulin. Binding was strongly inhibited by colchicine, podophyllotoxin and the benzimidazole nocodazole, while vinblastine had little effect. Since it is known that colchicine, podophyllotoxin and benzimidazoles bind to a common site on β-tubulin, while vinblastine binds to a different region, these results are consistent with binding of antitubilin benzamides to the β-tubulin subunit (Young, 1998).

3. Observations in humans

No specific surveys of personnel of manufacturing plants were available. There were two adverse reports linked to exposure to a diluted formulation of mancozeb/zoxamide. One relates to dermal irritation, the other to non-specific "flu-like" symptoms. The Meeting considered it to be unlikely that these findings were directly related to exposure to zoxamide.

Comments

Biochemical aspects

In rats given zoxamide, approximately 60% of a dose of 10 mg/kg bw was absorbed, with peak plasma concentrations of radioactivity occurring at 8 h after dosing. Zoxamide was extensively distributed among organs and tissues with highest concentrations reported in the liver.

Table 17. Inhibition of microtubule assembly and growth of mouse lymphoma cells by RH-54032, zoxamide and other antimicrotubule agents

Compound	Growth of mouse lymphoma cells		Microtubule assembly	
	EC_{50} (µmol/l)	EC_{50} (ppm)	IC_{50} (µmol/l)	IC_{50} (ppm)
Zoxamide	3.7	1.24	23.5	7.90
RH-54032	1.1	0.35	9.0	2.89
Colchicine	0.01	0.004	1.0	0.40
Taxol	0.007	0.006	NT	NT
Vinblastine	0.0008	0.0007	1.8	1.64
Carbendazim	9.0	1.72	29.2	5.58

From Young (1998)
EC_{50}, concentration having an effect on half the sample; IC_{50}, concentration inhibiting half the sample; NT, not tested.

Excretion was primarily in the faeces, via the bile. The overall elimination half-life was 13–14 h. At 1000 mg/kg bw, there was some evidence of saturation of absorption, with C_{max} and area under the curve of concentration–time (AUC) values being approximately 40–50 times those at 10 mg/kg bw, but with a similar elimination half-life. Females excreted approximately twice as much radiolabel in the urine as did males. Very little radioactivity remained in tissues (< 0.2% of the administered dose) or carcass (< 2% of the administered dose) at 5 days after dosing. Pre-treatment of animals with diets containing zoxamide for 2 weeks or with five daily gavage doses of radiolabelled zoxamide did not significantly alter the absorption or distribution of radiolabel compared with that in untreated animals.

The metabolism of zoxamide was extensive, involving a variety of pathways including hydrolysis, glutathione-mediated reactions, and reductive dehalogenation, secondary oxidation on both the aromatic methyl and the aliphatic side-chain, limited deamidation; and terminal glucuronic acid and amino-acid conjugation. Thirty-two separate metabolites were identified; no single metabolite accounted for more than 10% of the administered dose. After repeated doses, there was an indication of an increase in glutathione-mediated metabolism.

Toxicological data

Zoxamide was of low acute toxicity when administered orally (median lethal dose, LD_{50}, > 5000 mg/kg bw), dermally (LD_{50}, > 2000 mg/kg bw) or after a 4-h exposure by inhalation (LC_{50}, > 5.3 mg/l). Zoxamide is not a skin irritant, but is a slight, transient eye irritant. Zoxamide produced delayed contact hypersensitivity in guinea-pigs in the maximization and Buehler tests.

In repeat-dose studies, the main effects of zoxamide were reduced body-weight gain and liver hypertrophy. The reductions in body-weight gain were not consistent across studies. Investigative work performed as part of the study of reproductive toxicity indicated there might be palatability problems with diet containing zoxamide. However, food consumption was not reduced consistently in studies in which reduced body-weight gain was reported. Liver hypertrophy was not associated with any histopathological or clinical chemistry changes that indicated damage to liver cells. Therefore, in line with the guidance developed by the 2006 JMPR, increased liver weight and hepatocyte hypertrophy were considered to be adaptive rather than adverse effects of exposure to zoxamide.

In a 90-day study of toxicity in mice, the NOAEL was 2500 ppm, equal to 574 mg/kg bw per day, on the basis of reduced body-weight gains in females at 7000 ppm, equal to 1606 mg/kg bw per day. Increases in relative liver weights (by approximately 10%) were not associated with any pathological or clinical chemistry changes and are not considered to be adverse. In a 90-day study of toxicity and neurotoxicity in rats, the NOAEL was 20 000 ppm, equal to 1509 mg/kg bw per day, the highest dose tested.

In a 28-day study of toxicity in dogs, the NOAEL was 30 000 ppm, equal to 1045 mg/kg bw per day, the highest dose tested. Soft stools were present at an increased incidence at doses of 5000 ppm, equal to 175 mg/kg bw per day, and above, but as this finding was not seen consistently in other studies in dogs given similar doses and the same formulated diet, this finding was not considered to be an adverse effect of treatment. In the 90-day study of toxicity in dogs, the NOAEL was 7500 ppm, equal to 281 mg/kg bw per day, on the basis of reductions in body-weight gain, serum albumin concentrations and erythrocyte counts in both sexes at 30 000 ppm, equal to 1055 mg/kg bw per day. Increases in liver weights (by approximately 25%) in females at 7500 ppm were not associated with any histopathological or clinical chemistry changes and were not considered to be adverse. In the 1-year study of toxicity in dogs, reduced body-weight gain (45%) was present from the beginning of the study in females at 7500 ppm, equal to 255 mg/kg bw per day, and a deficit in body-weight gain (20%) was still present at the end of the study. Males receiving zoxamide at 7500 ppm also had reduced body-weight gain during the early stages of the study, but these animals had terminal body weights that

were higher than those of the controls. Although food consumption was reduced transiently, there was no clear link between body weights of individual animals and food consumption. At the highest dose of 30 000 ppm, there were marked effects on body weight and food consumption, with females taking up to 7 weeks to regain their pre-test body weight. Reduced concentrations of serum albumin, and increases in liver and thyroid weights and serum alkaline phosphatase activities were also seen in both sexes at 30 000 ppm. The NOAEL in the 1-year study was 1500 ppm, equal to 48 mg/kg bw per day.

In the 90-day and 1-year studies in dogs, cases of CJPS were seen in the groups receiving zoxamide, but not in the controls. CJPS is reported to be specific to beagle dogs, occurring spontaneously but with unknown etiology. A genetic link has been postulated, which might explain the occurrence in the 90-day and 1-year studies, which were started at the same time and used animals from the same supplier. Therefore, CJPS was not considered to be related to exposure to zoxamide.

In a 28-day study of dermal toxicity in rats, zoxamide produced significant local effects at doses of 107 mg/kg bw per day and greater. Findings of systemic toxicity were most likely to be secondary to the local effects and the NOAEL for systemic effects was 714 mg/kg bw per day

Negative results were obtained in assays for gene mutation in vitro and in assays for micronucleus formation in bone marrow of rats and mice in vivo. Zoxamide was found to induce polyploidy in an assay for chromosomal aberration in Chinese hamster ovary cells in vitro. These findings are consistent with the mechanism of fungicidal action of zoxamide, involving binding to the β-subunit of tubulin. Zoxamide also inhibits microtubule assembly in mouse lymphoma cells (IC_{50}, 23.5 µmol/l). The induction of polploidy after inhibition of tubulin polymerization and disruption of microtubule formation has been investigated for other compounds and is considered to be a threshold-mediated effect. The assay for micronucleus formation in rats included kinetochore staining and produced negative results for micronuclei and chromosomal damage. A supplementary kinetic study in mice demonstrated that there was exposure of the bone marrow after administration of zoxamide.

The Meeting concluded that zoxamide was unlikely to pose a genotoxic risk to humans at levels typical of dietary exposures.

In long-term studies of toxicity in mice and rats, zoxamide exhibited no general toxicity and was not carcinogenic in either species. Increased liver weights (approximately 20%) in female rats killed after a 1-year exposure to zoxamide at a dietary concentration of 5000 ppm and greater were not considered to be adverse as there were no associated histopathological or clinical chemistry findings at any time during the study. An apparent increase in thyroid C-cell lesions in male rats at the highest dose was not statistically significant, did not exhibit a dose–response relationship, was not reproduced in females and was within the range for historical controls. The NOAEL in mice was 7000 ppm, equal to1021 mg/kg bw per day, and the NOAEL in rats was 20 000 ppm, equal to 1058 mg/kg bw per day, both values being identified on the basis of the absence of treatment-related toxicity at the highest doses tested.

In view of the absence of carcinogenic potential in rodents and the lack of genotoxicity in vivo, the Meeting concluded that zoxamide was unlikely to pose a carcinogenic risk to humans.

The reproductive toxicity of zoxamide has been investigated in a two-generation study in rats and studies of developmental toxicity in rats and rabbits. In the study of reproductive toxicity in rats, the NOAEL for effects on fertility, parental toxicity and pup development was 20 000 ppm, equal to 1474 mg/kg bw per day. Reductions in pup body-weight gain and spleen weights and reduced extramedullary haematopoiesis in the spleen were seen in F_{1a} F_{1b} and F_{2a} offspring, but these effects appeared to be related to palatability as they were not evident in the F_{2b} generation, when pups and dams received equivalent exposures of zoxamide by gavage, rather than from the diet, from postnatal

days 14 to 21. Increased relative liver weight was noted at doses of 5000 ppm and greater in males and females, and in absolute liver weight only in males at 20 000 ppm. The changes in liver weight were not associated with any histopathological or clinical chemistry change and were not considered to be adverse.

There was no evidence of toxicity in the studies of prenatal developmental toxicity in rats or rabbits. The NOAEL in both studies was 1000 mg/kg bw per day on the basis of absence of toxicity to dams or fetuses at the highest dose tested. Zoxamide was not teratogenic in rats or rabbits.

Zoxamide was not neurotoxic in a study of acute neurotoxicity at doses of up to 2000 mg/kg bw. No adverse effects were seen during neurological and behavioural examinations performed during routine repeat-dose studies with zoxamide.

Studies on two plant metabolites of zoxamide—RH-141,452 (3,5-dichloro-4-hydroxymethyl benzoic acid) RH-141,455 (3,5-dichloro-1,4-benzene-dicarboxylic acid)—formed to a limited extent in rats, showed them to be rapidly absorbed and rapidly excreted, essentially unchanged; to have low acute oral toxicities to mice (LD_{50}s, > 5000 mg/kg bw), and to give negative results in assays for gene mutation with strains of *Salmonella typhimurium*.

There were two reports of mild adverse effects after exposure to a diluted formulation containing zoxamide and mancozeb. In one case there was a report of skin irritation, in the other "flu-like" symptoms were reported. The Meeting considered it to be unlikely that these effects were related directly to exposure to zoxamide.

Toxicological evaluation

An acceptable daily intake (ADI) of 0–0.5 mg/kg bw was established for zoxamide based on the NOAEL of 48 mg/kg bw per day in the 1-year study in dogs, on the basis of reduced body-weight gain in females at 255 mg/kg bw per day.

An acute reference dose (ARfD) was considered to be unnecessary for zoxamide as zoxamide is of low acute toxicity, did not produce developmental effects and did not produce any other significant effects after acute exposures.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	7000 ppm, equal to 1021 mg/kg bw per day[c]	—
		Carcinogenicity	7000 ppm, equal to 1021 mg/kg bw per day[c]	—
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	20 000 ppm, equal to 1058 mg/kg bw per day[c]	—
		Carcinogenicity	20 000 ppm, equal to 1058 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Reproductive toxicity	30 000 ppm, equal to 1474 mg/kg bw per day[c]	—
		Parental toxicity	30 000 ppm, equal to 1474 mg/kg bw per day[c]	—
		Offspring toxicity	30 000 ppm, equal to 1474 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day[c]	—
		Embryo/fetotoxicity	1000 mg/kg bw per day[c]	—

	Acute neurotoxicity[b]		2000 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[a]	Maternal toxicity	1000 mg/kg bw per day[c]	—
		Embryo/fetotoxicity	1000 mg/kg bw per day[c]	—
Dog	One-year study of toxicity[a]	Reduced body-weight gain	1500 ppm, equal to 48 mg/kg bw per day	7500 ppm, equal to 255 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.

Estimate of acceptable daily intake for humans

0–0.5 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Moderate (C_{max}, 8 h); approximately 60% absorbed at 10 mg/kg bw
Dermal absorption	Approximately 1% from concentrate; 6% from dilution
Distribution	Extensive. Highest levels in liver.
Potential for accumulation	Low
Rate and extent of excretion	> 85% in 48 h. Urine (approximately 10–20%); bile (approximately 45%); faeces (approximately 50–80%).
Metabolism in animals	Extensive. Primarily via hydrolysis, dehalogenation, oxidation and conjugation.
Toxicologically significant compounds in animals, plants and the environment	Zoxamide.
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.3 mg/l
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Slight transient irritant
Guinea-pig, skin sensitization (test method used)	A skin sensitizer (Buehler; Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Body-weight gain
Lowest relevant oral NOAEL	1500 ppm (48 mg/kg bw per day) in a 1-year study in dogs
Lowest relevant dermal NOAEL	< 107 mg/kg bw for local effects; 714 mg/kg bw per day for systemic effects.

Lowest relevant inhalation NOAEC	No data (not required)
Genotoxicity	
	Not genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	None
Lowest relevant NOAEL	7000 ppm (1021mg/kg bw per day) in mice (highest dose tested)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	20 000 ppm (1047 mg/kg bw per day) in rats (highest dose tested)
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	1000 mg/kg bw per day in rats and rabbits (highest dose tested)
Neurotoxicity/delayed neurotoxicity	
	No indications of neurotoxicity in studies of acute toxicity or repeat-doses
Acute neurotoxicity	NOAEL was 2000 mg/kg bw in rats (highest dose tested)
Other toxicological studies	
	RH-141,452 Rapid excretion; essentially unmetabolized. Oral LD_{50} in mice, > 5000 mg/kg bw Negative in an Ames test. RH-141,455 Rapid excretion; essentially unmetabolized. Oral LD_{50} in mice, > 5000 mg/kg bw Negative in an Ames test
Medical data	
	Two reports (one case of irritation & one of flu-like symptoms) after exposure to a diluted formulation of mancozeb/zoxamide. Unlikely to be directly related to zoxamide.

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.5 mg/kg bw	Dog, 1-year study	100
ARfD	Unnecessary	—	—

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