

TRIFLUMIZOLE

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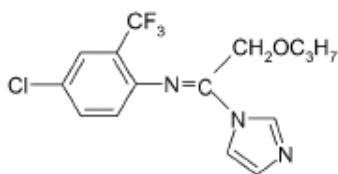
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Explanation

Triflumizole (Fig. 1) is the common name provisionally approved by the International Organization for Standardization for (*E*)-4-chloro- α,α,α -trifluoro-*N*-(1-imidazol-1-yl-2-propoxyethylidene)-*o*-toluidine (International Union of Pure and Applied Chemistry), for which the Chemical Abstracts Service number is 68694-11-1. Triflumizole is a fungicide used for the control of powdery mildew, such as *Sphaerotheca fuliginea*, *Sphaerotheca pannosa*, *Erysiphe cichoracearum* and others. As a consequence of ergosterol biosynthesis inhibition, spore germination, mycelial growth and the spread of the fungi within the plants are inhibited.

Fig. 1. Structure of triflumizole (NF-114)

Triflumizole has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All studies evaluated in this monograph were performed by good laboratory practice (GLP)–certified laboratories and complied with the relevant Organisation for Economic Co-operation and Development (OECD) and/or United States Environmental Protection Agency test guidelines, unless indicated otherwise.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

The absorption, distribution and excretion of triflumizole in Sprague-Dawley rats following oral administration have been assessed in a quantitative single low-dose study, a single high-dose study and a 14-day repeated-dose study (Table 1). The experiments were performed by dosing rats with a mixture of (phenyl-U-¹⁴C)-labelled triflumizole and triflumizole technical, which was subsequently diluted with dimethyl sulfoxide (DMSO). No specific information is available on biliary excretion, as bile duct–cannulated rats were not used.

Table 1. Overview of absorption, distribution and excretion studies

	Single low dose	Repeated low dose	Single high dose
Dose (mg/kg bw)	10	10 (days 1–13 unlabelled test substance, labelled test substance on day 14)	300
No. of animals	5 M, 5 F	5 M, 5 F	5 M, 5 F
Route	Oral, gavage	Oral, gavage	Oral, gavage
Purity			
- Radiochemical	98%	98%	98%
- Unlabelled	99.8%	98.3%	98.7%
Study duration	2 days	16 days	4 days
GLP	No	Yes	No
Reference	Soeda (1983)	Soeda & Mizuno (1988)	Soeda (1984)

bw: body weight; F: female; GLP: good laboratory practice; M: male

In a preliminary experiment with two male rats in which ¹⁴CO₂ was trapped, over a period of 4 days, approximately 0.07% of the administered dose was recovered from volatiles, approximately 72% from urine, approximately 27% from faeces and approximately 1% from the carcass (recovery approximately 97%). Based on these results, volatiles were not trapped in the main experiment, because the amount of radioactivity recovered from volatiles was considered to be negligible.

During the initial 48 hours (or 96 hours, single high-dose study) after dosing of labelled test substance, blood, urine and faeces were collected for analysis of the radioactivity and identification of metabolites. Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 6, 12 and 24 hours after dose administration and daily thereafter, and urine and faeces were collected at 24-hour intervals. Two days after dose administration, the animals were killed, and various tissues were excised for analysis: fat (adipose tissue), gonads (testes and epididymides from males, ovaries and uterus from females), spleen, kidney, liver, heart, lung, thymus, brain, pancreas, adrenal, thyroid gland, urinary bladder, femoral muscle and femoral bone, a part of dorsal skin, stomach, small intestine, large intestine, blind intestine and the digestive organ contents from each of these, and the remaining body structures ("carcass"). All samples were analysed for radioactivity by (combustion) liquid scintillation counting. Pharmacokinetic analysis was based on a one-compartment model with a first-order rate. Individual 0- to 1-day urine and faecal samples from each sex were pooled and analysed for metabolites. Metabolites present in urine were concentrated, separated in fractions by column chromatography and analysed by thin-layer chromatography (TLC) after being mixed with authentic standards. The standards were detected under ultraviolet light, and the corresponding test spots were analysed for radioactivity by liquid scintillation counting. Metabolites present in faeces were extracted with methanol, concentrated under vacuum (rotary evaporator) and dissolved in water, then analysed as described for urine samples.

There were no clear sex differences in oral absorption. Based on the radiolabel recovered from urine, tissues and carcass 48 hours after administration, the oral absorption following single or repeated dosing with 10 mg/kg body weight (bw) was at least 72% (see Table 2). The oral absorption was at least 72% at 48 hours after administration of a single high dose (300 mg/kg bw), based on radiolabel recovered from urine, and at least 79% 96 hours after administration, based on radiolabel recovered from urine, tissues and carcass. The oral absorption was considerably slower after the high dose than after the low dose, as evidenced by the much longer time to reach the maximum concentration in plasma ($T_{\max}$) (see Table 3). Excretion after 10 mg/kg bw was relatively fast following both single- and repeated-dose treatments, with approximately 90% of the administered radiolabel being excreted in the first 24 hours and about 95% of the radiolabel being excreted by 48 hours. At 300 mg/kg bw, the proportion of the administered dose that was excreted by 24 hours was 45% (males) and 35% (females) of the administered dose, increasing to 99% (males) and 92% (females) at 96 hours. The majority of the radiolabel was excreted via the urinary route: approximately 75% of the administered dose at sacrifice compared with about 20% excreted via the faeces.

The radioactivity detected in tissues and the carcass 48 hours (low dose) or 96 hours (high dose) after administration was 2% in both male and female rats (Tables 4–6). In all instances, the highest concentration of radiolabel was in the liver (approximately 1 mg equivalent [eq]/kg after the low dose in both sexes; 14.5 and 8.5 mg eq/kg after the high dose in males and females, respectively). Retention in all other tissues and organs was approximately 50% or less of the concentration detected in the liver, with well perfused tissues tending to have higher concentrations. Fat was among the tissues retaining the lowest concentration of radiolabel: approximately one tenth of the liver concentration. It should be noted that the brain retained relatively high concentrations (one third to one half of the liver concentration). This was also the case with the thyroid; however, this organ was measured only in the single low-dose group.

1.2 Biotransformation

Rats

The metabolism of triflumizole was investigated by analysing faecal and urine samples retained from the previous studies: single oral administration of 10 or 300 mg/kg bw (Soeda, 1983, 1984) and 14 consecutive oral doses of 10 mg/kg bw per day (Soeda & Mizuno, 1988).

Table 2. Cumulative excretion and retention of total radioactivity following oral dosing with triflumizole in rats

Fraction	Cumulative excretion/retention (% of administered radiolabel) in males/females						
	0–24 h		0–48 h			0–96 h ^a	
	Single oral 10 mg/kg bw	Repeated oral 10 mg/kg bw per day	Single oral 300 mg/kg bw	Single oral 10 mg/kg bw	Repeated oral 10 mg/kg bw per day	Single oral 300 mg/kg bw	Single oral 300 mg/kg bw
Urine	71/70	72/66	36/29	74/75	74/70	77/72	79/77
Faeces	18/18	20/21	9/6	19/19	22/22	19/13	21/15
<i>Total excreted^b</i>	89/89	92/87	45/35	93/94	96/91	99/85	99/92
Tissues & carcass	NC	NC	NC	2/2	2/2	NC	1/2
<i>Total recovered^b</i>	89/89	92/87	45/35	95/96	98/94	99/85	101/94

NC: not calculated

^a Animals exposed to low dose were sacrificed after 48 hours; those exposed to high dose were sacrificed after 96 hours.^b Due to rounding, totals may differ slightly from additions in the table.

Source: Soeda (1983, 1984); Soeda & Mizuno (1988)

Table 3. Toxicokinetic parameters: plasma

Parameter	Single oral low (males/females)	Repeated oral low (males/females)	Single oral high (males/females)
$T_{\max}$ (h)	1.8/1.7	~1	~24
$C_{\max}$ (µg eq/mL)	3.3/2.4	2.3/2.2	19/21
Approximate terminal half-life (h)	13/15	NC	NC
Volume of distribution (mL)	657/715	NC	NC

 $C_{\max}$: maximum concentration in plasma; eq: equivalent; NC: not calculated; $T_{\max}$: time to reach $C_{\max}$

Source: Soeda (1983, 1984); Soeda & Mizuno (1988)

Table 4. Levels of triflumizole equivalent in rat tissue at 2 days after administration of 10 mg/kg bw (single low dose)

Organ/tissue	Levels (ppm) of triflumizole equivalent (mean ± SE)	
	Males	Females
Plasma	0.26 ± 0.03	0.20 -
Brain	0.42 ± 0.02	0.30 -
Liver	1.22 ± 0.04	0.92 ± 0.06
Kidney	0.50 -	0.26 ± 0.03
Femoral muscle	0.10 -	0.10 -
Femoral bone	0.10 -	0.10 -
Fat	0.10 -	0.02 ± 0.02
Dorsal skin	0.18 ± 0.07	0.18 ± 0.11
Spleen	0.30 -	0.22 ± 0.02
Heart	0.30 -	0.20 -
Lung	0.40 -	0.30 -
Pancreas	0.22 ± 0.02	0.18 ± 0.02

Organ/tissue	Levels (ppm) of triflumizole equivalent (mean $\pm$ SE)	
	Males	Females
Thyroid	0.62 $\pm$ 0.28	0.64 $\pm$ 0.17
Bladder	0.44 $\pm$ 0.11	0.29 $\pm$ 0.08
Thymus	0.10 -	0.10 -
Adrenal	0.36 $\pm$ 0.05	0.30 -
Carcass	0.12 $\pm$ 0.02	0.14 $\pm$ 0.03
Testis	0.16 $\pm$ 0.03	–
Epididymis	0.32 $\pm$ 0.04	–
Ovary	–	0.16 $\pm$ 0.03
Uterus	–	0.10 -
Stomach	0.34 $\pm$ 0.06	0.22 $\pm$ 0.04
Small intestine	0.10 -	0.08 $\pm$ 0.02
Large intestine	0.50 $\pm$ 0.19	0.10 -
Blind intestine	0.70 $\pm$ 0.06	0.06 $\pm$ 0.03
Stomach contents	0.40 $\pm$ 0.26	0.01 -
Small intestinal contents	0.36 $\pm$ 0.05	0.16 $\pm$ 0.05
Large intestinal contents	0.74 $\pm$ 0.09	0.58 $\pm$ 0.08
Blind intestinal contents	0.44 $\pm$ 0.05	0.46 $\pm$ 0.08

- indicates a standard error of < 0.01; ppm: parts per million; SE: standard error

Source: Soeda (1983)

Table 5. Levels of triflumizole equivalent in rat tissue at 2 days after administration of 300 mg/kg bw (single high dose)

Organ/tissue	Levels (ppm) of triflumizole equivalent (mean $\pm$ SE)	
	Males	Females
Plasma	2.04 $\pm$ 0.16	0.90 $\pm$ 0.18
Brain	5.18 $\pm$ 0.41	4.40 $\pm$ 1.30
Liver	14.52 $\pm$ 0.95	8.48 $\pm$ 1.50
Kidney	5.58 $\pm$ 0.25	4.18 $\pm$ 0.42
Femoral muscle	1.20 $\pm$ 0.15	1.04 $\pm$ 0.29
Femoral bone	0.78 $\pm$ 0.08	0.56 $\pm$ 0.07
Fat	1.36 $\pm$ 0.12	1.08 $\pm$ 0.28
Spleen	2.74 $\pm$ 0.26	2.36 $\pm$ 0.56
Heart	3.82 $\pm$ 0.50	2.76 $\pm$ 0.69
Lung	3.52 $\pm$ 0.22	2.44 $\pm$ 0.34
Pancreas	2.46 $\pm$ 0.25	1.92 $\pm$ 0.42
Adrenal	2.90 $\pm$ 0.29	2.76 $\pm$ 0.44
Carcass	2.28 $\pm$ 0.14	4.14 $\pm$ 0.61
Testis	1.46 $\pm$ 0.18	–
Ovary	–	1.24 $\pm$ 0.27
Uterus	–	1.36 $\pm$ 0.51
Digestive organs & their contents	1.82 $\pm$ 0.27	3.02 $\pm$ 1.05

ppm: parts per million; SE: standard error

Source: Soeda (1984)

Table 6. Levels of triflumizole equivalent in rat tissue at 2 days after administration of 10 mg/kg bw (repeated low dose)

Organ/tissue	Levels (ppm) of triflumizole equivalent (mean $\pm$ SE)	
	Males	Females
Plasma	0.18 -	0.13 -
Brain	0.42 $\pm$ 0.02	0.43 $\pm$ 0.04
Liver	1.01 $\pm$ 0.03	1.14 $\pm$ 0.05
Kidney	0.39 -	0.45 $\pm$ 0.02
Femoral muscle	0.08 -	0.10 -
Femoral bone	0.06 -	0.07 -
Fat	0.07 -	0.11 -
Spleen	0.20 -	0.24 $\pm$ 0.02
Heart	0.24 -	0.25 $\pm$ 0.02
Lung	0.31 -	0.32 $\pm$ 0.02
Pancreas	0.18 -	0.19 $\pm$ 0.02
Adrenal	0.27 -	0.28 $\pm$ 0.02
Carcass	0.11 -	0.16 $\pm$ 0.04
Testis	0.12 -	—
Ovary	—	0.14 -
Uterus	—	0.11 -
Digestive organs and their contents	0.28 -	0.33 $\pm$ 0.03

- indicates a standard error of < 0.01 ; ppm: parts per million; SE: standard error

Source: Soeda & Mizuno (1988)

Triflumizole is extensively metabolized: less than 2% of the radiolabel recovered from urine or faeces was identified as parent compound (Table 7). A few differences in metabolite pattern were observed between males and females after repeated low and single high doses, but not after a single low dose. The major urinary metabolites are the sulfate conjugates of FM-8-1 and FA-1-5, each representing approximately 20% of the radiolabel recovered after the single low dose from that matrix and, respectively, approximately 11% and 20% after the high dose. In faeces, FD-2-1 is a major metabolite in all dose regimens (~6–10% of the recovered radiolabel). Considerable differences between dose regimens exist with respect to other major metabolites. FM-2-1 is the major metabolite after a single oral low dose (~9% of radiolabel recovered in faeces), but represents less than 2% for the other dose regimens. FA-1-1 is a major metabolite after single and repeated low dosing (~5–10%), but not after single oral high dosing ($< 3\%$), whereas FD-1-1 is the major metabolite after a single oral high dose (~16%) and a minor metabolite after single and repeated low doses ($< 2\%$). The metabolite FM-6-1 was tentatively identified by TLC co-chromatography, and the radioactive band corresponding to the authentic FM-6-1 was obscure, because of its low radioactivity.

There was also a supplemental, non-GLP study to the main single-dose metabolism studies (Soeda, 1983, 1984). The purpose of this study was to confirm the presence of the metabolite FM-6-1 in rat faeces and urine recovered during the main metabolism studies. Portions of the methanol fraction of 0- to 2-day urine and methanol extract of 0- to 2-day faeces (~74 kBq) in each experimental group or sex of the high and low single-dose studies were combined and purified using silica gel column chromatography, fractioned according to the radioactive chromatograms and analysed by TLC. The fraction corresponding to FM-6-1 was subjected to preparative TLC, and the radioactive band was extracted with acetone and then analysed using gas chromatography–mass spectrometry (GC-MS) and compared with the FM-6-1 standard. The GC-MS results confirmed the presence of FM-6-1 in the urine and faecal samples (Soeda, 1985).

Table 7. Distribution of radiolabel from the parent compound and major metabolites

Compound (code)	% of radiolabel recovered from excreta in males/females								
	Single oral low			Repeated oral low			Single oral high		
	Urine ^a	Faeces ^a	Total ^b	Urine ^a	Faeces ^a	Total ^b	Urine ^a	Faeces ^a	Total ^b
NF-114 (parent compound)	0.7/0.6	1.5/1.5	0.9/0.7	0.1/0.1	0.3/0.4	0.1/0.2	0.8/1.4	0.9/0.8	0.8/1.3
FM-2-1	0.6/0.5	8.3/9.3	2.1/2.3	0.1/0.1	0.5/0.3	0.2/0.1	0.4/0.6	1.8/1.2	0.7/0.6
FM-6-1	0.4/0.3	1.3/1.5	0.6/0.5	0.1/0.2	1.4/1.0	0.4/0.4	0.5/2.1	0.9/1.2	0.5/1.9
FM-7-1	0.3/0.5	1.1/1.0	0.5/0.6	0.1/0.1	0.1/0.2	0.1/0.1	0.1/0.2	0.6/0.4	0.2/0.2
FM-8-1	2.6/2.4	4.2/4.5	2.9/2.8	3.0/2.9	5.6/4.8	3.5/3.2	0.4/0.5	2.2/1.8	0.8/0.7
FM-8-1-S	19/20	4.2/3.6	15/16	19/21	4.4/11	15/18	13/9	1.6/2.3	11/7.5
FD-1-1	0.1/0.2	1.8/1.5	0.5/0.5	0.1/0.1	0.7/0.8	0.2/0.3	3.0/3.2	14/18	5.2/5.4
FD-2-1	1.8/1.4	6.1/5.5	2.6/2.2	1.4/1.7	10/10	3.3/3.6	0.7/1.0	9.8/5.2	2.5/1.7
FD-2-1-S	2.1/2.3	3.1/3.1	2.3/2.5	5.4/5.1	3.0/4.2	4.8/4.8	5.2/4.8	0.7/0.6	4.2/4.0
FD-2-1-G	5.6/6.4	1.3/1.1	4.6/5.2	3.0/4.3	2.2/1.6	2.7/3.5	4.9/1.6	1.5/1.6	4.2/3.2
FD-4-1	0.8/0.8	1.0/1.1	0.8/0.8	0.6/0.6	1.4/1.2	0.7/0.8	0.4/1.7	0.6/1.7	0.5/1.0
FD-6-1	1.0/1.0	3.4/2.2	1.4/1.3	0.6/1.0	5.5/3.7	1.7/1.6	0.8/0.8	4.7/1.9	1.6/0.9
FD-7-1	7.5/6.4	1.8/1.7	6.2/5.3	5.7/5.1	1.7/2.8	4.7/4.4	3.5/0.9	1.4/1.7	3.0/1.0
FA-1-1	1.0/0.7	7.8/7.6	2.3/2.1	4.0/0.4	9.6/4.6	5.2/1.4	0.6/1.2	2.7/1.0	1.0/1.2
FA-1-5	0.5/1.7	2.4/3.8	0.9/1.8	2.8/4.5	1.5/4.4	2.5/4.4	0.6/1.2	2.7/1.0	1.0/1.2
FA-1-5-S	20/19	—	16/15	24/23	2.7/2.8	5.9/5.9	17/24	2.8/4.3	14/21
FA-1-5-G	3.6/2.7	1.1/1.3	3.0/2.4	7.0/6.7	2.7/4.1	5.9/5.9	2.5/1.6	1.7/1.7	2.3/1.6
Unidentified metabolites	33/34	50/50	36/37	23/23	47/41	27/27	45/42	51/52	46/43
<i>Total</i>	<i>100/100</i>	<i>100/100</i>	<i>98.0/98.2</i>	<i>100/100</i>	<i>100/100</i>	<i>98.0/97.5</i>	<i>100/100</i>	<i>100/100</i>	<i>98.8/98.3</i>

G: glucuronide conjugate; S: sulfate conjugate

^a Percentage of radioactivity found in urine or faeces.^b Percentage of radioactivity administered.

Source: Soeda (1983, 1984); Soeda & Mizuno (1988)

In summary, all metabolites listed in Table 8 were identified in urine as well as in faeces of the rat. In total, 16 metabolites were identified in urine and faeces and represented 60–75% of the administered radiolabel dose.

The proposed metabolic pathway for triflumizole is illustrated in Fig. 2.

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of triflumizole is summarized in Table 9.

Table 8. Metabolites identified in urine and faeces of rat

Code	Metabolite
FM-2-1	2-(4-Chloro-2-trifluoromethylphenylimino)-2-imidazol-1-yl-ethanol
FM-6-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-propoxy-acetamidine
FM-7-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-propoxy-acetimidic acid methyl ester
FM-8-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamidine
FM-8-1-S	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamidine sulfate conjugate
FD-1-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-propoxy-acetamide
FD-2-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide
FD-2-1-S	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide sulfate conjugate
FD-2-1-G	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide glucuronide conjugate
FD-4-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-formamide
FD-6-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-(2-hydroxypropoxy)-acetamidine
FD-7-1	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-oxalamic acid
FA-1-1	4-Chloro-2-trifluoromethylphenylamine
FA-1-5	2-Amino-5-chloro-3-trifluoromethylphenol
FA-1-5-S	2-Amino-5-chloro-3-trifluoromethylphenol sulfate conjugate
FA-1-5-G	2-Amino-5-chloro-3-trifluoromethylphenol glucuronide conjugate

Table 9. Acute toxicity of triflumizole

Species	Strain	Sex	Route	LD ₅₀ (mg/kg bw)	LC ₅₀ (mg/L)	Reference	GLP
Rat	Wistar-SLC	M	Oral	1 057	–	Nishibe et al. (1983a)	No
		F		1 780	–		
Rat	SLC:SD	M	Dermal	> 5 000	–	Nishibe et al. (1983b)	No
		F		> 5 000	–		
Rat	Wistar Crl: WI	M & F	Inhalation	–	> 3.6	Janssen (2005)	Yes

F: female; GLP: good laboratory practice; LC₅₀: median lethal concentration; LD₅₀: median lethal dose; M: male

(a) *Oral administration*

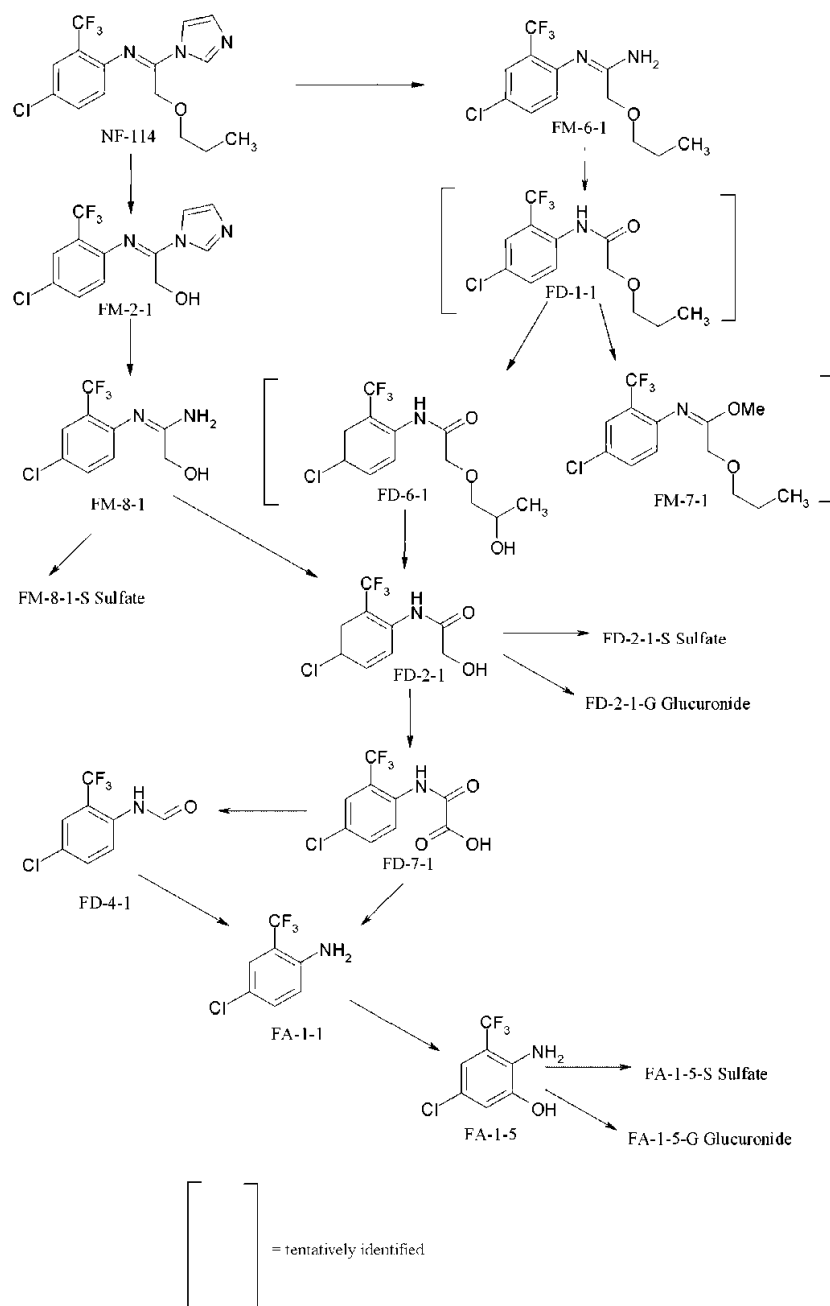
An acute oral toxicity test for triflumizole (purity 98.7%; lot no. YS-200) was conducted in rats. Mortality of both male and female rats was observed in the highest dose groups. Signs of toxicity from triflumizole observed in rats included ataxia, hypotonia, ventral position, lacrimation, urinary incontinence, decreased body temperature, decreased heart rate and respiration rate and ptosis. Body weights decreased slightly in the high-dose groups (2000 mg/kg bw and up) on the 1st day, although they had recovered by the 2nd day after dosing. Haemorrhages of intestinal mucosa, thymus and stomach mucosa and dark reddish lung were observed in dead rats. However, no gross pathological change was observed in the rats surviving for 14 days. The acute oral median lethal dose (LD₅₀) of the test substance was calculated to be 1057 (863–1297) mg/kg bw for male rats and 1780 (1369–2314) mg/kg bw for female rats (Nishibe et al., 1983a).

(b) *Dermal application*

An acute dermal toxicity test for triflumizole (purity 98.7%; lot no. YS-200) was conducted in rats. No deaths were observed during the study, nor were any gross pathological changes observed at necropsy. No signs of toxicity were observed in male rats. Urinary incontinence was observed only in female rats on the 2nd and 3rd days. In all groups, body weight decreased on the 1st day after

application, but recovered thereafter. No dose-response relationship was seen in these two observed signs. Therefore, it was concluded that these were not caused by triflumizole. No effects on feed consumption were observed. The acute dermal LD₅₀ of the test substance was found to be greater than 5000 mg/kg bw for male and female rats (Nishibe et al., 1983b).

Fig. 2. Proposed metabolic pathway



(c) *Exposure by inhalation*

An acute inhalation toxicity test for triflumizole (purity 99.7%; batch no. TDL-577) was conducted in rats. No mortalities occurred. Symptoms of toxicity included hunched posture, lethargy and chromodacryorrhoea (head and/or snout) among the majority of the animals between day 1 and day 4. Rales occurred in one animal on day 1, and periorbital alopecia occurred in another animal between days 4 and 13. No effects were seen on body weight or body weight gain; the body weight gain shown by the animals over the study period was considered to be normal for rats of this age and strain in this type of study. Macroscopic postmortem examination of the animals did not reveal abnormalities.

The study is considered acceptable. The acute 4-hour median lethal concentration (LC₅₀) in rats is greater than 3.6 mg/L, the maximum attainable exposure concentration (Janssen, 2005).

(d) *Dermal and ocular irritation*

A non-GLP dermal irritation study with triflumizole (purity 98.7%; lot no. YS-200) was performed using male Angola rabbits. No signs of erythema or oedema were observed after application of triflumizole on either intact or abraded skin (combined score = 0). Taking into account the absence of any skin reactions when applied to 9 cm², it is not expected that applying the same dose to 6 cm² will change the classification of the test substance (Nishibe et al., 1983c).

A non-GLP eye irritation study with triflumizole (purity 98.7%; lot no. YS-200) was performed using male Japanese White rabbits. At 24 hours after administration, conjunctival redness and discharge (grades 1–2) were observed in 5/6 animals. At 48 hours, no effects were observed except for conjunctival redness (grade 1) in 2/6 animals. Based upon the findings in this study, the test substance is mildly irritating to the eye of rabbits (Nishibe et al., 1983d).

(e) *Dermal sensitization*

In a non-GLP Magnusson and Kligman maximization test, triflumizole (purity 98.2%; lot no. YS-0155) was tested using 12 Hartley guinea-pigs per group. The study was performed partly in accordance with OECD Test Guideline 406; a negative control group should have been included. No results of the intradermal injection/topical induction are presented. Following challenge with triflumizole at 25% weight per weight (w/w), dermal responses were observed in 8/12 test animals. Sensitization of this strain of animals was positively tested with *N*-phenyl-*p*-phenylenediamine (positive control), which gave very severe allergic reactions in all areas treated.

Compared with the results found in the positive control group, the reactions elicited by triflumizole were very slight, and some of these disappeared after 48 hours. However, as no negative control group was included and no information on the dose selection was presented, it cannot be excluded that the reactions were due to sensitization instead of skin irritation. It is therefore concluded that triflumizole is sensitizing to the skin of guinea-pigs in this maximization study (Nishibe et al., 1983e).

2.2 *Short-term studies of toxicity*

(a) *Oral administration*

Mice

A 28-day feeding study in mice was not submitted. In the European Union monograph (The Netherlands, 2009), such a study is evaluated (Nishibe et al., 1980c). The no-observed-adverse-effect level (NOAEL) of that study was 200 ppm (equal to 40 mg/kg bw per day), which is comparable to the NOAEL in the 90-day feeding study described below.

In a non-GLP 90-day feeding study, groups of 20 male and 20 female Charles River ICR mice were treated with doses of triflumizole (purity 98.7%; lot no. YS-200) in the diet. The concentrations

in the feed were 0, 20, 200 and 2000 parts per million (ppm) (equal to 0, 3.2, 33 and 381 mg/kg bw per day for males and 0, 4.2, 43 and 466 mg/kg bw per day for females, respectively). An additional group of 10 animals of each sex was used for haematology and blood chemistry tests at the start of the dosing period. The procedure for obtaining blood for the blood chemistry tests was terminal. It should be noted that no cholinesterase activity was measured. The study was performed partly in accordance with OECD Test Guideline 408. Deviations from the guideline were that the animals were checked for morbidity and mortality once instead of twice a day and that no sensory stimuli tests or ophthalmological and functional observations were included. Further, blood clotting potential, urea and creatinine were not determined, and histopathological examinations of the spinal cord, aorta, female mammary gland and peripheral nerve were not performed. However, these deviations are not considered to have influenced the conclusions of the study.

In this study, a reduction in body weight gain (27% in males, 15% in females compared with controls; Table 10) and a slight increase in feed consumption (< 10% in males and females) was found in the highest dose group. Haemoglobin (males) and mean corpuscular haemoglobin concentrations (MCHC) (both sexes) were also significantly decreased (4–6%). Statistically increased levels of potassium were measured in both sexes (12–17%). In females, an increase in relative kidney weight (11% at 200 ppm and 8% at 2000 ppm) and ketone bodies in urine in 4/10 females at 2000 ppm were observed. Relative adrenal weights were also increased in females of all treated groups, but not in a dose-related manner (19–21% increase). Absolute and relative liver weights were increased in both sexes (Table 11), which corresponded with the microscopic finding of swelling of cytoplasm in the central zone of all male livers, at 2000 ppm. At 200 ppm, liver weights of males (absolute and relative weights) and relative liver, adrenal and kidney weights of females were increased, and haemoglobin levels of males were significantly decreased. Relative liver and adrenal weights were also increased in females at 20 ppm. The changes in liver weight at the middle and low doses are not considered toxicologically relevant, as the increases in relation to controls were less than 10%. The changes in blood parameters at the middle and high doses are also considered to be toxicologically irrelevant, as the relative changes are small, and the decrease in haemoglobin level is not more severe at the high dose. The significance of effects on adrenal weight observed in all short-term toxicity studies with rodents was evaluated, taking into account all submitted data, and it was concluded that the effects are not considered to be toxicologically relevant.

Table 10. Mean body weight gain in mice following dietary exposure for 13 weeks

	Mean body weight gain (g)							
	0 ppm		20 ppm		200 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Mean	18.0	14.2	14.9*	13.3	16.5	12.9	13.1*** (–27%)	12.1 (–15%)
Standard deviation	4.4	4.2	4.3	4.4	4.2	3.8	2.4	3.8

*: $P < 0.05$; ***: $P < 0.001$

Source: Nishibe et al. (1980a)

In conclusion, based on decreased body weight gain, increased feed consumption and effects on the liver (increased liver weight, swelling of cytoplasm) at 2000 ppm (equal to 381 mg/kg bw per day), the NOAEL for triflumizole in mice was 200 ppm (equal to 33 mg/kg bw per day) (Nishibe et al., 1980a).

Rats

A 28-day feeding study in rats was not submitted for evaluation. However, in the European Union monograph (The Netherlands, 2009), such a study was evaluated (Nishibe et al., 1980d); the NOAEL was 20 ppm (equal to 2.3 mg/kg bw per day), based on increased relative ovary weight at 200 ppm (equal to 22 mg/kg bw per day), which is lower than the NOAEL achieved in the 90-day feeding study described below.

Table 11. Liver weight in mice following dietary exposure for 13 weeks

	0 ppm		20 ppm		200 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Mean	1.658	1.399	1.630	1.407	1.788*	1.423	1.969***	1.677***
					(+8%)		(+19%)	(+20%)
Standard deviation	0.193	0.204	0.171	0.158	0.212	0.230	0.200	0.226
Relative liver weight (%)								
Mean	3.632	3.604	3.770	3.852*	3.972***	3.937**	4.703***	4.750***
				(+7%)	(+9%)	(+9%)	(+29%)	(+32%)
Standard deviation	0.253	0.329	0.181	0.333	0.250	0.368	0.310	0.413

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Source: Nishibe et al. (1980a)

In a non-GLP 90-day feeding study, groups of 20 male and 20 female Charles River Sprague-Dawley rats were treated with doses of triflumizole (purity 98.7%; lot no. YS-200) in the diet. The concentrations in the feed were 0, 20, 200 and 2000 ppm (equal to 0, 1.4, 15 and 177 mg/kg bw per day for males and 0, 1.8, 17 and 218 mg/kg bw per day for females, respectively). An additional group of 10 animals of each sex was used for haematology and blood chemistry tests at the start of the dosing period. At the end of the study, all animals in the test were subjected to haematology and blood chemistry tests. Blood for the blood chemistry tests was sampled after overnight fasting. The procedure for obtaining blood for the blood chemistry tests was terminal. Storage of the samples until testing and the time elapsed between sampling and testing were not specified. Plasma cholinesterase activity was measured using the 5,5'-dithiobis-2-nitrobenzoic acid method with *S*-butyrylthiocholine iodide as substrate. The study was performed partly in accordance with OECD Test Guideline 408. Deviations from the guideline were that the animals were checked for morbidity and mortality once instead of twice a day and that no sensory stimuli tests or ophthalmological and functional observations were included. Further, blood clotting potential, urea and creatinine were not determined, and histopathological examinations of the spinal cord, aorta, female mammary gland and peripheral nerve were not performed. However, these deviations are not considered to have influenced the conclusions of the study.

Oral exposure of rats to triflumizole at a concentration of 2000 ppm for 13 weeks resulted in a significantly lower body weight gain of females over the entire study period (16% at week 13; Table 12) and of males during weeks 1, 2 and 3 of dosing (10% at week 3) and an increased feed consumption in both sexes, mainly in the first weeks of the study (up to 14% in males and 45% in females in weeks 1–4). This may indicate a rise in catabolism, which may explain the increased concentrations of blood urea nitrogen, cholesterol, total protein and albumin observed in females at the highest dose levels. Further, female red blood cell parameters (decrease in red blood cells, haemoglobin and MCHC and increase in mean cell volume [MCV]) and plasma cholinesterase activity were affected at the high dose (Table 13). Kidney weights were increased in both sexes. Decreased adrenal weights were found in males, and decreased thymus and increased spleen weights were seen in females. Absolute and relative weights of the liver were increased in both sexes, which correlated with the microscopic finding of fatty metamorphosis in the livers of all animals in this dose group (Table 14).

In males, increased liver weights were also seen in the 20 and 200 ppm groups, as well as increased kidney weights at 200 ppm. The increases in absolute weights may be attributable to the higher body weights (~5%) in these dose groups compared with controls. The increases in relative weights were low (< 10%), and no changes in related parameters were present. Therefore, these deviations are not considered to be toxicologically relevant in the low- and mid-dose groups.

Table 12. Mean body weight gain in rats following dietary exposure for 13 weeks

	Mean body weight gain (g)							
	0 ppm		20 ppm		200 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Mean	335.1	171.7	361.5	164.0	360.5	158.3 (-8%)	321.0	144.9*** (-16%)
Standard deviation	35.0	25.0	54.0	21.0	49.3	16.4	46.6	22.0

***: $P < 0.001$

Source: Nishibe et al. (1980b)

Table 13. Plasma cholinesterase activity in rats following dietary exposure for 13 weeks

Dose (ppm)	Activity (U/mL)			
	0 months		3 months	
	Males	Females	Males	Females
0	0.18 ± 0.03	0.77 ± 0.31	0.10 ± 0.02	1.02 ± 0.24
20	—	—	0.12 ± 0.03	1.03 ± 0.33
200	—	—	0.10 ± 0.03	0.99 ± 0.27
2 000	—	—	0.10 ± 0.02	0.76 ± 0.27**

**: $P < 0.01$

Source: Nishibe et al. (1980b)

Table 14. Liver weight in rats following dietary exposure for 13 weeks

	0 ppm		20 ppm		200 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Mean	11.69	7.17	13.10* (+12%)	7.31	13.03* (+11%)	7.08	14.89*** (+27%)	8.49*** (+18%)
Standard deviation	1.45	0.56	1.99	0.92	1.93	0.84	2.73	1.00
Relative liver weight (%)								
Mean	2.29	2.34	2.47	2.42	2.44* (+7%)	2.38	3.00*** (+31%)	3.02*** (+29%)
Standard deviation	0.18	0.17	0.35	0.23	0.22	0.24	0.31	0.20

*: $P < 0.05$; *** $P < 0.001$

Source: Nishibe et al. (1980b)

Based on the decreased body weight gain, increased feed consumption, liver effects (increased liver weight, fatty changes) and increased kidney weights at 2000 ppm (equal to 177 mg/kg bw per day), the NOAEL for triflumizole in rats was 200 ppm (equal to 15 mg/kg bw per day) (Nishibe et al., 1980b).

Dogs

In a 1-year toxicity study, groups of six male and six female Beagle dogs were treated with doses of triflumizole (purity 98.7%; lot no. TK-1114) in the diet. The study was performed in accordance with OECD Test Guideline 409. In this study, groups of purebred Beagle dogs (six of each sex per dose) were dosed with triflumizole at a dietary concentration of 0, 100, 300 or 1000 ppm

(equal to 0, 3, 10 and 34 mg/kg bw per day for males and 0, 3, 11 and 35 mg/kg bw per day for females, respectively). Feed consumption was determined each working day, and body weight once a week. At least once a day, animals were observed for signs of toxicity. Ophthalmoscopic examinations were carried out prior to treatment and during weeks 12 and 51. Clinical chemistry, haematological examinations and urine analyses were carried out once before treatment and then during dose weeks 6, 12, 26 and 51. At interim sacrifice (at 13 weeks), two animals of each sex per dose were killed. The remaining animals were killed after 52 weeks. After necropsy, all animals were subjected to gross pathological assessment, followed by histopathological examination.

Oral exposure of dogs to triflumizole at a concentration of 1000 ppm for 1 year resulted in slightly but statistically significantly decreased packed cell volume, haemoglobin and red blood cells and increased MCV levels in males only. Alkaline phosphatase levels were statistically significantly increased in males (79%) and females (63%), and liver weights were increased in males (18%) and females (25%) (Table 15). Moreover, macroscopic changes in the liver, defined as lobular pattern and granular texture, were observed in one animal in each of the male and female high-dose main and interim groups. At a dose level of 300 ppm, no adverse effects were observed. Therefore, the NOAEL was 300 ppm (equal to 10 mg/kg bw per day) (Chesterman et al., 1984).

Table 15. Liver weight in dogs^a following 1-year dietary exposure

	0 ppm		100 ppm		300 ppm		1 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Interim (13 weeks) ^{a,b}		343.3		333.2		375.3		399.8*
Interim (13 weeks) ^{b,c}	362.2	304.9	358.7	302.1	398.9	363.5	430.7	382.4
Terminal (52 weeks)	388.7	321.9	421.1	375.8	419.3	380.2	460.0**	402.5*
Relative liver weight (%)^a								
Interim (13 weeks) ^{a,c}		3.13		3.02		3.42		3.66
Interim (13 weeks) ^c	3.10	3.16	3.12	2.94	3.52	3.32	3.72	3.75
Terminal (52 weeks) ^c	3.15	2.97	3.43	2.96	3.42	3.30	3.77	3.50

*: $P < 0.05$; **: $P < 0.01$

^a $n = 2$ of each sex at 13 weeks and 4 of each sex at 52 weeks. For meaningful statistics, the males and females were taken together at 13 weeks.

^b Adjusted for body weight.

^c Statistical analysis was not performed.

Source: Chesterman et al. (1984)

(b) Dermal application

Rats

In a 21-day dermal toxicity study, groups of six male and six female Charles River CD rats were treated with doses of triflumizole (purity 97%; lot no. 2112) on the skin. The test substance was moistened with distilled water into a paste. This was applied to the clipped dorsal skin (10% of the body surface) of the rats, at dose levels of 10, 100 and 1000 mg/kg bw per day. The application site was then wrapped with porous gauze bandages fastened with non-irritating tape. Following the 6-hour period of daily exposure, the bandages were removed, and the dosed skin areas were washed with tap water. A control group was handled in a similar way, except that no test substance was administered. The total dose administered to each rat was adjusted weekly based on the most recently recorded body weights. Daily observations on mortality and clinical signs and weekly recordings of body weights and feed consumption were performed. At the end of the study (21 days), blood and urine samples were collected, and macropathological and micropathological examinations took place. The study was

performed in accordance with OECD Test Guideline 410, except that only the treated skin, liver and kidney were examined histopathologically. As the liver is the target organ in the short-term oral toxicity studies, this deviation probably did not affect the derivation of a NOAEL.

Dermal exposure of rats to triflumizole at a concentration of 1000 mg/kg bw per day for 21 days resulted in a significant increase (16%) in relative liver weight of males. A slight increase in the incidence of vacuolar fatty change in the livers of females of the high-dose group was seen, as well as an increase in the severity of the effect. However, as the effects were not accompanied by any other effects in the liver in the same sex and as the effects in females were restricted to only mild fatty change, these liver effects in males and females are considered not to be adverse. The number of animals with skin inflammation (score: trace) was slightly higher in the high-dose groups than in the control groups. One low-dose female showed macroscopic skin effects; the skin was therefore examined microscopically and scored as mild inflammation. There is no clear dose-response relationship in severity, and the effects observed in the high-dose group were marginal (trace). The effects could be due to the application procedure. They are not considered to be related to the test substance (Table 16).

Table 16. Summary of effects in rats following dermal exposure for 6 hours/day for 21 days

	0 mg/kg bw per day		10 mg/kg bw per day		100 mg/kg bw per day		1 000 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Organ weights								
<i>Liver</i>								
- Absolute (g)	12.78	7.92	14.42	7.64	14.67	7.60	14.60	8.97
- Relative (%)	4.18	4.49	4.54	4.23	4.67	4.45	4.83*	4.94
							(+16%)	
Microscopic pathology								
<i>Liver</i>								
- Vacuolar fatty change (trace–mild)	0/6	1/6	–	–	–	–	0/6	3/6
<i>Skin</i>								
- Inflammation (trace)	2/6	1/6	–	1/1	–	–	3/6	3/6

*: $P < 0.05$

Source: Goldenthal (1990)

Based on the absence of adverse effects in the highest dose group, the NOAEL for triflumizole in rats was 1000 mg/kg bw per day, the highest dose tested (Goldenthal, 1990).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a combined chronic toxicity and carcinogenicity study, groups of 50 male and 50 female B6C3F1 mice were treated with doses of triflumizole (purity 98.6%; lot no. TK-1116) in the diet for 104 weeks. The study was performed mainly in accordance with OECD Test Guideline 453. Triflumizole was administered in feed for 104 consecutive weeks at four concentrations: 0, 100, 400 and 1600 ppm (equal to 0, 16, 67 and 296 mg/kg bw per day for males and 0, 22, 88 and 362 mg/kg bw per day for females, respectively). Additional groups of 10 male and 10 female mice per dose were used for each of three interim kills, performed in weeks 26, 52 and 78 after initiation of

exposure. Baseline blood parameters were established at the start of the study, using an additional 20 males and 20 females. Clinical observations and measurements of body weight, feed consumption and water consumption were performed during the test period. Clinical chemistry parameters were measured in 6–10 of the sacrificed animals in weeks 0, 26, 52 and 78 after the study started. No blood samples or urine samples were taken 3 months after dosing started. No ophthalmoscopy was performed. Gross pathology and histopathology were performed on all animals dying spontaneously or killed in a moribund condition, as well as those killed at the scheduled necropsies. Organs of all sacrificed animals at the scheduled necropsies were weighed.

Chronic oral administration of triflumizole to mice at doses of 400 ppm and above caused primarily liver effects. A decrease in body weight gain was noted at the highest dose in males (significant, –44%) and females (not significant, –10%). Effects on liver enzymes were observed as increased levels of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) in males administered 1600 ppm. The absolute and/or relative liver weights were increased in animals in the mid-dose and high-dose groups (Table 17). An increased number of animals in the high-dose group, compared with the control group, had macroscopic liver effects, such as nodules, white zone and enlargement. Some of these effects were also seen to a lesser degree in males and/or females in the 400 ppm group. The histopathological non-neoplastic lesions were found primarily in the liver, where several effects increased in a dose-related manner in the mid- and high-dose groups (Table 18). These histological findings included hepatic nodules and fatty metamorphosis (change) at all dose levels. Additionally, cytological alterations, pigmentation and necrosis of the liver were observed in males at 1600 ppm and, to a lesser degree, at 400 ppm.

The increase in liver fatty metamorphosis observed in males administered 100 ppm is not considered to be a toxicologically relevant effect, in the absence of additional liver effects. The decreases in the number of white blood cells in males of all dose groups do not show a consistent pattern across time or dose groups. In the absence of effects on lymphoid organs, its toxicological significance is not clear. As neither its relation to treatment nor its toxicological significance is clear, the reduction in the number of white blood cells observed in this study is not considered relevant.

An increase in the number of hepatic nodules was observed in males in all dose groups and in females in the mid- and high-dose groups, in comparison with the control group. The author of the study report considered it a non-neoplastic lesion. In view of the absence of a clear dose–effect relationship in males at the two lower doses and the non-significant increase in incidence observed in females of these dose groups, only the highest dose is considered to have resulted in a treatment-related increase in hepatic nodules. No increase in tumour incidence was observed (Tables 19 and 20).

In conclusion, the NOAEL was 100 ppm (equal to 16 mg/kg bw per day), based on non-neoplastic liver effects, such as increased organ weight and histopathological findings (nodules and fatty changes in both sexes as well as granulomatous inflammation, cytological alterations, pigmentation and necrosis in some males), in mice at 400 ppm (equal to 67 mg/kg bw per day) (Inoue, 1984).

Rats

In a combined chronic toxicity and carcinogenicity study, groups of 80 male and 80 female CD rats were treated with doses of triflumizole (purity 98.6%; lot no. TK-1116) in the diet for 104 weeks. The groups of 80 animals were split into an oncogenicity group (50 rats of each sex) and a toxicity group (30 rats of each sex, of which 10 were killed at 52 weeks). Triflumizole was administered at four concentrations: 0, 100, 400 and 1600 ppm (equal to 0, 3.5, 14 and 59 mg/kg bw per day for males and 0, 4.5, 18 and 77 mg/kg bw per day for females, respectively). Clinical observations and measurements of body weight, feed consumption and water consumption were performed during the test period. Clinical pathology parameters and urine samples were measured in 10 of the sacrificed animals of each sex in weeks 0, 26, 50 and 77/78 after the study started. Ophthalmoscopy was performed in controls and high-dose animals before treatment and in weeks 6, 13, 27, 51, 78 and 102. Gross pathology and histopathology were performed on all animals dying spontaneously or killed in a moribund condition, as well as those killed at the scheduled necropsies.

Table 17. Liver weight findings in mice following chronic dietary exposure

	0 ppm		100 ppm		400 ppm		1 600 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Week 26								
- Mean	1.33	1.12	1.37	1.22	1.36	1.27*	1.83***	1.57***
- Standard deviation	0.08	0.12	0.12	0.11	0.10	0.13	0.21	0.16
Week 52								
- Mean	1.57	1.38	1.59	1.40	1.79	1.50*	2.22***	1.83***
- Standard deviation	0.20	0.11	0.28	0.16	0.37	0.10	0.21	0.09
Week 78								
- Mean	1.74	1.46	1.90	1.48	1.57	1.51	2.32**	1.93***
- Standard deviation	0.51	0.30	1.16	0.13	0.12	0.17	0.31	0.21
Week 104								
- Mean	1.80	1.70	1.83	1.48	1.91	1.51	2.51***	1.86
- Standard deviation	0.82	0.81	1.01	0.40	0.69	0.37	0.80	0.53
Relative liver weight (%)								
Week 26								
- Mean	3.545	3.947	3.587	3.880	4.030***	4.139	5.651***	5.515***
- Standard deviation	0.160	0.335	0.157	0.204	0.213	0.337	0.513	0.303
Week 52								
- Mean	3.678	3.959	3.654	3.801	4.093*	4.137	5.905***	5.512***
- Standard deviation	0.323	0.492	0.293	0.451	0.376	0.628	0.252	0.140
Week 78								
- Mean	4.547	4.178	4.721	4.040	4.018	4.309	6.629**	6.086***
- Standard deviation	1.541	1.018	3.612	0.470	0.352	0.651	0.579	0.648
Week 104								
- Mean	4.455	5.104	4.551	4.266*	4.738	4.549	7.496***	5.882
- Standard deviation	2.564	2.503	3.101	0.906	1.853	1.121	2.410	1.892

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

Source: Inoue (1984)

Table 18. Histopathology: non-neoplastic lesions in mice following chronic dietary exposure

	0 ppm		100 ppm		400 ppm		1 600 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver								
Hepatic nodule	7/60	5/60	16/60	7/60	13/60	9/60	22/60	17/60
Fatty metamorphosis	17/60	9/60	23/60	10/60	27/60	17/60	40/60	25/60

Table 19. Time-related occurrence of tumours in male mice following chronic dietary exposure

Summary of histopathological findings	Weeks 0–26				Weeks 27–52				Weeks 53–78				Weeks 79–104			
	A ^a	B ^a	C ^a	D ^a	A	B	C	D	A	B	C	D	A	B	C	D
No. of benign tumours	0	0	0	0	0	0	0	1	0	0	1	0	21	33	27	30
No. of malignant tumours	0	0	0	0	0	0	0	0	1	1	1	0	9	7	13	10
No. of total tumours	0	0	0	0	0	0	0	1	1	1	2	0	30	40	40	40
No. of animals with a single tumour	0	0	0	0	0	0	0	1	1	1	2	0	22	20	16	27
No. of animals with multiple tumours	0	0	0	0	0	0	0	0	0	0	0	0	4	8	11	6
No. of animals examined	11	10	10	10	10	10	10	10	11	12	12	10	48	48	48	50

^a A: 0 ppm; B: 100 ppm; C: 400 ppm; D: 1600 ppm.

Source: Inoue (1984)

Table 20. Time-related occurrence of tumours in female mice following chronic dietary exposure

Summary of histopathological findings	Weeks 0–26				Weeks 27–52				Weeks 53–78				Weeks 79–104			
	A ^a	B ^a	C ^a	D ^a	A	B	C	D	A	B	C	D	A	B	C	D
No. of benign tumours	0	0	0	0	1	0	0	0	0	1	0	0	27	16	19	6
No. of malignant tumours	0	0	0	0	0	0	0	0	0	0	0	1	9	8	8	14
No. of total tumours	0	0	0	0	1	0	0	0	0	1	0	1	36	24	27	20
No. of animals with a single tumour	0	0	0	0	1	0	0	0	0	1	0	1	15	15	16	14
No. of animals with multiple tumours	0	0	0	0	0	0	0	0	0	0	0	0	10	4	5	2
No. of animals examined	10	10	10	10	10	10	10	10	11	11	10	11	49	49	50	49

^a A: 0 ppm; B: 100 ppm; C: 400 ppm; D: 1600 ppm.

Source: Inoue (1984)

Organs of all sacrificed animals at the scheduled necropsies were weighed. The study was mainly conducted in accordance with OECD Test Guideline 453, with the following exceptions. The mortality exceeded 50% in the male control, low-dose and medium-dose groups and the female low-dose group. No blood samples or urine samples were taken 3 months after dosing started. Cholinesterase activity was measured immediately after blood sampling and centrifugation, using the Technicon method no. 354-75 P/A. Plasma cholinesterase activity was measured using acetyl and butyryl substrate, erythrocyte cholinesterase activity using acetyl substrate only. Brain cholinesterase activities were measured in the fresh homogenates of the mid-line sagittal section of the brain, using the same assays as for plasma cholinesterases. Rats were apparently not fasted prior to blood sampling or sacrifice.

The study is considered acceptable, even though the mortality was higher than 50% (OECD Test Guideline 453) in male rats in the control, low-dose and mid-dose groups and in female rats in the low-dose group (Table 21). First, comparisons with historical control data indicate that the mortality was within the background range for these rats in the laboratory where the study was performed, except for male rats of the high-dose group, where the mortality was low, and female rats of the low-dose group, where the mortality was rather high. Additionally, there was no indication of an increase in tumour incidence even when including the results of the animals that died between 18 and 24 months. Moreover, the survival rate increased with increasing dose and was not too high at the highest dose level.

Table 21. Mortality rates in rats following chronic dietary exposure

	0 ppm		100 ppm		400 ppm		1 600 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Mortality	52/70	31/70	50/70	47/70	46/70	25/70	32/70	22/70

Source: Virgo (1984)

Chronic oral administration of triflumizole to rats also provoked neurotoxic effects at the high dose. Convulsions (violent jerking movement, ataxia, tremors) were noted in all dose groups except the female controls (Table 22). When looking at the historical control incidences of convulsions in CD rats for this laboratory, the incidences in males ranged from 0% to 6.7% and in females ranged from 0% to 2.0%. At doses of 0, 100, 400 and 1600 ppm, the percentages affected in males were 3.8%, 5.0%, 2.5% and 7.5%, respectively, and in females were 0%, 2.5%, 2.5% and 18.8%, respectively. Therefore, the incidence of convulsive episodes among controls and animals receiving 100 or 400 ppm was consistent with that reported in a range of similar studies. However, the incidence of convulsions at the highest concentration of 1600 ppm was above the background range, particularly in females, and is considered an adverse effect of treatment.

Table 22. Convulsive episodes in rats following chronic dietary exposure

Sex	Dose (ppm)	Incidence (number of convulsing animals/total number) (% affected)	Week of onset of convulsions (mean $\pm$ SD)
M	0	3/80 (3.8%)	81 $\pm$ 31
	100	4/80 (5.0%)	52 $\pm$ 20
	400	2/80 (2.5%)	58 $\pm$ 30
	1 600	6/80 (7.5%)	42 $\pm$ 24
F	0	0/80	—
	100	2/80 (2.5%)	62 $\pm$ 31
	400	2/80 (2.5%)	65 $\pm$ 51
	1 600	15/80* (18.8%)	30 $\pm$ 17

F: females; M: males; SD: standard deviation; *: $P < 0.05$

Source: Virgo (1984)

Body weight gain and feed consumption were decreased in high-dose animals. Erythrocyte acetylcholinesterase activity was decreased in males (400 and 1600 ppm) and in females (100 and 400 ppm) transiently (one or two occasions) without a dose–response relationship (Table 23). Brain acetylcholinesterase activity (Table 24) was not changed on any occasions in any dose groups, except for a transient decrease in males from the 400 ppm group. It is considered that triflumizole does not inhibit acetylcholinesterase activities because of no dose-related response or no more severe change with time. Brain butyrylcholinesterase (butyrylcholine as a substrate) was decreased compared with controls in males (400 and 1600 ppm) and in females (all dose groups) at week 54 (Table 24). After administration for 104 weeks, its activity was increased in both sexes (significant in female) of the 1600 ppm group. Due to the lack of consistency and lack of a dose–response relationship, these changes are assessed as being incidental.

Plasma acetylcholinesterase (acetylcholine as a substrate) was increased in males (1600 ppm) and decreased in females (1600 ppm) at week 26. This was not observed at week 77 or 102 (Table 25). Plasma butyrylcholinesterase (butyrylcholine as a substrate) revealed a similar trend. As no consistent decrease was observed, it is concluded that triflumizole does not inhibit brain, erythrocyte or plasma cholinesterases.

Table 23. Erythrocyte acetylcholinesterase activity in rats following chronic dietary exposure

Dose (ppm)	Activity (IU/L)							
	26 weeks		50 weeks		77 weeks		102 weeks	
	Males	Females	Males	Females	Males	Females	Males	Females
0	1 265 ± 139	1 013 ± 143	658 ± 61	587 ± 48	946 ± 124	984 ± 86	1 175 ± 146	987 ± 156
100	1 212 ± 139	1 031 ± 158	691 ± 89	573 ± 38	838 ± 205	799 ± 106*	1 130 ± 186	1 140 ± 164
400	1 153 ± 134	1 001 ± 142	584 ± 80*	539 ± 56*	924 ± 112	802 ± 93*	1 048 ± 238	1 084 ± 226
1 600	1 090 ± 174*	1 054 ± 170	565 ± 68*	614 ± 63	995 ± 137	951 ± 92	1 234 ± 294	1 148 ± 188

IU: International Units; *: $P < 0.05$

Source: Virgo (1984)

Table 24. Brain acetylcholinesterase and butyrylcholinesterase activity in rats following chronic dietary exposure

Dose (ppm)	Activity (10 ³ IU/L)							
	54 weeks				104 weeks			
	Acetylcholinesterase				Butyrylcholinesterase			
	Males	Females	Males	Females	Males	Females	Males	Females
0	10.9 ± 0.7	10.0 ± 0.7	8.1 ± 1.1	8.5 ± 0.5	2.0 ± 0.2	3.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.2
100	10.6 ± 1.0	10.0 ± 0.5	8.2 ± 0.4	8.2 ± 0.8	2.1 ± 0.4	2.4 ± 0.3*	1.2 ± 0.1	1.0 ± 0.2
400	10.0 ± 0.6*	10.1 ± 0.6	8.5 ± 0.6	8.2 ± 0.7	1.4 ± 0.2*	2.5 ± 0.3*	1.2 ± 0.2	1.2 ± 0.2
1 600	10.7 ± 1.3	9.7 ± 0.7	8.5 ± 0.6	8.6 ± 0.4	1.5 ± 0.4*	2.7 ± 0.2*	1.3 ± 0.2	1.3 ± 0.3*

IU: International Units; *: $P < 0.05$

Source: Virgo (1984)

Table 25. Plasma acetylcholinesterase activity in rats following chronic dietary exposure

Dose (ppm)	Activity (IU/L)							
	26 weeks		50 weeks		77 weeks		102 weeks	
	Males	Females	Males	Females	Males	Females	Males	Females
0	639 ± 78	3 638 ± 656	429 ± 69	1 506 ± 259	804 ± 285	2 575 ± 543	1 264 ± 741	1 790 ± 624
100	639 ± 80	3 108 ± 283*	370 ± 68	1 545 ± 221	698 ± 124	2 324 ± 524	780 ± 184	1 972 ± 676
400	717 ± 112	3 152 ± 583	494 ± 87	1 435 ± 213	660 ± 114	2 281 ± 428	1 262 ± 441	2 266 ± 573
1 600	765 ± 201*	2 599 ± 590*	457 ± 126	1 445 ± 243	774 ± 303	2 470 ± 462	1 121 ± 801	2 076 ± 420

IU: International Units; *: $P < 0.05$

Source: Virgo (1984)

The main target organ was the liver. Relative liver weight was increased in both males and females in the high-dose groups and in males administered 400 ppm (Table 26). Females administered 400 or 1600 ppm had more macroscopic liver lesions than females in the control group, including swollen or pale livers and dark, depressed areas in the liver. Microscopically, the number of observed effects increased with increasing dose, and the effects were more prominent in females than in males. The incidence of diffuse hepatocytic fatty vacuolation, as shown in Table 27, indicated that the liver damage increased with time. At 104 weeks, the severity also increased with dose. Centriacinar, periacinar and midzonal vacuolation was also observed, but without a clear dose-related pattern. The effects were reflected in the liver enzyme levels, notably an increase in ALAT in males in the high-dose group. Other findings in the livers of females at the high dose and, to a lesser extent, at the middle dose were periacinar hepatocytic hypertrophy, basophilic and eosinophilic foci, focal inflammation/necrosis and hyaline degradation/fibrosis of the bile ducts. Additionally, females at 1600 ppm also showed an increased incidence of cystic ovaries macroscopically, which was confirmed histopathologically by follicular cysts. No increase in tumour incidence was observed (Fig. 3). In the absence of a clear dose-response relationship and additional adverse effects, the periacinar hepatocytic hypertrophy observed in females given 100 ppm (Table 27) is not considered to be toxicologically relevant. The other non-neoplastic effects observed at 100 ppm in males and females were considered comparable with those seen in controls.

Table 26. Liver weight in rats following chronic dietary exposure

	0 ppm		100 ppm		400 ppm		1 600 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Week 54								
- Mean	27.9	15.1	30.0	16.2	33.6**	16.5	35.3***	18.3
- Standard deviation	4.0	2.5	4.4	4.1	4.3	4.1	3.7	5.1
Week 104 onco ^a								
- Mean	27.8	23.2	29.5	22.3	29.5	24.9	30.3	24.7
- Standard deviation	3.6	5.6	4.5	5.6	6.6	6.6	4.6	4.6
Week 104 tox ^b								
- Mean	31.0	21.1	28.7	22.3	30.3	23.8	31.0	22.0
- Standard deviation	6.9	3.3	0.6	5.7	3.4	4.4	6.5	4.8
Relative liver weight (%)								
Week 54								
- Mean	3.3	3.3	3.5	3.5	4.2***	4.2**	4.8***	4.8***
- Standard deviation	0.4	0.2	0.4	0.5	0.7	0.9	0.5	0.9
Week 104 onco ^a								
- Mean	3.1	3.8	3.4	3.8	3.7*	4.1	4.5***	5.2***
- Standard deviation	0.6	0.6	0.4	0.5	0.8	0.7	1.0	0.7
Week 104 tox ^b								
- Mean	3.8	3.6	3.7	3.2	3.8	4.1	4.7*	5.2***
- Standard deviation	1.0	0.7	0.6	0.3	0.9	0.9	0.5	0.6

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

^a Oncogenicity study.

^b Toxicity study.

Source: Virgo (1984)

Table 27. Liver histopathology findings in rats following chronic dietary exposure

	Males				Females			
	Control	100 ppm	400 ppm	1 600 ppm	Control	100 ppm	400 ppm	1 600 ppm
Diffuse hepatocytic fatty vacuolation								
- Week 54	0/10	1/10	3/10	4/10	0/10	0/10	2/10	3/10
- Week 104	10/69	15/70	26/70	35/70	13/70	15/70	43/70	55/70
Periacinar hepatocytic hypertrophy	0/69	5/70	10/70	17/70	0/70	18/70	13/70	28/70

Source: Virgo (1984)

After 54 weeks, 10 animals of each sex per group were killed (satellite group). In these small groups, no treatment-related effect on neoplasm incidence was found. In addition, in animals that were killed or that died during the treatment period, no treatment-related effects on tumour incidence occurred. There was a lower incidence of fibromas in the subcutis of males that had received the highest dose of triflumizole ($P < 0.01$) compared with controls. In animals killed after 104 weeks of treatment, the incidence of neoplasms did not suggest an effect of the administration of triflumizole. There were, however, lower than control incidences of both benign and malignant mammary gland fibroepithelial tumours and pituitary adenomas in females that had received the highest dose of triflumizole (statistically significant in all cases).

It is concluded that triflumizole, when fed to CD rats for 104 weeks, did not increase the incidence of tumours, shorten the induction period of tumours or alter the type of tumours found; indeed, there were generally fewer tumours in rats treated at 1600 ppm than in controls. This is probably a result of the lower body weight gain and feed consumption observed in rats that had received the highest dose of triflumizole.

In conclusion, the NOAEL was 100 ppm (equal to 3.5 mg/kg bw per day), based on hepatotoxicity (increased liver weight and macroscopic and microscopic hepatic changes) observed at 400 ppm (equal to 14 mg/kg bw per day). No carcinogenic potential of triflumizole was observed in this study (Virgo, 1984).

2.4 Genotoxicity

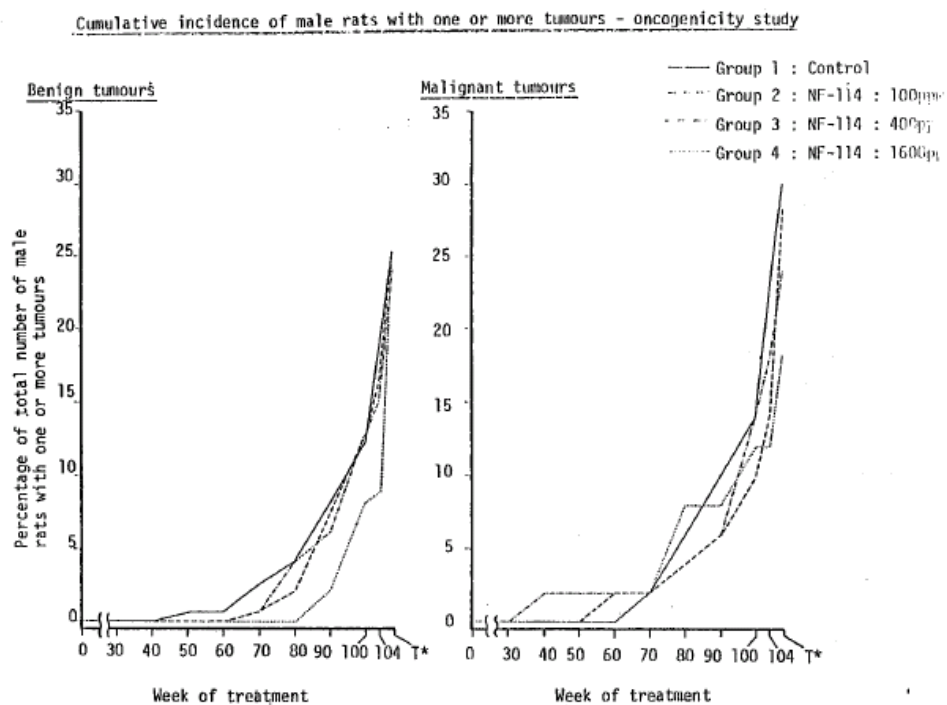
Triflumizole has been tested in a battery of genotoxicity studies comprising several in vitro assays and two in vivo assays, the results of which are summarized in Table 28. Triflumizole was not genotoxic or mutagenic in any of the assays tested.

2.5 Reproductive and developmental toxicity

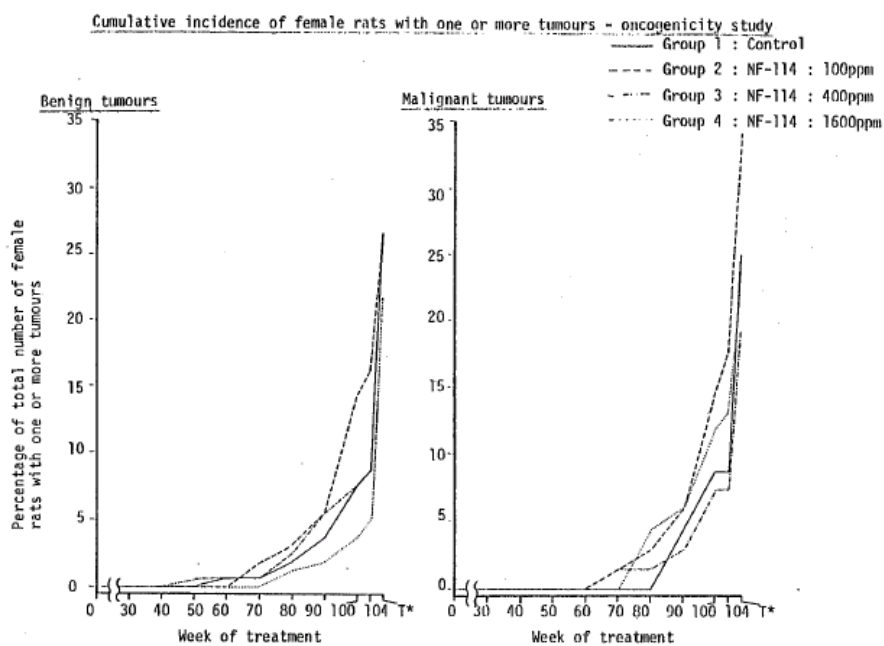
(a) Multigeneration studies

In a non-GLP range-finding reproductive toxicity study, groups of six male and six female Sprague-Dawley rats were treated with doses of triflumizole (purity not stated; batch no. TK-1116) in the diet at a dose level of 0, 400 or 1200 ppm (equal to approximately 0, 20 and 60 mg/kg bw per day, respectively) for 2 weeks prior to mating, throughout the mating period, gestation and lactation, and up to termination after day 21 postpartum. Rats were paired for mating on day 15 of treatment, and females were checked for evidence of mating each morning after pairing. The day on which evidence of mating was found was designated as day 1 of gestation. Animals were examined daily for clinical signs. Males and females were weighed at twice weekly intervals until mating occurred; females were additionally weighed every 2–3 days postcoitum and every 3–4 days postpartum. All does were allowed to deliver their litters and rear their offspring until postpartum day 21. Litter observations were recorded at birth and/or throughout the postpartum interval. On postpartum day 21, female rats

Fig. 3. Cumulative incidence of rats with one or more tumours in the carcinogenicity study



* Terminal kill



*Terminal kill

Source: Virgo (1984)

Table 28. Results of genotoxicity studies on triflumizole

End-point	Test system	Concentration range	Purity (%)	Results	Reference
In vitro					
Reverse mutation ^a	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	5–5 000 µg/plate in DMSO	98.2	Negative (±S9 mix)	Nishibe (1987)
Reverse mutation ^b	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, and <i>Escherichia coli</i> WP2uvrA	8–8 000 µg/plate in DMSO	98.6	Negative (±S9 mix)	Inoue (1983)
Gene mutation (HPRT) ^c	CHL cells (V79)	1.22–19.5 µg/mL (–S9 mix) in DMSO 9.75–156 µg/mL (+S9 mix) in DMSO	98.3	Negative (±S9 mix)	Seeberg & Forster (1989)
Chromosomal aberration ^d	CHL cells	5–40 µg/mL in DMSO	98.2	Negative (±S9 mix)	Nishibe (1988)
DNA repair (unscheduled DNA synthesis) ^e	Primary rat hepatocytes (male Fischer 344)	12.5–40.0 µg/mL in DMSO	98.7	Negative	Cifone (1984)
In vivo					
Micronuclei (bone marrow) ^f	Mouse (CD-1)	160–1 600 mg/kg bw in DMSO	98.7	Negative	Ivett (1984)
Chromosomal aberrations (bone marrow) ^g	Chinese hamster	1 000–4 000 mg/kg bw in 0.5% carboxymethyl cellulose sodium salt	98.3	Negative	Mosesso (1989)

CHL: Chinese hamster lung; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; HPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × g supernatant fraction from rat liver homogenate

^a Test in duplicate; positive controls included; S9 fraction of phenobarbital and 5,6-benzoflavone-induced rat liver; GLP and quality assurance (QA) statements included. Cytotoxicity observed at ≥ 50 µg/plate; precipitation observed at ≥ 1575 µg/plate.

^b Test in duplicate; positive controls included; S9 fraction of phenobarbital and 5,6-benzoflavone-induced rat (Sprague-Dawley) liver. Cytotoxicity observed at ≥ 80 µg/plate.

^c Test in duplicate; positive controls included; S9 fraction of phenobarbital and 5,6-benzoflavone-induced rat (Sprague-Dawley) liver; GLP and QA statements included. Cytotoxicity observed at ≥ 39.1 µg/mL (–S9 mix) and ≥ 313 µg/mL (+S9 mix), observed in a separate toxicity test using dose levels of 1–2500 µg/mL; precipitation observed at ≥ 2500 µg/mL.

^d Test in duplicate; positive controls included; S9 fraction of phenobarbital and 5,6-benzoflavone-induced rat liver; GLP and QA statements included. Only 100 cells/dose scored instead of 200 cells/dose as required by OECD. Cytotoxicity observed at ≥ 40 µg/mL.

^e Single test; positive control included; GLP and QA statements included. Cytotoxicity observed at ≥ 40 µg/mL.

^f Administered by single oral gavage in three dose groups with five male and five female animals per dose; positive control included; sacrifice 24 or 48 hours after dosing; GLP and QA statements included. Clinical signs: 1st hour after dosing, the mice in the medium- and high-dose groups had difficulties breathing, one female animal in the high-dose group was observed to convulse after 24 hours, two male mice in the high-dose group died before the second sacrifice, and all other animals in the high-dose group seemed barely alive.

^g Administered by single oral application in three dose groups with five male and five female animals per dose; positive control included; sacrifice at 12, 24 or 48 hours after dosing; GLP and QA statements included. Clinical signs: decreased activity in all treatment groups. Only 50 cells scored instead of the 100 cells/animal required by OECD.

and their litters were killed and subjected to gross necropsy. Males were killed and subjected to gross necropsy after the females had successfully littered.

Some evidence of toxicity was observed in rats fed diet containing 400 ppm triflumizole. Body weight gain in females was reduced compared with controls over gestation days (GDs) 0–13 (approximately 25% less than control body weight gain; $P < 0.01$). Gestation length was increased by 1 day compared with controls, but gestation index (number of live litters born/number pregnant $\times 100$) was not affected. The mean litter size was slightly reduced (10.5 versus 13.3 for controls; statistical significance not known), but the number of live births and viability were the same as for controls. No other differences were noted in the 400 ppm group compared with controls.

Treatment with 1200 ppm triflumizole in the diet produced significant toxicity. Males showed a significant reduction in body weight gain compared with controls in the interval before pairing (–39%; $P < 0.05$); body weight gain was normal in males for the remainder of the study. Females had a decrease in body weight gain compared with controls before pairing (–29%; not statistically significant) and over GDs 0–13 (–47%; $P < 0.001$). Body weight gain increased for the remainder of gestation and was comparable to or greater than that of controls during the lactation period. The precoital interval (time between initial pairing and evidence of mating) was increased in 3/6 females. Controls mated within 4 days. In the high-dose group, three females mated within 4 days, whereas two mated within 5–8 days and one within 9–12 days. The estrous cycle appeared to have shifted to a 5-day cycle instead of the normal 4-day cycle in the high-dose group. Gestation length was extended by approximately 1 day, and the gestation index (the number of live litters born/number pregnant $\times 100$) was reduced (33% versus 100% for controls). Of the six females in the high-dose group, only one gave birth to live fetuses and survived. Of the three female rats that gave birth, one gave birth to live fetuses but was killed in extremis on postpartum day 1; necropsy revealed retained dead fetuses in utero. The other female gave birth to dead fetuses. Of the three females that did not give birth, one female was found dead on day 23 postcoitum, and one female was killed in extremis on day 24 postcoitum. Both of these females had dead fetuses in utero. The remaining female did not deliver a litter, and necropsy revealed evidence of one early resorption. Necropsy of animals that died or were killed in extremis did not reveal any abnormalities other than the dead fetuses. In surviving fetuses in the high-dose group, fetal birth weight was reduced compared with controls, but increased during lactation.

In conclusion, the parental systemic lowest-observed-adverse-effect level (LOAEL) was 400 ppm (equal to approximately 20 mg/kg bw per day in males and females), the lowest dose tested, based on reduced maternal weight gain over GDs 0–13. A parental systemic NOAEL could not be identified.

The offspring LOAEL was 1200 ppm (equal to approximately 60 mg/kg bw per day), based on dead fetuses and reduced body weight at birth. The offspring NOAEL was 400 ppm (equal to approximately 20 mg/kg bw per day).

The reproductive LOAEL was 1200 ppm (equal to approximately 60 mg/kg bw per day), based on increased gestation length, reduced gestation index and retention of dead fetuses. The reproductive NOAEL was 400 ppm (equal to approximately 20 mg/kg bw per day) (Tesh & Willoughby, 1982).

In a three-generation reproductive toxicity study, groups of 30 male and 30 female CD rats were treated with doses of triflumizole (purity not stated; lot no. TK 1116) in the diet at a dose level of 0, 70, 170 or 420 ppm (equal to 0, 4.8, 11.7 and 29.0 mg/kg bw per day for F_0 males and 0, 5.5, 13.5 and 33.3 mg/kg bw per day for F_0 females, respectively). Thirty F_0 animals were treated for 13 weeks before pairing and continuously until premature termination of the study after weaning of the F_{1A} litters, following adverse reaction at the highest dose level. Feed consumption and water consumption were recorded weekly for both sexes until animals were paired for mating. Males were weighed weekly throughout the study. Females were weighed weekly until mating occurred, on days 1, 3, 7, 14 and 21 postcoitum and on days 1, 4, 10, 14, 21 and 25 postpartum. Observations were made for estrous cycle length, mating performance, fertility, gestation length and parturition. Litter size and

offspring viability were recorded. Offspring were weighed as litters on days 1, 4, 10, 14, 21 and 25 postpartum. All animals were examined externally and internally at necropsy for macroscopic abnormalities. Limited histopathology was conducted on selected F₀ adults.

Continuous dietary administration of triflumizole at 420 ppm resulted in slight decreases in body weight gain, increased length of estrous cycles, reduced vaginal cornification and extended precoital interval. Eventual mating performance and conception rate were unaffected, but gestation length was extended, and severe parturition difficulties resulted in maternal death and high perinatal mortality of offspring. In surviving offspring, body weight gain to weaning was reduced. At a dose of 170 ppm, similar but less severe effects on gestation length, parturition and perinatal mortality were observed. At a dose of 70 ppm, only slight increases in gestation length were observed. There were no organ weight changes or pathological findings in males at any dose that were considered related to treatment. There was a marginal increase in relative liver weights in females in the 420 ppm dose group, but no microscopic findings. The only treatment-related macroscopic finding in offspring was a statistically significant increase in the incidence of hydronephrosis at 420 ppm.

In conclusion, the NOAEL for parental and reproductive effects was 70 ppm (equal to 4.8 mg/kg bw per day). Based on the results observed at 170 and 420 ppm, the decision was made to terminate this study after weaning of the F_{1A} litters and to conduct a second study at levels of 0, 30, 70 and 170 ppm in the diet (see below) (Tesh, Willoughby & Whitney, 1984).

In a three-generation reproductive toxicity study, groups of 30 male and 30 female CD rats were treated with doses of triflumizole (purity 98.6%; lot no. TK 1116) in the diet at a dose level of 0, 30, 70 or 170 ppm (equal to 0, 2.1, 4.8 and 12 mg/kg bw per day for F₀ males and 0, 2.5, 5.8 and 14 mg/kg bw per day for F₀ females; 0, 2.6, 5.8 and 13 mg/kg bw per day for F₁ males and 0, 2.8, 6.6, and 16 mg/kg bw per day for F₁ females; and 0, 2.6, 6.0 and 15 mg/kg bw per day for F₂ males and 0, 3.0, 6.9 and 16 mg/kg bw per day for F₂ females, respectively).

Thirty F₀ animals were treated for 13 weeks before pairing twice in succession. The first pairing produced the F_{1A} litters, which were discarded at weaning. After the second pairing, half of the females ($n = 15$) were killed on day 21 postcoitum to permit teratological examination, and the remainder were allowed to litter (F_{1B} litters), from which the F₁ generation was selected. This procedure was repeated for the F₁ generation: 30 F₁ animals were treated for 13 weeks before pairing twice in succession. The first pairing produced the F_{2A} litters, which were discarded at weaning. After the second pairing, half of the females were killed on day 21 postcoitum to permit teratological examination, and the remainder of the females were allowed to litter (F_{2B} litters). From the F_{2B} litter, 10 animals of each sex per dose were selected and treated for 13 weeks after weaning. Another group of 30 animals of each sex per dose from the F_{2B} litter underwent the above-mentioned procedure. Physical development and auditory and visual function were examined in the F_{1B}, F_{2B} and F_{3B} litters. The data on the F₀, F₁ and F₂ females ($n = 15$) selected for the teratogenicity part of the study are included in the summary/conclusions below; however, litter data on these females were omitted. Selected animals (10 of each sex per dose) from the F₀ and F₁ parents (after 29 weeks of treatment) and all F₂ parents, as well as some of the F₂ adults (13 weeks after weaning), were subjected to a detailed necropsy procedure, and several organs were weighed. Microscopic examination was performed as shown in Table 29.

All other animals were examined externally and internally for macroscopic abnormalities. The study was performed partly in accordance with OECD Test Guideline 416 (1983); the main deviations were that 1) the reproductive organs of not all parental animals were subjected to a full histopathological examination and 2) feed consumption was measured only until the animals were mated for the first pairing. In addition to OECD Test Guideline 416 from 1983, organ weights of several organs were determined, and more tissues were examined than indicated in the guideline.

Table 29. Summary of schedule for microscopic examination of tissues of rats in a three-generation study

	Number/sex/dose	Dose groups	Tissues examined
F ₀ (parent)	5	Control, high-dose group	Full tissue list
	5	Control, high-dose group	Reproductive organs
F ₁ (parent)	5	Control, high-dose group	Full tissue list
	5	Control, high-dose group	Reproductive organs
F ₂ (13 weeks)	10	All dose groups	Full tissue list
F ₂ (parent)	5	Control, high-dose group	Full tissue list
	25 males/dose	Control, high-dose group	Reproductive organs
	12 females/dose		
F _{3B} (weanlings)	3	Control, high-dose group	Full tissue list

Source: Tesh, Willoughby & Secker (1986)

Parental toxicity consisted of increased kidney weights at 70 and 170 ppm and increased liver weights at 170 ppm (Table 30). Moreover, placental weights were increased at 170 ppm in all generations (Table 30). One pregnant female in the high-dose group died of dystocia, and a relationship to treatment cannot be excluded. The parental effects at 70 ppm were rather slight, significantly affecting only absolute kidney weights of females of the F₁ generation. Therefore, effects at this dose level are not considered adverse.

At 170 ppm, an increased gestation length was observed in the first two generations. In each generation, there tended to be decreased conception rate, fertility and/or percentage mating at the highest dose, although sometimes without clear dose–response relationships (Table 31). At 170 ppm, the litter size of the F_{1A} generation was decreased; however, this was not seen in any generation thereafter (Table 32).

At 170 ppm in the F₃ generation, several statistically significant effects were seen on birth weight, live birth index and viability index (Table 32). However, these effects were seen only in this third generation and were not consistent or dose related in the other generations, or the incidences were within the range observed in other generations.

The NOAEL for parental toxicity was 70 ppm (equal to 4.8 mg/kg bw per day), based on increased placental weights and increased liver and kidney weights at the high dose (170 ppm, equal to 12 mg/kg bw per day). The NOAEL for reproductive toxicity was 70 ppm (equal to 4.8 mg/kg bw per day), based on reduced mating, fertility and litter size. The NOAEL for offspring toxicity was 170 ppm (equal to 12 mg/kg bw per day), the highest dose tested (Tesh, Willoughby & Secker, 1986).

(b) *Developmental toxicity*

Rats

In a non-GLP developmental toxicity study in rats, groups of 24 pregnant female Sprague-Dawley (Crj:CD) rats were treated with doses of triflumizole (purity 98.7%; lot no. YS200) by gavage at a dose level of 0, 10, 35 or 120 mg/kg bw per day. The study was performed partly in accordance with OECD Test Guideline 414. Rats were dosed up to GD 16, but caesarean sections were not performed until GD 20. OECD guidelines state that doses should be administered daily from implantation to the day before caesarean section. Females were examined daily from GD 6 to GD 20 for mortality and clinical signs. Body weights and feed consumption were recorded daily on GDs 1–20. Water consumption was recorded on GDs 1, 6, 11, 16 and 20. On GD 20, the rats were killed and subjected to a caesarean section and gross necropsy. The uterus and ovaries were exposed, and the number and location of viable and non-viable fetuses, number of resorptions and total numbers of implantations and corpora lutea were recorded. The uterus was removed and weighed, and fetuses were removed. The uterus of each apparently non-pregnant female was opened and stained with

Table 30. Organ weights in adult rats in a three-generation reproductive toxicity study (n = 10)

	0 ppm		30 ppm		70 ppm		170 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
F₀ animals								
Placental weight (g)								
- F _{1A}		—		—		—		—
- F _{1B}		0.49		0.52		0.51		0.56**
F₁ animals								
Liver weight								
- Absolute (g)	21.7	13.8	22.7	14.5	23.2	14.6	24.0 (+11%)	15.8* (+14%)
- Relative (%)	3.3	4.0	3.5	4.1	3.5	3.9	3.8 (+15%)	4.4* (+10%)
Kidney weight								
- Absolute (g)	4.11	2.53	4.29	2.62	4.17	2.83* (+12%)	4.16 (+9%)	2.86* (+13%)
- Relative (%)	0.62	0.72	0.66	0.73	0.63	0.77	0.66 (+10%)	0.80* (+11%)
Placental weight (g)								
- F _{2A}		—		—		—		—
- F _{2B}		0.53		0.53		0.54		0.59*
F₂ animals (13 weeks)								
Kidney weight								
- Absolute (g)	3.55	2.18	3.54	2.08	3.34	2.03	3.77 (+6%)	2.45 (+12%)
- Relative (%)	0.67	0.74	0.69	0.73	0.70	0.73	0.73* (+9%)	0.80 (+8%)
F₂ animals (parent)								
Liver weight								
- Absolute (g)	23.9	13.0	22.9	13.4	24.3	13.1	26.3* (+10%)	13.7
- Relative (%)	3.4	3.7	3.5	3.8	3.5	3.7	3.8** (+11%)	3.8
Kidney weight								
- Absolute (g)	4.17	2.44	3.94	2.51	4.20	2.57	4.56* (+9%)	2.67* (+9%)
- Relative (%)	0.60	0.70	0.60	0.71	0.61	0.71	0.66** (+10%)	0.75 (+7%)
Placental weight (g)								
- F _{3A}		—		—		—		—
- F _{3B}		0.54		0.53		0.56		0.66**

—: not assessed; *: $P < 0.05$; **: $P < 0.01$

Source: Tesh, Willoughby & Secker (1986)

Table 31. Effects on mating/fertility/gestation in rats in a three-generation reproductive toxicity study

	0 ppm		30 ppm		70 ppm		170 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
F₀ animals								
% mating								
- First pairing (F _{1A})	100	100	97	100	97	97	93	97
- Second pairing (F _{1B})	100	100	100	100	97	100	97	100
Conception rate (%)								
- First pairing (F _{1A})	97	97	97	97	90	90	93	93
- Second pairing (F _{1B})	100	100	100	100	97	97	93	93
Fertility index (%)								
- First pairing (F _{1A})	97	97	93	97	87	87	87	90
- Second pairing (F _{1B})	100	100	100	100	93	97	90	93
Mean gestation length (days)								
- First pairing (F _{1A})	–	22.6	–	22.7	–	22.6	–	23.0***
- Second pairing (F _{1B})	–	22.6	–	22.7	–	22.7	–	22.8
F₁ animals								
% mating								
- First pairing (F _{2A})	97	100	97	97	100	100	93	97
- Second pairing (F _{2B})	97	100	87	90	97	97	86	97
Conception rate (%)								
- First pairing (F _{2A})	83	83	79	79	87	87	71	72
- Second pairing (F _{2B})	89	90	96	96	90	90	92	89
Fertility index (%)								
- First pairing (F _{2A})	80	83	77	77	87	87	67	70
- Second pairing (F _{2B})	86	90	83	87	87	87	79	86
Mean gestation length (days)								
- First pairing (F _{2A})	–	22.7	–	22.6	–	22.8	–	23.0
- Second pairing (F _{2B})	–	22.6	–	22.6	–	22.7	–	22.7
F₂ animals								
Bleeding from vagina before parturition (first pairing F _{3A})	–	1	–	2	–	2	–	6
% mating								
- First pairing (F _{3A})	93	97	90	100	90	100	87	97
- Second pairing (F _{3B})	83	93	83	90	97	100	70	90
Conception rate (%)								
- First pairing (F _{3A})	89	90	100	100	93	93	81	83
- Second pairing (F _{3B})	92	86	83	81	86	83	100	100
Fertility index (%)								
- First pairing (F _{3A})	83	87	90	100	83	93	70	80
- Second pairing (F _{3B})	77	80	69	73	83	83	70	90

Table 31 (continued)

	0 ppm		30 ppm		70 ppm		170 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Mean gestation length (days)								
- First pairing (F _{3A})	–	22.6	–	22.9	–	22.9	–	23.0
- Second pairing (F _{3B})	–	22.6	–	22.7	–	22.8	–	22.8

–: not applicable; *** *P*: < 0.001

Source: Tesh, Willoughby & Secker (1986)

Table 32. Effects in rat pups in a three-generation reproductive toxicity study

	0 ppm		30 ppm		70 ppm		170 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
F₁ pups								
Litter size (day 1)								
- F _{1A}		13.6		13.1		13.1		12.0*
- F _{1B}		13.6		12.9		12.8		14.2
Live birth index (%)								
- F _{1A}		98		98		97		93
- F _{1B}		93		93		97		98
Viability index day 25 (%)								
- F _{1A}		84		86		92		90
- F _{1B}		77		94		81		84
Birth weight (g)								
- F _{1A}		5.8		5.8		5.9		6.1
- F _{1B}		5.8		6.0		6.1		6.0
F₂ pups								
Litter size (day 1)								
- F _{2A}		12.1		12.6		12.9		12.6
- F _{2B}		12.9		12.6		12.7		12.6
Live birth index (%)								
- F _{2A}		97		95		92		95
- F _{2B}		100		100		93		89
Viability index day 25 (%)								
- F _{2A}		82		79		69		82
- F _{2B}		87		85		80		90
Birth weight (g)								
- F _{2A}		6.3		6.3		6.2		6.2
- F _{2B}		6.1		5.9		5.9		6.3
F₃ pups								
Litter size (day 1)								
- F _{3A}		11.8		11.7		12.5		11.9
- F _{3B}		11.5		12.5		12.3		12.4

	0 ppm		30 ppm		70 ppm		170 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Live birth index (%)								
- F _{3A}		99		95		91 (-8%)		72*** (-27%)
- F _{3B}		97		99		92 (-5%)		85 (-12%)
Viability index day 25 (%)								
- F _{3A}		94		90		80 (-13%)		57*** (-37%)
- F _{3B}		88		93		97		66* (-25%)
Birth weight (g)								
- F _{3A}		6.2		6.4		5.9 (-5%)		5.7* (-8%)
- F _{3B}		6.7		6.4		6.4 (-4%)		5.8* (-13%)

*: $P < 0.05$; ***: $P < 0.001$

Source: Tesh, Willoughby & Secker (1986)

ammonium sulfide to detect implantations. All viable fetuses were weighed, sexed and examined for external malformations and variations. Approximately half of each litter was placed in Bouin's fixative and examined for visceral malformations and variations, and the remaining half of the fetuses were fixed in ethanol and stained for examination for skeletal malformations and variations. In addition, the following organs of the dams were weighed: liver, kidney, spleen, ovaries and adrenals.

Maternal effects were observed at dose levels of 35 and 120 mg/kg bw per day and consisted of significant reductions in body weight gain, feed consumption and water intake and significant increases in spleen and liver weights (Table 33). In the 120 mg/kg bw per day dose group, a statistically significant decrease in body weight gain was observed for several days immediately after the start of dosing (GD 6). Such an effect was not observed at 35 mg/kg bw per day. At these dose levels, a reduction in the number of viable fetuses, a reduction in fetal weight, an increase in the number of late resorptions and increased placental weight were also observed. At the highest dose, there was an increase in fetuses with 14th rudimentary rib; in the low- and mid-dose groups, the percentage of fetuses affected was comparable to that in controls. The incidences of renal pelvic dilatation showed no dose-response relationship in the percentage of fetuses affected. Therefore, these histopathological findings were not considered adverse.

The NOAEL for maternal toxicity was 10 mg/kg bw per day, based on reduced body weight gain and feed consumption, increased placental weight and increased liver and spleen weights at 35 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 10 mg/kg bw per day, based on a reduction in the number of viable fetuses, a reduction in fetal weight and an increase in the number of late resorptions at 35 mg/kg bw per day.

The total litter loss, mainly due to late resorptions in one high-dose female, might potentially have been caused by a teratological effect in the fetuses. However, as placental weight and the number of late resorptions increased as the dose level increased, it was considered that the late resorption had occurred as a consequence of damage to the placenta by the test substance, rather than a direct effect on fetuses. Therefore, it is considered that triflumizole did not produce a teratogenic response in this study (Nishibe et al., 1983f).

In a developmental toxicity study in rats, groups of 24 pregnant female Sprague-Dawley (Crj:CD) rats were treated with triflumizole (purity 98.3%; lot no. TK4121) by gavage at a dose level of 0, 3, 7 or 35 mg/kg bw per day. The study was performed partly in accordance with OECD Test Guideline 414. Rats were dosed from GD 6 up to GD 16, but a caesarean section was not performed until GD 20. OECD guidelines state that doses should be administered daily from implantation to the day before caesarean section. Females were examined daily throughout the study for mortality and clinical signs. Body weight and feed consumption were recorded daily on GDs 0–20. Water intake

Table 33. Maternal and fetal toxicity in a developmental toxicity study in rats

Parameter	0 mg/kg bw per day	10 mg/kg bw per day	35 mg/kg bw per day	120 mg/kg bw per day
Maternal effects				
Body weight gain days 0–20 (g)	139.4	137.1 (–2%)	117.1*** (–16%)	111.5*** (–20%)
Organ weights				
- Liver, absolute (g)	14.93	14.91	14.97	15.38
- Liver, relative (%)	4.55	4.62	4.81*	5.07***
- Spleen, absolute (g)	0.680	0.659	0.751*	0.774***
- Spleen, relative (%)	0.207	0.204	0.242***	0.256***
Litter response				
Live fetuses/ pregnant female	14.3	15.0	11.5** (–20%)	11.5** (–20%)
Fetal weight (g)				
- Males	3.8	3.7	3.5** (–6%)	3.5** (–7%)
- Females	3.5	3.5	3.3 (–5%)	3.2** (–8%)
Placental weight (g)	0.46	0.49 (+7%)	0.79*** (+73%)	0.85*** (+86%)
Dead or resorbed fetuses ^a	15 (4.2%)	16 (4.3%)	83*** (23.2%)	87*** (24.0%)
- Early deaths	14 (3.9%)	16 (4.3%)	17 (4.7%)	14 (3.9%)
- Late deaths	1 (0.3%)	0 (0%)	66*** (18.4%)	73*** (20.1%)
Examination of the fetuses^a				
Skeletal findings				
- 14th rudimentary rib (% of fetuses)	11 (6%)	16 (9%)	16 (11%)	55* (37%)
Visceral findings				
- Renal pelvic dilatation (% of fetuses)	13 (8%)	29* (17%)	24 (19%)	25* (20%)

*: $P < 0.05$; **: $P < 0.01$; *** $P < 0.001$ ^a Number of fetuses, with percentage of fetuses in parentheses.

Source: Nishibe et al. (1983f)

was measured on GDs 1, 6, 11, 16 and 20. On GD 20, females were killed and subjected to a caesarean section and gross necropsy. The uterus was removed and opened, and fetuses were removed. Numbers of viable fetuses, early and late resorptions, total implantations and number of corpora lutea were recorded. Uteri from apparently non-pregnant females were stained with ammonium sulfide solution for confirmation of pregnancy status. All viable fetuses were weighed, sexed and examined for external malformations and variations. The total numbers of fetuses examined (number of litters) were 339 (24), 343 (23), 342 (24) and 317 (24) for the 0, 3, 7 and 35 mg/kg bw per day groups, respectively. Approximately half of the fetuses were placed in Bouin's fixative and examined for visceral malformations and variations, and the remaining half of the fetuses were fixed in ethanol and stained for examination for skeletal malformations and variations.

Maternal toxicity was evident at 35 mg/kg bw per day. Body weight gain was reduced compared with controls over GDs 17–18 and over the dosing interval (–18% and –15%, respectively; $P < 0.05$). The reduction in mean body weight gain was accompanied by reductions in feed consumption on GD 7 and daily over GDs 12–19, ranging from –9% to –16% of control values. No statistically significant differences were noted in absolute body weight or water intake. Changes in organ weights were not definitive of adverse effects of treatment. The mean absolute right adrenal weight was significantly decreased, but was not accompanied by changes in relative weight. The mean left ovary weight relative to body weight was significantly increased, but the right relative ovary weight was comparable to that of controls. The placental weight was significantly increased compared with controls (+45%; $P < 0.01$). Gross necropsy did not reveal any adverse effects of treatment. No adverse effects of treatment were observed in females treated with 3 or 7 mg/kg bw per day.

Evidence of developmental toxicity was seen at 35 mg/kg bw per day. The incidence of late resorptions/dead fetuses was significantly ($P < 0.05$) increased (17 versus 0 for controls; 4.8% versus 0% for controls). Although the number of viable fetuses was slightly reduced compared with controls (13.2 versus 14.1 for controls), this reduction did not attain statistical significance. Fetal weight was not affected (Table 34). No statistically significant, treatment-related external, visceral or skeletal malformations or variations were noted.

Table 34. Fetal toxicity in a developmental toxicity study in rats

	0 mg/kg bw per day	3 mg/kg bw per day	7 mg/kg bw per day	35 mg/kg bw per day
Litter response				
Live fetuses/ pregnant female	14.1	14.9	14.3	13.2 (–6%)
Dead or resorbed fetuses	23	13 (–43%)	24	35 (+52%)
- Early deaths	23	12	21	18
- Late deaths	0	1	3	17*

*: $P < 0.05$; **: $P < 0.01$

Source: Gotoh (1986)

The maternal toxicity NOAEL was 7 mg/kg bw per day, based on reductions in body weight gain and feed consumption and increased placental weights at 35 mg/kg bw per day. The embryo/fetal toxicity NOAEL was 7 mg/kg bw per day, based on increased numbers of late resorptions/dead fetuses at 35 mg/kg bw per day (Gotoh, 1986).

Rabbits

In a non-GLP developmental toxicity study in rabbits, groups of 15 pregnant female New Zealand White rabbits were treated with triflumizole (purity 98.7%; lot no. TK3081) by gavage at a dose level of 0, 50, 100 or 200 mg/kg bw per day. The study was performed partly in accordance with OECD Test Guideline 414. Deviations from the guideline were the following: 1) only 15 female rabbits per group were used instead of the recommended 20; and 2) guidelines state that doses should be administered daily from implantation to the day before caesarean section; in this study, the last day of dosing was GD 18, but the caesarean section was not conducted until GD 29. The rabbits were observed for clinical signs and weighed every 2 days, except during GDs 6–18, when they were weighed daily. Feed consumption was generally measured over 3-day intervals spanning GDs 1–29, and water consumption was generally measured over 2-day intervals between GDs 2 and 29. On GD 29, the rabbits were killed and subjected to a caesarean section and gross necropsy. The uterus was removed and opened, and the types of implantations (live and dead fetuses, resorptions) were counted and their relative positions recorded. The ovaries were also removed, and corpora lutea were counted and recorded. Viability of the fetuses was monitored for 24 hours after the caesarean section. Then all

fetuses were processed and examined for visceral and skeletal malformations and variations. The total numbers of fetuses examined (number of litters) were 100 (13), 87 (14), 105 (13) and 109 (14) for the 0, 50, 100 and 200 mg/kg bw per day groups, respectively.

Treatment with triflumizole produced maternal toxicity, as shown by reduced body weight and feed consumption at 200 mg/kg bw per day. A slight, temporary depression of feed consumption was also recorded at 100 mg/kg bw per day. Absolute body weight in the high-dose group was significantly decreased ($P \leq 0.05$) compared with controls on GD 8 and again on GD 11, continuing to study termination on GD 29. The reductions in absolute body weight ranged from 4% on GD 11 to a maximum of 6% on GD 18. Body weight gain over the study period of GDs 0–29 was reduced by 25% compared with that of controls (not statistically significant). Calculation of body weight gain over the dosing interval of GDs 6–18 revealed a 57% reduction in body weight gain of high-dose females compared with the controls. The reductions in absolute body weight and body weight gain in high-dose females were accompanied by reduced feed consumption over the dosing intervals of GDs 8–10, 12–14 and 16–18 (22%, 30% and 35%, respectively). Feed consumption was also reduced in the 50 mg/kg bw per day group on GDs 8–10 (13%) and in the 100 mg/kg bw per day group over GDs 12–14 (32%), but these reductions are not considered an adverse effect of treatment because they were not correlated with reductions in body weight. Gross necropsy revealed changes in the high-dose group, including increased absolute liver weight (15%), reduced absolute ovary weights (22%) and reduced placental weights (–16%) (Table 35).

Table 35. Maternal and fetal toxicity in a developmental toxicity study in rabbits

	0 mg/kg bw per day	50 mg/kg bw per day	100 mg/kg bw per day	200 mg/kg bw per day
Maternal effects				
Weight (g)				
- Final body weight	3 853	3 725	3 753	3 645**
- Liver weight	92	94	92	106** (+15%)
- Ovary weight	0.96	0.83	0.90	0.75** (–22%)
Placental weight (g)				
- Male fetuses	5.1	4.7	4.5	4.3* (–16%)
- Female fetuses	4.9	4.7	4.4	4.1* (–16%)
Litter response				
Pup weight (g)				
- Males	37.5	35.9	36.1	32.3** (–13%)
- Females	36.2	35.5	34.3	31.6* (–14%)
24 h survival rate (%)	98.0	90.8	86.7	77.1* (–21%)

*, $P < 0.05$; **, $P < 0.01$

Source: Furuhashi (1985)

Treatment with triflumizole did not result in any external, visceral or skeletal malformations. Pups from the high-dose group had a reduced 24-hour survival rate (77% compared with 98% for controls) and statistically significantly reduced pup weight ($P < 0.01$ for males; $P < 0.05$ for females)

when compared with controls (Table 35). Values were also low when compared with the laboratory historical control data. No other effects on reproduction or fetal development were noted.

In conclusion, the maternal toxicity NOAEL was 100 mg/kg bw per day, based on reductions in body weight gain and feed consumption, increased liver weight and decreased ovary and placental weights compared with the controls at 200 mg/kg bw per day. The embryo/fetal toxicity NOAEL was 100 mg/kg bw per day, based on a reduced 24-hour survival rate and reductions in pup weight compared with controls at 200 mg/kg bw per day (Furuhashi, 1985).

In a developmental toxicity study in rabbits, groups of 16 pregnant female New Zealand White rabbits were treated with triflumizole (purity 98.3%; lot no. TK4121) by gavage at a dose level of 0, 5, 25 or 50 mg/kg bw per day at GDs 7–19. The study was performed partly in accordance with OECD Test Guideline 414. Deviations from the guideline included the following: 1) only 16 female rabbits per group were used instead of the recommended 20; 2) feed consumption was not measured; and 3) OECD guidelines state that doses should be administered daily from implantation to the day before caesarean section; in this study, the last day of dosing was GD 19, with a caesarean section conducted on GD 29. Females were examined twice daily throughout the study for mortality and clinical signs. Body weights were recorded on GDs 0, 7, 13, 20, 24 and 29; feed consumption was not recorded. On GD 29, the rabbits were killed and subjected to a caesarean section and gross necropsy. The uterus and ovaries were exposed, and the number and location of viable and non-viable fetuses, resorptions and total numbers of implantations and corpora lutea were recorded. The uterus was removed and weighed, and fetuses were removed. The uterus of each apparently non-pregnant female was opened and stained with ammonium sulfide to detect implantations. All fetuses were weighed, sexed and examined for external malformations and variations. Viable fetuses were retained for 24 hours for viability determination, after which they were sacrificed for examination. All fetuses were then processed and examined for visceral and skeletal malformations/variations. The total numbers of fetuses examined (number of litters) were 128 (15), 110 (15), 116 (15) and 118 (16) for the 0, 5, 25 and 50 mg/kg bw per day groups, respectively.

There was no clear evidence of maternal toxicity at any of the doses tested. All the female rabbits survived to study termination, and no adverse clinical signs were observed; although there was evidence of reduction in body weight gain in the high-dose group over days 7–13, this was not statistically significant. Overall, no statistically significant differences were noted in mean absolute body weight or body weight gain of treated female rabbits; feed consumption was not recorded. No changes were evident during examination at gross necropsy.

Treatment with triflumizole did not result in any adverse effects on reproduction and did not produce external, visceral or skeletal malformations. Two rabbits from the low-dose group and one rabbit from the high-dose group delivered early, but this was not considered to be related to treatment because of the lack of a dose–response relationship and low incidence. No effects on fetal development were noted.

In conclusion, based on the lack of statistically significant effects seen in this limited study, the maternal toxicity NOAEL was set at 50 mg/kg bw per day, the highest dose tested; a LOAEL could not be determined. The embryo/fetal toxicity NOAEL was 50 mg/kg bw per day, the highest dose tested; a LOAEL could not be determined (Keller, 1988).

2.6 *Special studies*

(a) *Neurotoxicity*

In an acute neurotoxicity study, groups of 11 male and 11 female CD (CrI: CD[®] (SD)IGS BR) rats were treated with a single dose of triflumizole (purity 99.2%; lot no. TBC-343) by gavage. The study was performed in accordance with OECD Test Guideline 424. The rats were segregated by sex, divided into groups of 11 and administered the following doses orally: 25, 100 and 400 mg/kg bw for males and 25, 100 and 200 mg/kg bw for females. Detailed clinical observations and functional

observational battery evaluations were performed on days -4, 1 (before and 2 hours after test substance administration), 8 and 15. Motor activity evaluations were conducted immediately following each functional observational battery interval. Individual body weights were measured on day -1 and weekly during the study. Following neurobehavioural evaluations, six animals of each sex per dose were randomly selected for neuropathology.

One male in the 400 mg/kg bw group and one female in the 200 mg/kg bw group were found dead on day 4 of the study (Table 36). Postmortem evaluations did not reveal the cause of death of the two deceased animals.

A few animals at 100 mg/kg bw had decreased activity within 3–6 hours after dosing. At 400 mg/kg bw in males and 200 mg/kg bw in females, decreased activity, shallow/slow breathing and black/yellow discoloured hair in the anogenital or scrotal region were observed in some animals within 3–6 hours after dosing, and some males were noted with skin cold to touch. The male that died had clinical signs similar to those of other males in that dose group on days 1 and 2. The female that died had similar clinical signs; in addition, impaired limb function and impaired righting reflex were found at 3 hours post-dosing on days 1 and 2. One other female at 200 mg/kg bw had impaired limb function at 3 hours after dosing. One male at 400 mg/kg bw had decreased activity on day 8 (Table 36). No other clinical findings were seen. Body weight was not affected by exposure to triflumizole.

Functional observational battery evaluations showed some statistically significant and dose-dependent changes on day 1. At 100 mg/kg bw, the following signs were observed: decreased rearing counts (females), decreases in mean forelimb and/or hindlimb grip strength (males and females) and decreased body temperature (females). Effects in the high-dose males and females (400 mg/kg bw for males and 200 mg/kg bw for females) were decreased rearing counts, changes in gait, mobility and righting reflex, decreases in mean forelimb and/or hindlimb grip strength, increase in thermal response and decreased body temperature. Motor activity evaluations revealed statistically significant and dose-dependent decreases in all motor activity parameters (horizontal activity, vertical activity, stereotypy activity, total distance) in mid- and high-dose animals on day 1. On day 8, high-dose males demonstrated increases in motor activity parameters (Table 36).

No clinical signs of acute neurotoxicity were observed in animals dosed with 25 mg/kg bw. The NOAEL was 25 mg/kg bw, based on the clinical findings and the functional and motor activity effects observed at the next higher dose (Goldenthal, 2003).

In a repeated-dose neurotoxicity study, groups of 16 male and 16 female CD (CrI: CD[®] (SD)IGS BR) rats were treated with doses of triflumizole (purity 99.2%; lot no. TBC-343) in the diet for 13 weeks. The study was performed in accordance with OECD Test Guideline 424. Dose levels were 0, 70, 700 and 2000 ppm (equal to 0, 4.1, 41 and 117 mg/kg bw per day for males and 0, 4.9, 48 and 133 mg/kg bw per day for females, respectively) and were based on the results of unspecified previous studies. All animals were observed twice a day for morbidity, injury and availability of food and water. A detailed clinical examination of each animal was performed once during each study week. Ophthalmoscopic examinations were performed pretest and prior to scheduled euthanasia, and body weight and feed consumption measurements were conducted weekly. Blood was collected pretest, in week 4 and at study termination from 10 animals of each sex per group. Functional observational battery examinations were conducted on 10 animals of each sex per group prior to initiation of exposure to the test article and during weeks 4, 8 and 13 of test article administration. The same animals were tested at all time points. All 10 animals of each sex per group designated for behavioural testing were also tested for motor activity prior to initiation of exposure to the test article and during weeks 4, 8 and 13. Following the functional observational battery and motor activity evaluations, six rats of each sex per group were randomly selected for neuropathological evaluation from the 10 rats of each sex per group designated as behavioural test animals. Complete necropsies were performed on these animals. The brain (including cerebrum, cerebellum/pons and medulla

Table 36. Effects in rats following single oral administration

	0 mg/kg bw per day		25 mg/kg bw per day		100 mg/kg bw per day		400 mg/kg bw per day	200 mg/kg bw per day
	Males	Females	Males	Females	Males	Females	Males	Females
Mortality	0/11	0/11	0/11	0/11	0/11	0/11	1/11	1/11
Clinical signs^a								
Decreased activity	1/11	0/11	1/11	0/11	3/11	4/11	9/11	9/11
Shallow breathing	0/11	0/11	0/11	0/11	0/11	0/11	5/11	3/11
Slow breathing	0/11	0/11	0/11	0/11	0/11	0/11	4/11	4/11
Discoloured hair	0/11	0/11	1/11	1/11	0/11	0/11	4/11	2/11
Skin cold to touch	0/11	0/11	0/11	0/11	0/11	0/11	4/11	0/11
Righting reflex impaired	0/11	0/11	0/11	0/11	0/11	0/11	0/11	1/11
Limb function impaired	0/11	0/11	0/11	0/11	0/11	0/11	0/11	2/11
Functional observational battery^a								
Rearing counts	–	–	No effect	No effect	No effect	dc (28%)	d (77%)	dc (63%)
Gait, mobility, righting reflex	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
Grip strength forelimbs	–	–	No effect	No effect	dc (27%)	dc (19%)	dc (68%)	dc (38%)
Grip strength hindlimbs	–	–	No effect	No effect	dc (35%)	d (11%)	dc (46%)	d (21%)
Thermal response time	–	–	No effect	No effect	No effect	No effect	ic (132%)	ic (158%)
Tail pinch response	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0
Body temperature	–	–	No effect	No effect	No effect	dc (5%)	dc (7%)	dc (8%)
Motor activity measurements								
<i>Day 1</i>								
All parameters ^b	–	–	No effect	No effect	dc (~43–84%)	dc (~50–70%)	dc (~80–97%)	dc (~80–95%)
<i>Day 8</i>								
Horizontal activity (11–30 min)	–	–	No effect	No effect	No effect	No effect	ic (~79%)	No effect
Stereotypy (11–30 min)	–	–	No effect	No effect	No effect	No effect	ic (~86%)	No effect
Total distance (11–20 min)	–	–	No effect	No effect	No effect	No effect	ic (~119%)	No effect
<i>Day 15</i>	No treatment-related effects							

d: decreased, but not statistically significantly, compared with the controls; dc: statistically significantly decreased compared with the controls; ic: statistically significantly increased compared with the controls

^a Incidences on day 1, hardly any effects on days 8 and 15.

^b Horizontal and vertical activity, stereotypy and total distance.

oblongata), proximal sciatic nerve (2), sural nerve (2), tibial nerve (2), spinal cord including cervical swelling (C3–C6) and lumbar swelling (L1–L4), trigeminal ganglia (2), dorsal root ganglia (C3–C6, L1–L4) and dorsal and ventral root fibres (C3–C6, L1–L4) were collected. After fixation, both sciatic nerves with tibial, fibular and sural extensions were dissected free from the carcass from control and high-dose animals. No neuropathology animals died on study or were euthanized in extremis. For the remaining animals (10 of each sex per group), complete necropsy examinations were performed. No cholinesterase activities were measured.

All rats survived to scheduled termination. Body weights were slightly higher (10%) in males at 700 ppm and lower (8%) in females at 2000 ppm. Feed consumption was lower (9%) in females at 2000 ppm. Slight decreases in red blood cells, haemoglobin and haematocrit were observed in males and females at 2000 ppm at both intervals of analysis and in females at 700 ppm only in week 4. Increases in reticulocytes were observed in males and females at 2000 ppm at 4 and 13 weeks and at 700 ppm in week 4. Changes in locomotor activity, general arousal and rearing in males were observed without a clear dose–response relationship (Table 37). Increased liver weights were observed in males and females at 700 and 2000 ppm (Table 38). The decrease (> 10%) in uterus weight observed in the mid- and high-dose groups could not be explained by a change in body weight, as only the body weight in the high-dose group was decreased. In contrast, none of the other repeated-dose studies in rats showed an effect on the uterus. Therefore, this decrease in uterus weight, in the absence of any histopathology, is considered not treatment related. The increase in relative kidney weight was only slight and therefore not considered treatment related. Liver hypertrophy was seen in all males at 70, 700 and 2000 ppm and in females at 700 and 2000 ppm. Periportal hepatocellular vacuolation was observed at 700 and 2000 ppm (Table 39).

Table 37. Effects in rats following 13-week dietary exposure

Parameter	0 ppm		70 ppm		700 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Functional observational battery								
Rearing								
- Week 4	4.2 ± 3.5	No effects	8.4 ± 5.4	No effects	9.3 ± 6.0	No effects	12.6 ± 6.3*	No effects
- Week 13	3.7 ± 4.8	No effects	6.4 ± 4.0	No effects	12.9 ± 2.6*	No effects	8.5 ± 5.7	No effects
Motor activity measurements^a								
Horizontal activity	–	–	ic (87%)	No effects	ic (86%)	No effects	ic (92%)	No effects
Stereotypy activity	–	–	ic (94%)	No effects	ic (92%)	No effects	ic (88%)	No effects
Total distance	–	–	ic (136%)	No effects	ic (119%)	No effects	ic (157%)	No effects
Vertical activity	–	–	ic (58%)	ic (20%)	ic (58%)	ic (27%)	ic (46%)	ic (22%)

ic: statistically significantly increased compared with the controls; *: $P < 0.05$

^a Data from week 13, consistent statistically significant changes observed only in week 13.

Source: Goldenthal (2004)

Table 38. Liver weights in rats following 13-week dietary exposure

	0 ppm		70 ppm		700 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Absolute liver weight (g)								
Week 13								
- Mean	14.378	8.194	16.291	9.033	19.810** (+38%)	9.762** (+19%)	22.767** (+58%)	10.812** (+32%)
- Standard deviation	2.857	0.483	2.075	1.233	2.766	0.972	3.839	1.353
Relative liver weight (%)								
Week 13								
- Mean	2.971	3.137	3.050	3.312	3.589** (+21%)	3.623** (+15%)	4.504** (+53%)	4.439** (+41%)
- Standard deviation	0.275	0.170	0.261	0.278	0.364	0.294	0.533	0.389

**: $P < 0.01$

Source: Goldenthal (2004)

Table 39. Pathology findings in rats following 13-week dietary exposure

	0 ppm		70 ppm		700 ppm		2 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
Pathology								
<i>Kidney</i>								
Chronic progressive nephropathy								
- Minimal	1/10	0/10	3/10	2/10	5/10	0/10	5/10	0/10
<i>Liver</i>								
Hypertrophy centrilobular								
- Minimal	0/10	0/10	10/10	0/10	5/10	8/10	0/10	0/10
- Mild	0/10	0/10	0/10	0/10	5/10	2/10	0/10	10/10
Hypertrophy panlobular								
- Minimal	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10
- Mild	0/10	0/10	0/10	0/10	0/10	0/10	8/10	0/10
Vacuolation periportal								
- Minimal	0/10	0/10	0/10	0/10	1/10	3/10	7/10	6/10
- Mild	0/10	0/10	0/10	0/10	0/10	1/10	3/10	2/10
Neuropathology								
<i>Tibial nerve</i>								
Axonal/myelin degeneration								
- Minimal	2/6	0/6	Not assessed		Not assessed		2/6	1/6

Source: Goldenthal (2004)

When the results of the motor activity measurement are viewed, it is apparent that habituation has occurred. Habituation, defined as a decrement in some natural response to a stimulus after repeated presentations, is apparent when an animal's locomotor behaviour declines with experience in the test cage within a single test session. However, habituation is also apparent with a decline in locomotion between sessions. This inter-session decline in activity counts provides evidence of previous experience in the test environment. In this study, there appears to be both within-session habituation as well as across-session habituation in the series of four sessions (especially in control rats). The male treatment groups showed a within-session decrement in activity. However, in contrast to male and female control rats or to the female test article-treated rats, male test article-treated rats failed to show a very robust across-session habituation. A clear inter-session habituation was, however, also not observed in the female control and treatment groups.

For motor activity measurements, consistent statistically significant changes were observed only in week 13. However, although the dose level increased from 70 to 2000 ppm, there was no dose-response relationship with regard to increased motor activity. Additionally, historical control data from other laboratories (no historical control data available from the performing laboratory) indicate that the motor activity of treated rats falls within the historical control range.

A statistically significant change in rearing was noted in males at 700 and 2000 ppm at weeks 13 and 4, respectively. These effects were most likely related to the pretest difference in total number of rears between control rats and do not reflect test article-related changes in the functional behaviour. There was no trend in the data, and there was no dose or time dependency. These effects, although statistically significant, are not considered to be physiologically relevant. In conclusion, the effects on motor activity are considered not adverse because there is no dose-response relationship, there is no effect in females and the locomotor changes are within the normal range of behaviour. Furthermore, it should be noted that in the 2-year rat study, female rats appeared to be more sensitive than males to the neurotoxic effects of triflumizole: a statistically significant increase of convulsive episodes was observed in females of the highest-dose group, but not in males.

The study is generally considered acceptable, as it was designed and executed in accordance with OECD Test Guideline 424, except that cholinesterase activity was not measured. Additionally, electroencephalograms have been not made, which could give an early indication of the occurrence of convulsions. It should be noted that overt convulsions were observed only in the chronic study more than 13 weeks after initiation of exposure.

Based on the liver effects at 700 ppm (equal to 41 mg/kg bw per day), the NOAEL for non-neurotoxic effects was 70 ppm (equal to 4.1 mg/kg bw per day). The NOAEL for neurotoxicity was 2000 ppm (equal to 117 mg/kg bw per day), the highest dose tested (Goldenthal, 2004).

(b) *Immunotoxicity*

In an immunotoxicity study, triflumizole (purity 99.8%; lot no. THJ-933) was administered for 28 days to BALB/c female mice in the diet at a dose level of 0, 20, 200 or 2000 ppm (equal to 0, 4.4, 43 and 413 mg/kg bw per day). Groups of 10 female mice per dose were used in the sheep red blood cell (sRBC) assay, whereas groups of 8 female mice per dose were used for the natural killer (NK) cell assay. The 1st day of dietary administration was designated as day 1. Mice were monitored daily for toxicity and were weighed on day 1 and weekly thereafter. Mice in the sRBC cohort were immunized on day 24 with sRBCs (0.2 mL/mouse of 1×10^8 sRBCs/mL). On day 29, all animals were euthanized, and body, spleen and thymus weights were recorded. Mice in the sRBC cohort had blood collected in tubes without anticoagulant. Blood was allowed to clot for approximately 30 minutes, followed by centrifugation at $2000 \times g$ for 10 minutes. Serum was collected and stored at -20°C until used for the assay. For mice in the NK cell assay cohort, the spleen was collected and prepared for evaluation of NK activity. Positive controls for the sRBC assay were treated with cyclophosphamide monohydrate at 15 mg/kg bw by intraperitoneal administration once daily for 5 days (days 24–28). Positive controls for the NK assay were treated with 10 μL reconstituted polyclonal antibody in 0.9% saline (per 2 mL dose) by intravenous administration on day 28.

All animals survived to study termination, and no clinical signs were noted. There did not appear to be any definitive treatment-related changes in water consumption; it appeared that significant amounts of spillage occurred during the movement of the cages or water bottles. Water consumption in the high-dose group was slightly reduced compared with controls, ranging from -8% to -13% over weeks 2-4, but it is not known if these values are statistically significant. Feed consumption was not affected by treatment. Mean body weight was slightly reduced in the high-dose group compared with controls over days 8-29, but the decreases were not biologically significant, ranging from -3% to -7% of controls. Although differences in mean absolute body weight did not appear to be biologically significant, body weight gain of mice in the high-dose group was biologically significant, with a reduction of -53% when compared with control weight gain. Absolute and relative spleen and thymus weights were not affected by treatment with triflumizole. Anti-sRBC immunoglobulin M (IgM) was significantly decreased in the high-dose group (-45%, $P < 0.05$). Although IgM was also decreased in the 200 ppm group, the difference did not attain statistical significance. NK cell activity was not affected by treatment. The cyclophosphamide positive control group had significantly decreased absolute and relative spleen and thymus weights and reduced IgM. The polyclonal antibody positive control group had significantly decreased absolute and relative spleen weights and reduced NK cell activity.

In conclusion, the NOAEL for systemic toxicity was 200 ppm (equal to 43 mg/kg bw per day), based on significantly reduced body weight gain at 2000 ppm (equal to 413 mg/kg bw per day). The NOAEL for immunotoxicity was 200 ppm (equal to 43 mg/kg bw per day), based on a significant reduction in anti-sRBC IgM in mice immunized with sRBCs at 2000 ppm (equal to 413 mg/kg bw per day) (Burlison, 2010).

(c) *Effects on enzymes and other biochemical parameters*

In a study designed to assess hepatic microsomal enzyme induction in rats, groups of five rats (rat strain not provided) of each sex per dose were administered triflumizole (purity 98.3%; lot no. TK-4121) in 0.5% carboxymethyl cellulose by oral gavage for 5 consecutive days at a dose of 0 or 200 mg/kg bw per day. Groups of five positive control rats of each sex were administered phenobarbital sodium (PB) dissolved in water at 75 mg/kg bw per day or 3-methylcholanthrene (3-MC) dissolved in sesame oil at 25 mg/kg bw per day by intraperitoneal injection on 4 consecutive days. Following the treatment period, each rat was anaesthetized and the liver perfused in situ for microsomal assays. Following perfusion, the liver was removed and weighed, and a section was removed from the left lateral lobe and weighed for use in hepatic microsomal determinations. Measurements included total amount of microsomal protein, amounts of cytochrome P448/P450 on a per milligram protein basis as well as a per gram liver basis, and activities of the following enzymes: *p*-nitroanisole *O*-demethylase (PNAS), 7-ethoxycoumarin *O*-deethylase (7-EC) and 7-ethoxyresorufin *O*-deethylase (EROD).

No mortality was observed in any of the groups. Ataxia was noted on days 1-3 of treatment in the PB group. No clinical signs were noted in any other groups. Treatment with triflumizole did not result in any definitive adverse effects on body weight.

Mean absolute and relative liver weights were increased ($P < 0.05$) in the triflumizole-treated males and females (+32% and +24%, respectively), males and females in the PB group (+17% and +24%, respectively) and males and females in the 3-MC group (+31% and +38%, respectively), compared with the control group (Table 40).

Other increases in the triflumizole, PB and 3-MC groups compared with the control group included microsomal P448/P450 concentrations per milligram protein (males: +73%, +67% and +60%, respectively; females: +15%, +39% and +58%, respectively); concentration of P450 per gram liver (males: +90%, +122% and +75%, respectively; females: +86%, +108% and +88%, respectively); and concentration of P450 on a total liver basis (males: +142%, +176% and +136%, respectively; females: +135%, +175% and +169%, respectively) (Table 41).

Table 40. Body and liver weights in rats

	Control		Triflumizole 200 mg/kg bw per day		PB 75 mg/kg bw per day		3-MC 25 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Body weight (g)	299	226	289	228	319	241	309	234
Absolute liver weight (g)	12.36	9.34	15.62**	11.81*	15.36**	12.37*	16.66*	13.37*
Liver weight relative to body weight (%)	4.11	4.14	5.41*	5.15*	4.81**	5.13*	5.39*	5.72*

3-MC: 3-methylcholanthrene; PB: phenobarbital sodium; *: $P < 0.01$; **: $P < 0.05$

Source: Johnson (1988)

Table 41. Hepatic microsomal enzyme induction in rats

	Control		Triflumizole 200 mg/kg bw per day		PB 75 mg/kg bw per day		3-MC 25 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Lowry protein (mg/mL)	2.24	2.15	3.34	3.32*	3.32	3.39*	3.40	2.57
Cytochrome P448/450 ($\mu\text{mol}/\text{mg}$ protein)	0.63	0.65	1.09	0.85	1.05	1.03	1.01	1.17
P450 ($\mu\text{mol}/\text{g}$ liver)	3.75	3.14	7.15	6.35	8.31	7.10	6.53	6.40

3-MC: 3-methylcholanthrene; PB: phenobarbital sodium; *: $P < 0.01$

Source: Johnson (1988)

When the effects of treatment on the activities of the enzymes are examined, triflumizole, PB and 3-MC increased the activity levels of PNAS (overall group activity increased by 2.1-, 2.4- and 2.8-fold, respectively) and 7-EC (increased by 1.9-, 2.2- and 8.3-fold, respectively). Triflumizole and PB did not increase the activity of EROD, whereas 3-MC treatment increased the overall activity by 48-fold (Table 42).

In summary, the results of this study demonstrated that treatment with triflumizole at 200 mg/kg bw per day for 5 days increased liver weight and microsomal P448/P450 levels, although the increases in microsomal levels on a per milligram protein, per gram of liver and per total liver basis did not often attain statistical significance. The increased liver weight and P448/P450 activity levels indicate that triflumizole treatment increased metabolic capacity. PNAS activity, indicative of P450, and 7-EC activity, indicative of P448/P450, were significantly increased, whereas triflumizole did not affect the activity levels of EROD (a P448 indicator). This suggests that triflumizole acts more like a PB-type inducer than a 3-MC-type inducer (Johnson, 1988).

(d) Studies on metabolites

The acute oral toxicity of 10 metabolites of triflumizole was assessed in Slc:SD rats in a non-GLP study. The test substances were administered in a single dose by gavage. The tested metabolites were FD-1-1, FD-2-1, FD-6-1, FD-7-1, FM-2-1, FM-5-1, FM-6-1, FM-8-1, FA-1-1 and FA-1-5. Of these metabolites, FA-1-1 and FD-1-1 were defined as metabolites relevant for the environment. Metabolites FM-5-1, FM-6-1, FD-1-1, FM-8-1, FD-6-1, FD-2-1, FD-7-1 and FA-1-1 were identified in plants (Table 43).

Table 42. Mean enzyme activities in rats^a

Group	Sex	PNAS	7-EC	EROD
Control	M	48.1 ± 13.1	231.6 ± 106.1	0.99 ± 0.62
	F	34.3 ± 5.1	106.8 ± 32.2	0.90 ± 0.48
Overall		41.2 ± 11.9	169.2 ± 98.9	0.95 ± 0.55
Triflumizole 200 mg/kg bw per day	M	113.8 ± 6.8	504.7 ± 114.3	1.21 ± 0.36
	F	59.9 ± 9.7	150.6 ± 41.2	1.25 ± 0.63
Overall		86.8* ± 29.5	327.6* ± 203.4	1.23 ± 0.49
PB 75 mg/kg bw per day	M	125.5 ± 24.9	534.8 ± 132.1	1.50 ± 0.55
	F	72.7 ± 17.0	217.8 ± 86.7	1.06 ± 6.27
Overall		99.1* ± 34.3	376.3* ± 197.5	1.28 ± 0.47
3-MC 25 mg/kg bw per day	M	130.1 ± 16.0	1 477.2 ± 375.4	52.72 ± 15.22
	F	103.1 ± 10.6	1 339.5 ± 295.2	38.06 ± 7.83
Overall		116.6* ± 19.2	1 408.3* ± 325.7	45.39* ± 13.78

7-EC: 7-ethoxycoumarin *O*-deethylase; EROD: 7-ethoxyresorufin *O*-deethylase; F: females; M: males; 3-MC: 3-methylcholanthrene; PB: phenobarbital sodium; PNAS: *p*-nitroanisole *O*-demethylase; *: $P \leq 0.05$

^a Data presented as mean ± standard deviation of nanomoles per hour per milligram protein.

Source: Johnson (1988)

Table 43. Overview of major metabolites of triflumizole

Metabolite	Source	Chemical name
FD-1-1	Environment, plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-propoxy-acetamide
FD-2-1	Plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide
FD-6-1	Plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-(2-hydroxypropoxy)-acetamidine
FD-7-1	Plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-oxalamic acid
FM-2-1	–	2-(4-Chloro-2-trifluoromethylphenylimino)-2-imidazol-1-yl-ethanol
FM-5-1	Plant	(<i>E</i>)- <i>N</i> -(4-Chloro-2-trifluoromethylphenyl)- <i>N'</i> -formylpropoxy-acetamidine
FM-6-1	Plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-propoxy-acetamidine
FM-8-1	Plant	<i>N</i> -(4-Chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamidine
FA-1-1	Environment, plant	4-Chloro-2-trifluoromethylphenylamine
FA-1-5	–	2-Amino-5-chloro-3-trifluoromethylphenol

The studies were performed in accordance with OECD Test Guideline 401. For doses, refer to Table 44 below.

FD-1-1

Mortality: Death occurred in almost all groups of both sexes on the 1st and 3rd days.

Symptoms of toxicity: Decreased motor activity, ventral position, hypotonia, ataxia, decreased body temperature, lacrimation and haematuria were observed in both sexes. In addition to these toxic signs, decreased righting reflex, low sensitivity, urinary incontinence and bradypnoea in female rats and diarrhoea and decreased heart rate in male rats were observed.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: Haemorrhages in intestinal and bladder mucosa were observed in both sexes. In addition, dilated bladder was observed in dead female rats.

FD-2-1

Mortality: No deaths occurred in male rats. One death was observed on the 1st day in females.

Symptoms of toxicity: Decreased motor activity, low sensitivity, ventral position, hypotonia, bradypnoea, decreased righting reflex, decreased body temperature and ataxia were observed in both sexes. In addition to these toxic signs, lacrimation was observed in female rats.

Body weight: No unusual changes in body weight were found in either sex.

Pathology: No abnormality was observed in either sex.

FD-6-1

Mortality: No deaths occurred in male rats. In females, death occurred in the 3000 mg/kg bw dose group on the 1st or 2nd day.

Symptoms of toxicity: Decreased motor activity, ventral position, hypotonia, low sensitivity, decreased righting reflex, ataxia, bradypnoea and decreased body temperature were observed in both sexes. In addition to these toxic signs, lacrimation, ptosis, urinary incontinence, salivation and decreased heart rate were observed in female rats.

Body weight: A temporary body weight decrease was found in female rats.

Pathology: Haemorrhages in intestinal mucosa were found in dead female rats.

FD-7-1

Mortality: Death occurred in the 1000 mg/kg bw and higher dose groups in males and in all groups in females on the 1st or 2nd day.

Symptoms of toxicity: Decreased motor activity, ventral position, hypotonia, ataxia, low sensitivity, decreased righting reflex, urinary incontinence, bradypnoea and decreased body temperature were observed in both sexes.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: Haemorrhages in intestinal and stomach mucosa were observed in dead rats of both sexes.

FM-2-1

Mortality: No death occurred in either sex.

Symptoms of toxicity: Decreased motor activity, hypotonia and ataxia were observed in both sexes. In addition to these toxic signs, ventral position, low sensitivity, decreased righting reflex, decreased body temperature and urinary incontinence were observed in female rats.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: No abnormality was observed in either sex.

FM-5-1

Mortality: No deaths occurred in male rats. In females, a death was observed in the 3000 mg/kg bw dose group on the 2nd day.

Symptoms of toxicity: Decreased motor activity, ventral position, hypotonia, ataxia, low sensitivity and decreased righting reflex were observed in both sexes. In addition to these toxic signs, urinary incontinence, bradypnoea and decreased body temperature were observed in female rats.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: No abnormality was observed in either sex.

FM-6-1

Mortality: Death occurred in male rats of the 4167 mg/kg bw and higher dose groups on the 1st to 5th days. In females, deaths occurred on the 1st and 3rd days in all groups.

Symptoms of toxicity: Decreased motor activity, hypotonia, ventral position, ataxia, lacrimation and decreased body temperature were observed in both sexes. In addition to these toxic signs, low sensitivity, decreased righting reflex and bradypnoea in female rats and haematuria in male rats were observed.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: Haemorrhages in intestinal and stomach mucosa were observed in both sexes. In addition, haemorrhages in bladder mucosa in males and dilated bladder in females were observed.

FM-8-1

Mortality: Death occurred in the 1400 mg/kg bw and higher dose groups in males and the 1593 mg/kg bw and higher dose groups in females on the 1st to 3rd days.

Symptoms of toxicity: Decreased motor activity, ventral position, hypotonia, ataxia, low sensitivity, decreased righting reflex, bradypnoea, lacrimation, urinary incontinence and decreased body temperature were observed in both sexes.

Body weight: A temporary body weight decrease was found in both sexes.

Pathology: Haemorrhages in intestinal mucosa were observed in dead rats of both sexes. Haemorrhages in stomach mucosa and ascites were observed in dead male rats.

FA-1-1

Mortality: Deaths occurred in the 819 mg/kg bw and higher dose groups in males on the 1st to 3rd days and the 579 mg/kg bw and higher dose groups in females on the 1st to 3rd days.

Symptoms of toxicity: Decreased motor activity, low sensitivity, decreased righting reflex, hypotonia, ventral position, ataxia, bradypnoea and lacrimation were observed in both sexes. In addition to these toxic signs, decreased body temperature and diarrhoea in male rats and salivation, urinary incontinence and vocalization in female rats were observed.

Body weight: A temporary body weight decrease was found in male rats.

Pathology: Haemorrhages in intestinal mucosa were observed in dead rats of both sexes. In addition, dilated bladder and ascites were observed in dead male rats.

FA-1-5

Mortality: No deaths were seen in males. In females, a death was observed in the 3000 mg/kg bw dose group on the 1st day.

Symptoms of toxicity: Decreased motor activity, hypotonia, ataxia, urinary incontinence and low sensitivity were observed in both sexes. In addition to these toxic signs, decreased righting reflex was observed in female rats.

Body weight: A temporary body weight decrease was found in male rats.

Pathology: No abnormality was observed in either sex.

Conclusions on acute toxicity of metabolites

The acute oral LD₅₀s of the test substances are given in **Error! Reference source not found.**44. Compared with the parent compound (triflumizole), for which the LD₅₀ values were 1057 mg/kg bw in males and 1780 mg/kg bw in females, FA-1-1 and FD-7-1 are slightly more toxic, whereas the other metabolites FD-1-1, FD-2-1, FD-6-1, FM-2-1, FM-5-1, FM-6-1, FM-8-1 and FA-1-5 are less toxic (Nishibe et al., 1985).

Table 44. Summary of acute oral toxicity of triflumizole metabolites

Test substance	Purity (%)	Lot no.	Appearance	Group size	Doses (mg/kg bw)	LD ₅₀ (mg/kg bw)
FD-1-1	99.1	GLP 82-1	Colourless liquid	10 M/dose	3 471, 4 167, 5 000, 6 000, 7 200, 8 640	5 882 (5 729–6 132)
				5 F/dose	2 785, 3 482, 4 352, 5 440, 6 800, 8 500	3 405 (2 965–3 910)
FD-2-1	99.9	31-2294-SY	White crystal	5 M/dose	500, 1 000, 2 000	> 2 000
				5 F/dose	1 000, 2 000, 3 000	> 3 000
FD-6-1	> 98	31-8329-MH	Yellow crystal	5 M/dose	500, 1 000, 2 000	> 2 000
				5 F/dose	1 000, 2 000, 3 000	2 000–3 000
FD-7-1	> 96	31-3158	White crystal	5 M/dose	500, 1 000, 2 000	~1 000
				5 F/dose	1 000, 2 000, 3 000	~1 000
FM-2-1	99.9	31-2299-SY	Colourless crystal	5 M/dose	500, 1 000, 2 000	> 2 000
				5 F/dose	1 000, 2 000, 3 000	> 3 000
FM-5-1	98.8	31-2381-YK	Colourless liquid	5 M/dose	500, 1 000, 2 000	> 2 000
				5 F/dose	1 000, 2 000, 3 000	> 3 000
FM-6-1	99.8	GLP 82-2	White crystal	10 M/dose	3 471, 4 167, 5 000, 6 000, 7 200	4 987 (4 887–5 087)
FM-6-1	> 99	MH-8319	White crystal	10 F/dose	1 561, 2 107, 2 845, 3 841, 5 185, 7 000	2 131 (1 398–2 874)
FM-8-1	99.9	31-2349-YK	Colourless crystal	10 M/dose	1 077, 1 400, 1 820, 2 367, 3 077, 4 000	1 935 (1 651–2 261)
FM-8-1	> 99	31-4187-MH	Light yellow crystal	10 F/dose	942, 1 225, 1 593, 2 071, 2 629, 3 500	2 144 (1 894–2 429)
FA-1-1	98.5	33764	Colourless liquid	10 M/dose	655, 819, 1 024, 1 280, 1 600, 2 000	961 (872–1 060)
				10 F/dose	402, 482, 579, 694, 833, 1 000	771 (711–839)
FA-1-5	99.7	31-2384-YK	Light brown crystal	5 M/dose	500, 1 000, 2 000	> 2 000
				5 F/dose	1 000, 2 000, 3 000	> 3 000

F: females; M: males

Source: Nishibe et al. (1985)

3. Observations in humans

A report on the results of its yearly health examination of the personnel involved in the production of triflumizole at the Takaoka plant (Japan) in the period May 1996 to May 2002 was prepared as a consequence of the Japanese “Occupational Safety and Health Law” and has been submitted to the regulatory authority. Commercial production of triflumizole started in 1985 at this plant. The health examination consisted of physical examination, haematology, urine analysis and blood chemistry. No adverse health effects attributable to chemical exposure were observed. In

addition, it was reported that in the period covered, no events of acute poisoning by exposure or skin and/or eye irritation were observed (Takami, 2002).

Comments

Biochemical aspects

The absorption of (phenyl- $U-^{14}C$)-labelled triflumizole in rats was at least 72% of the administered single and repeated doses at 10 mg/kg bw after 48 hours and about 80% of the applied single dose at 300 mg/kg bw after 96 hours. Absorption was considerably slower after the high dose than after the low dose, as evidenced by the much longer time to reach C_{max} (T_{max}). The radioactivity detected in tissues was generally low (~2%), with highest concentrations found in the liver. Excretion of triflumizole occurred rapidly and independently of sex. Most of the administered low dose (~90%) was recovered within 24 hours. At the single high dose of 300 mg/kg bw, 35–45% of the administered dose was excreted by 24 hours, increasing to 92–99% at 96 hours. The majority of the radiolabel was excreted via the urinary route (~75% of the administered dose compared with ~20% excreted via the faeces).

Triflumizole is extensively metabolized. A few differences in metabolite pattern were observed between males and females after repeated low and single high doses, but not after a single low dose. The major urinary metabolites are the sulfate conjugates of *N*-(4-chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide (FM-8-1) and 2-amino-5-chloro-3-trifluoromethylphenol (FA-1-5). In faeces, *N*-(4-chloro-2-trifluoromethylphenyl)-2-hydroxy-acetamide (FD-2-1) is a major metabolite. Differences between dose regimens exist with respect to other major metabolites. 2-(4-Chloro-2-trifluoromethylphenylimino)-2-imidazol-1-yl-ethanol (FM-2-1) is the major metabolite after a single oral low dose. 4-Chloro-2-trifluoromethylphenylamine (FA-1-1) is a major metabolite after single and repeated low dosing, whereas *N*-(4-chloro-2-trifluoromethylphenyl)-2-propoxy-acetamide (FD-1-1) is the major metabolite after a single high dose. The presence of the metabolite *N*-(4-chloro-2-trifluoromethylphenyl)-2-propoxy-acetamide (FM-6-1) was confirmed in rat faeces and urine.

Toxicological data

Triflumizole has an oral LD_{50} of 1057 mg/kg bw in rats. The dermal LD_{50} is greater than 5000 mg/kg bw, and the inhalation LC_{50} is greater than 3.6 mg/L. No skin irritation and only mild eye irritation were observed. Based on a maximization test in guinea-pigs, triflumizole is considered a skin sensitizer. These studies were conducted prior to GLP, except for the acute inhalation study, but they were conducted to an acceptable standard.

In all short-term studies, decreased body weight gain combined with increased feed consumption and increased liver weights were observed. In a non-GLP 90-day dietary toxicity study, mice were exposed to 0, 20, 200 or 2000 ppm (equal to 0, 3.2, 33 and 381 mg/kg bw per day for males and 0, 4.2, 43 and 466 mg/kg bw per day for females, respectively). The NOAEL was 200 ppm (equal to 33 mg/kg bw per day), based on decreased body weight gain, increased feed consumption and liver effects (increased liver weight, swelling of cytoplasm) observed at 2000 ppm (equal to 381 mg/kg bw per day).

In a second non-GLP 90-day dietary toxicity study, rats were exposed to 0, 20, 200 or 2000 ppm (equal to 0, 1.4, 15 and 177 mg/kg bw per day for males and 0, 1.8, 17 and 218 mg/kg bw per day for females, respectively). Decreased body weight gain, increased feed consumption, liver effects (increased liver weight, fatty changes) and increased kidney weights were also observed at 2000 ppm in both sexes. Based on these effects at 2000 ppm (equal to 177 mg/kg bw per day), the NOAEL was 200 ppm (equal to 15 mg/kg bw per day).

In a 1-year oral toxicity study with dogs exposed to 0, 100, 300 or 1000 ppm (equal to 0, 3, 10 and 34 mg/kg bw per day for males and 0, 3, 11 and 35 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 10 mg/kg bw per day), based on liver effects (increased weights,

macroscopic changes) and increased alkaline phosphatase levels at 1000 ppm (equal to 34 mg/kg bw per day).

In a combined chronic toxicity and carcinogenicity study in mice, animals were exposed through the diet to triflumizole at a concentration of 0, 100, 400 or 1600 ppm (equal to 0, 16, 67 and 296 mg/kg bw per day for males and 0, 22, 88 and 362 mg/kg bw per day for females, respectively). At 400 ppm (equal to 67 mg/kg bw per day) and above, liver effects were observed, as demonstrated by increased organ weight and histopathological findings (nodules and fatty changes in both sexes as well as granulomatous inflammation, cytological alterations, pigmentation and necrosis in some males). Based on these liver effects, the NOAEL was 100 ppm (equal to 16 mg/kg bw per day).

In a combined chronic toxicity and carcinogenicity study in which rats were exposed through the diet to triflumizole at a concentration of 0, 100, 400 or 1600 ppm (equal to 0, 3.5, 14 and 59 mg/kg bw per day for males and 0, 4.5, 18 and 77 mg/kg bw per day for females, respectively), the liver was an important target organ, as demonstrated by increased organ weight and a wide range of histopathological findings at 400 ppm (equal to 14 mg/kg bw per day) and above. The highest dose group of females also showed an increased incidence of convulsive episodes and an increased incidence of ovarian follicular cysts. Based on the liver effects observed, the NOAEL was 100 ppm (equal to 3.5 mg/kg bw per day).

The Meeting concluded that triflumizole is not carcinogenic in mice or rats.

The genotoxic potential of triflumizole was tested in an adequate range of in vitro and in vivo studies. Triflumizole showed no evidence of genotoxicity in any assays.

The Meeting concluded that triflumizole is unlikely to be genotoxic.

Based on the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that triflumizole is unlikely to be carcinogenic in humans.

In a three-generation reproductive toxicity study, rats were exposed through the diet to triflumizole at a concentration of 0, 30, 70 or 170 ppm (equal to 0, 2.1, 4.8 and 12 mg/kg bw per day for males and 0, 2.5, 5.8 and 14 mg/kg bw per day for females, respectively). The NOAEL for parental toxicity was 70 ppm (equal to 4.8 mg/kg bw per day), based on increased placental weights and increased liver and kidney weights at the high dose (170 ppm, equal to 12 mg/kg bw per day). The NOAEL for reproductive toxicity was 70 ppm (equal to 4.8 mg/kg bw per day), based on reduced mating, fertility and litter size. The NOAEL for offspring toxicity was 170 ppm (equal to 12 mg/kg bw per day), the highest dose tested.

Two developmental toxicity studies were conducted in rats. In a non-GLP study, rats were exposed by gavage to 0, 10, 35 or 120 mg/kg bw per day, and in a GLP study, the dose levels were 0, 3, 7 and 35 mg/kg bw per day. Maternal toxic effects were similar in the two studies and comprised reduced body weight gain and feed consumption and increased placental weight at 35 mg/kg bw per day as well as increased liver and spleen weights at the same dose in the non-GLP study. Based on these effects, the overall NOAEL for maternal toxicity was 10 mg/kg bw per day, and the overall LOAEL was 35 mg/kg bw per day. The observed developmental effects were a reduction in the number of viable fetuses, a reduction in fetal weight and an increase in the number of late resorptions at 35 mg/kg bw per day. The overall NOAEL for embryo and fetal toxicity was 10 mg/kg bw per day, with an overall LOAEL of 35 mg/kg bw per day.

Two developmental toxicity studies were conducted in rabbits. In a non-GLP study, rabbits were exposed by gavage to 0, 50, 100 or 200 mg/kg bw per day, and in a GLP study, the dose levels were 0, 5, 25 and 50 mg/kg bw per day. Maternal toxic effects were reduced body weight gain and feed consumption, increased liver weights, decreased ovary weights and decreased placental weight at 200 mg/kg bw per day. The overall NOAEL for maternal toxicity was 100 mg/kg bw per day, with an overall LOAEL of 200 mg/kg bw per day. The observed developmental effects were a lower 24-hour pup survival rate and decreased pup weight at 200 mg/kg bw per day. Based on these effects, the overall NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, with an overall LOAEL of 200 mg/kg bw per day.

The Meeting concluded that triflumizole is not teratogenic in rats or rabbits.

The Meeting was aware of several mechanistic studies and unpublished reports that have been submitted to a regulatory authority. These studies aimed to clarify the effects seen in the reproductive and developmental toxicity studies in rats. These studies were not made available to the Meeting.

In an acute neurotoxicity study, rats were administered triflumizole by gavage at 0, 25, 100 or 400 (males)/200 (females) mg/kg bw. Based on the clinical findings and the functional and motor activity effects observed at the next higher dose (100 mg/kg bw), the NOAEL was 25 mg/kg bw.

In a 13-week neurotoxicity study, rats were exposed to triflumizole at 0, 70, 700 or 2000 ppm (equal to 0, 4.1, 41 and 117 mg/kg bw per day for males and 0, 4.9, 48 and 133 mg/kg bw per day for females, respectively). The systemic findings were similar to those obtained in the 90-day repeated-dose toxicity study, the liver clearly being the main target organ. Based on the liver effects at 700 ppm (equal to 41 mg/kg bw per day), the NOAEL for non-neurotoxic effects was 70 ppm (equal to 4.1 mg/kg bw per day). Observed effects on motor activity were considered not adverse because there was no dose-response relationship, there were no effects in females and the locomotor changes were within the normal range of behaviour. The NOAEL for neurotoxicity was 2000 ppm (equal to 117 mg/kg bw per day), the highest dose tested.

In an immunotoxicity study, triflumizole was administered for 28 days to female mice in diet containing concentrations of 0, 20, 200 and 2000 ppm (equal to 0, 4.4, 43 and 413 mg/kg bw per day, respectively). The NOAEL for systemic toxicity was 200 ppm (equal to 43 mg/kg bw per day), based on significantly reduced body weight gain at 2000 ppm (equal to 413 mg/kg bw per day). The NOAEL for immunotoxicity was 200 ppm (equal to 43 mg/kg bw per day), based on a significant reduction in anti-sRBC IgM in mice immunized with sRBCs at 2000 ppm (equal to 413 mg/kg bw per day).

Toxicological data on metabolites and/or degradates

Acute oral toxicity studies were performed with a number of triflumizole metabolites. For metabolites FD-1-1, FD-2-1, FD-6-1, FM-2-1, FM-5-1, FM-6-1, FM-8-1 and FA-1-5, the oral LD₅₀s were similar to or higher than that of the parent (1057 mg/kg bw). Only metabolites FA-1-1 and FD-7-1 were slightly more toxic than the parent (LD₅₀ = 771 mg/kg bw and ~1000 mg/kg bw, respectively).

Human data

A report on the health examination of production workers in the period May 1996 – May 2002 did not reveal any adverse health effects. No cases of acute poisoning or skin/eye irritation were observed in the same period.

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

Toxicological evaluation

An acceptable daily intake (ADI) of 0–0.04 mg/kg bw was established on the basis of the NOAEL of 3.5 mg/kg bw per day for hepatotoxicity (increased liver weight, macroscopic and microscopic hepatic changes) in the chronic toxicity study in the rat. A safety factor of 100 was applied. This is supported by the NOAEL of 4.8 mg/kg bw per day in the multigeneration study of reproductive toxicity in rats.

An acute reference dose (ARfD) of 0.3 mg/kg bw was established on the basis of the NOAEL of 25 mg/kg bw in the acute neurotoxicity study in the rat, based on clinical findings and effects on

function and motor activity at 100 mg/kg bw. A safety factor of 100 was applied. The effects observed in developmental toxicity studies in rats were not considered to be a consequence of a single dose.

The ADI and the ARfD are also applicable to the metabolites containing the 4-chloro-2-(trifluoromethyl)phenyl group.

Levels relevant to risk assessment of triflumizole

Species	Study	Effect	NOAEL	LOAEL
Mouse	Ninety-day study of toxicity ^a	Toxicity	200 ppm, equal to 33 mg/kg bw per day	2 000 ppm, equal to 381 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	100 ppm, equal to 16 mg/kg bw per day	400 ppm, equal to 67 mg/kg bw per day
		Carcinogenicity	1 600 ppm, equal to 296 mg/kg bw per day ^b	–
Rat	Acute neurotoxicity study ^c	Neurotoxicity	25 mg/kg bw	100 mg/kg bw
	Ninety-day studies of toxicity ^{a,d}	Toxicity	200 ppm, equal to 15 mg/kg bw per day	700 ppm, equal to 41 mg/kg bw per day ^d
	Two-year study of toxicity and carcinogenicity ^a	Toxicity	100 ppm, equal to 3.5 mg/kg bw per day	400 ppm, equal to 14 mg/kg bw per day
		Carcinogenicity	1 600 ppm, equal to 59 mg/kg bw per day ^b	–
	Three-generation study of reproductive toxicity ^a	Parental toxicity	70 ppm, equal to 4.8 mg/kg bw per day	170 ppm, equal to 12 mg/kg bw per day
		Offspring toxicity	170 ppm, equal to 12 mg/kg bw per day ^b	–
		Reproductive toxicity	70 ppm, equal to 4.8 mg/kg bw per day	170 ppm, equal to 12 mg/kg bw per day
	Developmental toxicity studies ^{c,d}	Maternal toxicity	10 mg/kg bw per day	35 mg/kg bw per day
		Embryo and fetal toxicity	10 mg/kg bw per day	35 mg/kg bw per day
Rabbit	Developmental toxicity studies ^{c,d}	Maternal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day	200 mg/kg bw per day
Dog	One-year study of toxicity ^a	Toxicity	300 ppm, equal to 10 mg/kg bw per day	1 000 ppm, equal to 34 mg/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

^d Two or more studies combined.

Estimate of acceptable daily intake

0–0.04 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

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

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

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

<i>Absorption, distribution, excretion and metabolism in mammals</i>	
Rate and extent of oral absorption	Rapid and extensive, 72–79%; 2-fold slower at 24 h at high dose than at low dose
Dermal absorption	No data
Distribution	Widely distributed
Potential for accumulation	No evidence for accumulation
Rate and extent of excretion	Rapid, ~90% excreted within first 24 h, mainly via urine (~75%)
Metabolism in animals	Extensively metabolized, < 2% excreted as parent compound
Toxicologically significant compounds in animals, plants and the environment	Triflumizole and metabolites containing the 4-chloro-2-(trifluoromethyl)phenyl group
<i>Acute toxicity</i>	
Rat, LD ₅₀ , oral	1 057 mg/kg bw
Rat, LD ₅₀ , dermal	> 5 000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 3.6 mg/L (4 h, nose only)
Rabbit, dermal irritation	Not a skin irritant
Rabbit, ocular irritation	Mild eye irritant
Guinea-pig, dermal sensitization	Sensitizer (maximization test)
<i>Short-term studies of toxicity</i>	
Target/critical effect	Liver
Lowest relevant oral NOAEL	15 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, the highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
<i>Long-term studies of toxicity and carcinogenicity</i>	
Target/critical effect	Liver
Lowest relevant NOAEL	3.5 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
<i>Genotoxicity</i>	
	Not genotoxic
<i>Reproductive toxicity</i>	
Reproduction target/critical effect	Reduced fertility and litter size at parentally toxic doses
Lowest relevant parental NOAEL	4.8 mg/kg bw per day

Lowest relevant offspring NOAEL	12 mg/kg bw per day, the highest dose tested
Lowest relevant reproductive NOAEL	4.8 mg/kg bw per day
<i>Developmental toxicity</i>	
Developmental target/critical effect	Placenta, fetal viability and weight, number of late resorptions at maternally toxic doses
Lowest relevant maternal NOAEL	10 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	10 mg/kg bw per day (rat)
<i>Neurotoxicity</i>	
Acute neurotoxicity NOAEL	25 mg/kg bw per day (rat)
Subchronic neurotoxicity	177 mg/kg bw per day, the highest dose tested (rat) ^a
<i>Other toxicological studies</i>	
Immunotoxicity NOAEL	43 mg/kg bw per day (mouse)
Studies on metabolites	FD-1-1, FD-2-1, FD-6-1, FM-2-1, FM-5-1, FM-6-1, FM-8-1, FA-1-5: oral LD ₅₀ s similar to or higher than that of parent FA-1-1 and FD-7-1: oral LD ₅₀ s slightly lower than that of parent
<i>Medical data</i>	
No effects in manufacturing personnel, no cases of poisoning	
LC ₅₀ : median lethal concentration; LD ₅₀ : median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level	
^a In the chronic study, possible neurotoxicity (convulsive periods) was observed in female rats from week 30 onwards.	

Summary

	Value	Study	Safety factor
ADI	0–0.04 mg/kg bw	Two-year study in rats	100
ARfD	0.3 mg/kg bw	Acute neurotoxicity study in rats	100

ADI: acceptable daily intake; ARfD: acute reference dose

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