

### Short-term intake

The international estimated short-term intake (IESTI) for fenitrothion was calculated for the food commodities (and their processing fractions) for which maximum residue levels, STMRs and HRs were estimated and for which consumption data was available. The results are shown in Annex 4. The IESTI varied from 0–80 % of the ARfD (0.04 mg/kg bw) for the general population. The IESTI varied from 0–110% of the ARfD for children 6 years and below. The intake of 110% was for unprocessed wheat bran. Since this is not the edible commodity and further processing is likely to reduce the level of residues, the Meeting assumed that the intake of fenitrothion from processed wheat bran would be below the ARfD. The Meeting concluded that the short-term intake of residues of fenitrothion from uses considered by the Meeting was unlikely to present a public health concern.

## 5.13 FENPYROXIMATE (193)

### TOXICOLOGY

#### Evaluation for an acute reference dose

Fenpyroximate is the ISO approved name for the phenoxypyrazole acaricide, *tert*-butyl (*E*)- $\alpha$ -(1,3-dimethyl-5-phenoxy-pyrazol-4-yl)methylene aminooxy)-*p*-toluate (International Union of Pure and Applied Chemistry, IUPAC), also known as 1,1-dimethylethyl 4-[[[(*E*)-[(1,3-dimethyl-5-phenoxy-1*H*-pyrazol-4-yl)methylene]amino]oxy]methyl] benzoate (CAS; CAS No. 134098-61-6). Fenpyroximate is a contact acaricide with a mode of action involving the inhibition of mitochondrial proton-translocating NADH-quinone oxidoreductase (complex I).<sup>32</sup>

Fenpyroximate was evaluated by the JMPR in 1995, when an ADI of 0–0.01 mg/kg bw was established based on the NOAEL for reduced body-weight gain in a 2-year study in rats. In 2004, the JMPR established an ARfD of 0.01 mg/kg bw based on the LOAEL of 2 mg/kg bw per day for the induction of diarrhoea at the beginning of a 13-week study of toxicity in dogs. It was unclear whether the diarrhoea was the result of a direct irritant or pharmacological effect of fenpyroximate. Since a NOAEL for diarrhoea was not identified, an additional safety factor of 2 was applied to the usual 100 to establish the ARfD. The Meeting concluded that the ARfD was conservative and could be refined if a suitable study became available.

The present Meeting reconsidered the ARfD following submission of a new study of acute toxicity in dogs. The Meeting also reconsidered the existing database on fenpyroximate, as previously evaluated. The submitted study in dogs complied with GLP requirements.

### Toxicological data

#### Previously evaluated study in dogs

In a 13-week study, groups of four male and four female dogs received capsules containing fenpyroximate (purity, 98.4–98.6%) at a dose of 2, 10 or 50 mg/kg bw per day. Controls (four dogs of each sex per group) received the empty gelatin capsules. Dogs were inspected throughout the working day and daily observation of each animal was carried out. A more detailed weekly examination was also carried out. A detailed veterinary examination was carried out before the start of the study and after 4, 8 and 12 weeks of treatment. Ophthalmoscopic examination of the eyes was undertaken after 4, 8 and 12 weeks of treatment. Debilitated animals were carefully observed and those in extremis were killed, blood samples having been taken ante-mortem. Body weight was measured at the start of the study, and then weekly and before death. Food consumption was measured daily. Water consumption was measured during 3 days in week 6. Electrocardiography was performed before the start of treatment and at weeks 6 and 12; at weeks 6 and 12, electrocardiography was performed 2 h

<sup>32</sup>Nakamaru-Ogiso, E., Sakamoto, K., Matsuno-Yagi, A., Miyoshi, H. & Yagi, T. (2003) The ND5 subunit was labelled by a photoaffinity analogue of fenpyroximate in bovine mitochondrial complex I. *Biochemistry*, **42**, 7.

and 24 h after dosing. Before the start of treatment and after 6 and 12 weeks of dosing, blood was taken for haematological investigations and clinical chemistry studies; during the treatment period, samples were taken before dosing. Urine analysis was carried out before the start of the study and after 11 weeks of treatment. Surviving dogs were killed at 12 weeks and a detailed necropsy undertaken. Selected organs were removed and weighed. Samples of selected organs and any macroscopical abnormalities were processed for histopathological examination.

Two females at the highest dose were killed in extremis during the study, because of severe weight loss and loss of appetite. Dogs in all treated groups had diarrhoea, and in the males this appeared to be dose-related and was apparent from week 1 (see Tables 9 and 10).

Table 9. The mean percentage<sup>a</sup> of dogs having diarrhoea after treatment with capsules containing fenpyroximate for 13 weeks

Sex	Dogs with diarrhoea (%)			
	Dose (mg/kg bw per day)			
	0	2	10	50
Males	8.5	22.7	21.2	70.0
Females	5.0	30.8	50.0	48.0

From Broadmeadow (1989)

<sup>a</sup> The percentage of dogs having diarrhoea was recorded each day. The mean percentage of dogs having diarrhoea was calculated by adding the daily percentage for each group and dividing by the number of days on which observations had been carried out.

Table 10. Incidence of diarrhoea in individual dogs before dosing and during week 1 of dosing with fenpyroximate<sup>a</sup>

Dog	Incidence of diarrhoea (days)															
	Dose (mg/kg bw per day)															
	Males								Females							
	0	2	10	50	0	2	10	50	0	2	10	50	0	2	10	50
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	0	3	0	4	0	4	0	7	0	0	0	5	0	5	0	3
2	0	0	0	2	0	6	0	7	0	0	1	6	0	6	1	7
3	0	0	0	0	1	0	0	2	3	3	0	2	0	0	0	2
4	0	0	0	1	0	1	0	6	0	0	0	0	0	1	0	0

From Broadmeadow (1989)

<sup>a</sup> 'Pre' refers to the number of days during the week before dosing that each beagle had diarrhoea; 'Post' refers to the number of days during the first week of dosing that each beagle had diarrhoea.

Emesis was seen in both sexes at 10 and 50 mg/kg bw per day. Emaciation was seen at 50 mg/kg bw per day (and in one female at 2 mg/kg bw per day). Lethargy (torpor) was seen in some females at 2 and 10 mg/kg bw per day, and in males and females at 50 mg/kg bw per day. Weight loss was seen in week 1, in females receiving fenpyroximate at 10 mg/kg bw per day and in males and females at 50 mg/kg bw per day. Body-weight gain was clearly depressed at 50 mg/kg bw per day in males, and at 50 and 10 mg/kg bw per day in females, compared with that of the controls. Body-weight gain in females was marginally depressed, compared with that of the controls, in the group receiving fenpyroximate at 2 mg/kg bw per day. Food consumption was unaffected by treatment in males, but was reduced by treatment in a dose-related fashion in females.

No treatment-related ocular lesions were noted. Slight bradycardia was seen in all treatment groups in both sexes, but especially in the groups receiving fenpyroximate at 10 and 50 mg/kg bw per day. There was no consistent difference between the measurements made 2 h after dosing and 24 h after dosing, and the bradycardia was not consistently present at 2 mg/kg bw per day. In males at all doses and in females at 2 and 10 mg/kg bw per day, no differences in haematological parameters were seen, compared with those of the concurrent controls. In females at 50 mg/kg bw per day, low total leukocyte counts at 6 weeks and 12 weeks, prolonged activated partial thromboplastin times at 6 weeks and high platelet counts at 12 weeks were recorded relative to these values for the concurrent controls. The two decedents (both females at 50 mg/kg bw per day) had low leukocyte counts. Raised concentrations of blood urea nitrogen were seen in females at 50 mg/kg bw per day at week 6, and at 2 and 50 mg/kg bw per day at week 12; it is unclear whether these effects were treatment-related as there was no clear dose–response relationship. Low concentrations of glucose were seen in males at 10 mg/kg bw per day and in both sexes at 50 mg/kg bw per day at weeks 6 and 12. The two decedents (both females at 50 mg/kg bw per day) had high blood urea concentrations and low plasma butyrylcholinesterase activities, and one of them had a low concentration of blood glucose. No inter-group differences were seen in the results of urine analysis.

Slightly higher absolute and relative weights of the adrenals were observed in males at 50 mg/kg bw per day and slightly higher relative weights of the adrenals in females at that dose. Relative weights of the liver were increased in both sexes at 50 mg/kg bw per day. Macroscopic examination post mortem showed emaciation in one surviving female at 50 mg/kg bw per day. The decedents showed emaciation. There was depleted hepatic glycogen and fine renal medullary cytoplasmic vacuolation in the two decedent females at the highest dose, as well as in one surviving female at 50 mg/kg bw per day. The lowest-observed-adverse-effect level (LOAEL) for the study was 2 mg/kg bw per day on the basis of clinical signs at that dose (diarrhoea in both sexes, and lethargy in females) and reduced body-weight gain in females. This LOAEL was probably close to the NOAEL.<sup>33, 34</sup>

*Study evaluated for the first time by the present Meeting*

In a study designed to establish the maximum tolerated dose and a NOAEL for acute toxicity, two male and two female beagle dogs (age 34–37 weeks) were given fenpyroximate (purity, 99.8%) at a concentration of 5 ml/kg (suspended in 0.5% w/v methylcellulose) by gavage. In the first phase of the study, the four dogs were given a single dose at 2 mg/kg bw on day 1, followed by 5 mg/kg bw on day 8, and finally 20 mg/kg bw on day 15. In the second phase, the same four dogs were dosed on day 23 at 5 mg/kg bw per day for five consecutive days.

Food consumption was measured daily and the body weight was measured before the day of dosing during the incremental dosing phase and then twice per week for the repeat-dosing phase until necropsy. Clinical monitoring, with an emphasis on neurobehavioural effects, was performed daily. Haematology parameters (erythrocyte volume fraction, haemoglobin, mean cell haemoglobin concentration (MCHC), mean corpuscular volume, erythrocyte count, leukocyte differential count, reticulocyte count, platelet count and prothrombin time) were determined before treatment (day–5) and again on the morning following each incremental change in dose before feeding, and at the end of the fixed-dose phase.

Clinical chemistry was carried out to measure blood glucose, blood urea nitrogen, total serum protein, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sodium, potassium, chloride, calcium, phosphorus, cholesterol, creatinine, albumin, gamma globulins and the

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<sup>33</sup> Broadmeadow, A. (1989) NNI-850: toxicity study by oral (capsule) administration to CD beagle dogs for 13 weeks. Unpublished report No. 89/NNH036/1111 (amended from 89/NNH036/614) from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Tokyo, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan. Guidelines: USEPA/FIFRA, 1982; MAFF Japan, 1985.

<sup>34</sup> Annex 5, reference 101.

albumin : globulin ratio. Surviving dogs were killed on day 28 and a detailed necropsy was undertaken. Selected organs were removed and weighed. Samples of selected organs and any macroscopic abnormalities were processed for histopathological examination.

There were no treatment-related deaths or changes in body weight at any dose. The only clinical sign observed was soft or liquid faeces in all dogs at 20 mg/kg bw. The diarrhoea started 2–3.5 h after dosing and lasted for up to 6 h. Surprisingly, two out of four dogs (one male, one female) who received fenpyroximate at a dose of 5 mg/kg bw in the second phase of the test on day 23 had diarrhoea 3 h after dosing, but no dogs exposed to the same dose on day 8 had any clinical signs. All four dogs had clinical signs at about 3–6 h after the second consecutive dose at 5 mg/kg bw on day 24. The investigators suggested that this may have been due to pre-exposure to fenpyroximate at a high dose (20 mg/kg bw) 8 days earlier. Although blood had been collected 1, 3, 6 and 24 h after dosing at 5 mg/kg bw (fixed-dose phase) for toxicokinetic purposes, it was not possible to test this assertion owing to the absence of any blood collection before dosing.

The haematological and clinical chemistry analyses revealed values that were well within the range for historical controls. Necropsy revealed no apparent effects on organ weights, but macroscopically there were lesions observed in the gastrointestinal tract of three dogs (two males, one female). In two of the dogs, the mucosa of the ileo-caecal junction was red and dark. In the third dog, the mucosa of the stomach fundus was reported to be pale. The NOAEL was 2 mg/kg bw on the basis of clinical signs (diarrhoea in both sexes) at the next higher dose of 5 mg/kg bw.<sup>35</sup>

### Toxicological evaluation

An examination of the existing toxicological database indicated that the toxic effects of fenpyroximate are diarrhoea, reduced body-weight gain and haematological and clinical chemistry changes. The most sensitive end-point, namely diarrhoea, was observed in all studies in dogs, but not in other species. In a 13-week study in dogs given capsules containing fenpyroximate, diarrhoea with reduced body-weight gain and food consumption was observed. A NOAEL was not identified in the 13-week study and the LOAEL was 2 mg/kg bw per day on the basis of diarrhoea occurring at all doses. In that study it was unclear whether the diarrhoea occurred after a single dose, since only the incidence per week was reported. In a follow-up study of acute toxicity, diarrhoea was again observed in some dogs at 5 mg/kg bw, but not at 2 mg/kg bw.

After considering previous evaluations of fenpyroximate and the new submitted study, the Meeting established an ARfD of 0.02 mg/kg bw based on the NOAEL of 2 mg/kg bw identified on the basis of induction of diarrhoea after a single dose in dogs and using a safety factor of 100. Since it remained unclear whether the diarrhoea observed in dogs was the result of a direct irritant or pharmacological effect of fenpyroximate, it was not possible to consider a modification in the safety factor.

A toxicological monograph was not prepared.

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<sup>35</sup> Harvey, P.W. (2006) Fenpyroximate: tolerated dose and 5-day repeat dose study by bolus oral gavage dosing in the dog to assist in setting the acute reference dose. Unpublished report No. 0608-073 from Covance Laboratories Ltd, North Yorkshire, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Osaka, Japan. GLP: UK DHSS; OECD (1981).

**Levels relevant to risk assessment**

Species	Study	Effect	NOAEL	LOAEL
Dog	Acute toxicity <sup>a</sup>	Diarrhoea	2 mg/kg bw	5 mg/kg bw
	Three-month studies of toxicity <sup>b</sup>	Clinical signs and reduced body-weight gain in females	—	2 mg/kg bw per day

<sup>a</sup> Gavage administration<sup>b</sup> Capsule administration.*Estimate of acute reference dose*

0.02 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.

**5.14 FLUSILAZOLE (165)****TOXICOLOGY**

Flusilazole is the ISO approved name for 1-[[bis(4-fluorophenyl)methyl]silyl]methyl]-1*H*-1,2,4-triazole (CAS No. 85509-19-9). It is a broad-spectrum fungicide that belongs to the triazole subclass of ergosterol biosynthesis inhibitors. Flusilazole was previously evaluated by the Joint Meeting in 1989 (Annex 5, references 56, 58) and in 1995. An ADI of 0–0.001 mg/kg bw was allocated in 1989, based on a NOAEL of 0.14 mg/kg bw per day (5 ppm) for liver toxicity in a 1-year feeding study in dogs. This was confirmed in 1995. The compound was re-examined by the present Meeting as part of the Periodic Re-evaluation Programme of CCPR. Three new studies were provided, two studies of developmental toxicity in rats (one of oral and one of dermal administration) and a 28-day mechanistic study in dogs.

Owing to the age of the database, some studies predate GLP; however, all critical studies complied with GLP.

**Biochemical aspects**

In rats, orally administered [<sup>14</sup>C] labelled flusilazole was readily absorbed from the gastrointestinal tract and rapidly excreted in urine (72% of triazole label) and faeces (up to 87% of phenyl label), with little or no radioactivity recovered in the expired air. The excretion half-life was approximately 34 h and > 90% of the administered dose was eliminated within 96 h. Tissue retention of radiolabelled material was low. Total tissue residues excluding the carcass (which accounted for approximately 2% of the administered dose) was < 1%, therefore demonstrating no evidence of bioaccumulation.

[<sup>14</sup>C]Flusilazole was extensively metabolized in rats. Recovered parent compound accounted for only 2–11% of the given dose, found predominantly in the faeces (urinary concentration, < 1%). After absorption, flusilazole was cleaved at the triazole ring. With phenyl-labelled test material, the major faecal metabolites identified were [bis(4-fluorophenyl)methyl] silanol, [bis(4-fluorophenyl)methylsilyl] methanol and its fatty acid conjugates, and disiloxane. Except for the fatty acid conjugates, the same metabolites were found in the urine. With triazole-labelled material, the main metabolite identified was 1*H*-1,2,4-triazole, which was found predominantly in the urine (63.8% of the administered dose in males, 51.6% in females); faeces contained only a small amount of the metabolite.