TERBUFOS

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Explanation

Terbufos is an organophosphorus compound, classified as a systemic insecticide and nematocide, and was last evaluated by the JMPR in 1989, when an ADI of 0–0.0002 mg/kg bw was established. Terbufos was considered by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

In an study of metabolism, 16 male Royal Hart Wistar rats were given a single dose of [^{14}C]terbufos (purity, uncertain; specific activity of 26.4 µCi/mg labelled at the carbon atom of the thiomethyl portion of the parent molecule (or methylene carbon) in ethanol and

water (1 : 1) at 0.8 mg/kg bw by gavage. Animals were housed individually in metabolism cages. Urine was collected on dry ice at 6, 12, 24, 48, 72, 96, 120, and 168 h after dosing, and faeces were collected and frozen at 12, 48, 72, 96, 120, and 168 h after dosing. After thawing, urine samples were pooled for each time interval. Groups of three animals were terminated at 6, 12, 24, and 48 h after dosing and the final group of four animals was terminated at 168 h after dosing. Livers, kidneys, gastrointestinal tract, muscle, skin, fat and blood were taken at these times.

Radioactivity was extracted from the urine, faeces and tissues, and metabolites in urine, faeces, liver, kidney and muscle were separated and identified. In addition, the amount of $^{14}CO_2$ in expired air over a 72 h period was determined in one animal that had received a dose of 0.2 mg/kg bw by gavage. There was no indication of overt toxicity. Elimination was relatively rapid and fairly complete. About 90% of the administered dose was recovered by the end of the study. Over the entire duration of the study, approximately 83% of the administered dose was found in the urine, which was thus the major route of elimination; about 72% of the administered dose was excreted by 24 h and 80% was excreted by 48 h. Over the course of the study, about 3.5% of the radiobel was found in the faeces. The recovery of the administered dose reached a peak of 31.2% in the urine by 24 h, and a peak of about 2% in the faeces by 48 h. Tissue concentrations of radiolabel reached a maximum at between 6 and 12 h after dosing. By 168 h after dosing, the concentration of radiolabel in each body tissue examined was <0.1 mg/kg. The total percentage of the administered dose recovered in tissues by 168 h was greatest in the liver (0.34%), followed by the gastrointestinal tract (0.087%), blood (0.036%), kidney (0.034%), muscle (0.024%), skin (0.017%) and fat (0.003%). No $^{14}CO_2$ was recovered during the designated interval of 72 h.

After extraction and thin-layer chromatography, metabolites that did not contain phosphorus were found to account for about 96% of the radiolabel present in urine. The predominant species appearing by 6 h after dosing was CL 202 474, with lesser amounts of CL 99 843, CL 99 844 and CL 99 875 (see Figure 1 and Table 1). Small amounts of parent compound and other species containing phosphorus accounted for most of the remainder of the radiolabel (2–3%) in the urine. Two peaks of radioactivity in urine were not definitively identified.

In the faeces, at 12 h after dosing, about 95% of the radiolabel comprised species containing phosphorus (mostly metabolites CL 92 320 and CL 94 221, with lesser amounts of CL 94 302 and CL 94 301). At subsequent time intervals, in addition to CL 92 320, non-phosphorus-containing metabolites CL 202 474, CL 99 843, and CL 99 875 predominated. Very little parent compound was found in the faeces.

The metabolites identified in urine and faeces were also observed in tissue extracts; the types of metabolite found at detectable levels depended on the tissue and time after dosing. In liver, kidney and muscle, the approximate ratio of species not containing phosphorus (four metabolites) to those containing phosphorus (e.g. five phosphorus-containing metabolites and small amounts of parent compound) at 6 h after dosing were 2.6, 11 and 6 in liver, kidney and muscle respectively, and about 9, 23 and 7 for these same tissues at 12 h after dosing. Possible sex differences in the metabolic fate of terbufos were not addressed by this study (North, 1973). No statements of compliance with quality assurance (QA) or good laboratory practice (GLP) were provided. The study was not performed according to a specific guideline. Despite the limitations of the study some useful information can be extracted.

Radiolabelled terbufos (purity, >98%; specific activity, 61.4 mCi/g), labelled with ^{14}C at the methylene carbon position of the parent molecule, was administered in corn oil by gavage to groups of fasted male and female Crl:CD®(SD)BR rats. Groups of five male and five female rats were given single oral doses of 0.1 mg/kg bw (lowest dose) or 0.4 mg/kg bw (highest dose) and additional groups of one male and one female were similarly treated and used for collection of volatiles. In the multiple-dose segment of the study, groups of rats were given non-radiolabelled terbufos (purity, 97.8%) as single oral doses of 0.1 mg/kg bw in corn oil for 14 days, followed by a single dose of radiolabelled material of 0.1 mg/kg bw on day 15. Additional groups of one male and one female were similarly treated with terbufos at a dose of 0.1 and used for collection of volatiles. Urine and faeces for all groups were collected 0–6, 6–12, and 12–24 h after the administration of radiolabelled terbufos and daily thereafter until termination at 168 h. Cage rinses were collected as necessary. Volatiles were collected at intervals of up to 7 days after dosing. Radioactivity was extracted from urine, faeces and tissues (blood, bone, brain, fat, ovaries, testes, heart, liver, kidneys, lungs, muscle, spleen, uterus and residual carcass). Metabolites were characterized and identified only in the urine and faeces and only for the 12–24 h interval after dosing.

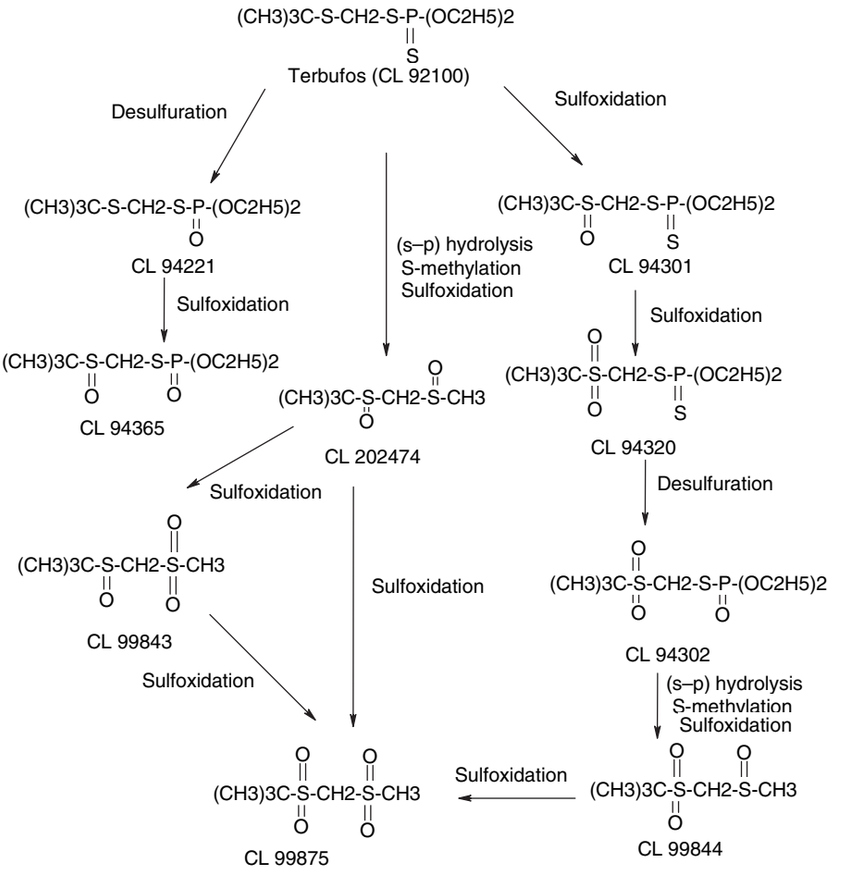
No toxicity was reported. In all the treated groups, total recoveries of the administered radiolabel was about 93–99% in males and 89–96% in females at 168 h after dosing. Relatively rapid elimination, primarily in the urine, indicated fairly fast and appreciable absorption via the gastrointestinal tract. Urinary excretion of radiolabel was 76% and 79% in males and females treated with single low doses of terbufos, and 79% and 69% in males and females treated with single high doses of terbufos, at 168 h after dosing. In animals receiving repeated low doses of terbufos, urinary excretion of radiolabel was 86% and 85% of the administered dose in males and females. Faecal elimination in groups of males and females treated with low and high single doses was 13–17% of the administered dose and in the groups receiving repeated doses was about 5–7% in both sexes. In males and females in all treated groups, much smaller amounts of radiolabel, as a percentage of the administered dose, were found in the tissues (about 0.09–0.15%), in the carcass (about 0.90–2%), in expired CO_2 (about 2–4%) and in volatiles (0.06–0.5%), and most of the radiolabel was eliminated in excreta by 24 h after dosing.

The highest concentrations of residues in tissues at 168 h after dosing were found in animals receiving single, high doses. For all dosing regimens, the highest tissue residues were found in the lungs of both sexes (single low doses, 0.003–0.005 mg/kg; repeated doses, 0.005–0.007 mg/kg; and single high doses, 0.018–0.022 mg/kg) and there was no indication of bioaccumulation.

Metabolites in urine and faeces were characterized only for the period between 12 and 24 h after dosing (Figure 1 and Table 1). Since appreciable amounts of radiolabel were excreted before this interval, the metabolites cannot be reliably quantified relative to percentage of administered dose. In addition, variation in the radiolabel detected in cage rinses for individual animals (about 6–56% of the administered dose) contributed to uncertainty in metabolite quantification during the 12–24 h interval.

For all dosing regimens and for both sexes, about 70–90% of the urinary residue of radiolabel was reported to have been characterized for the 12–24 h period. Of this, about 67–80% of the residue was described as non-phosphorus-containing metabolites, about 1–4% as phosphorus-containing metabolites and about 2–9% as unknown substances (two substances). Of the non-phosphorus-containing metabolites, the major metabolite was CL

Figure 1. Proposed metabolic pathway of terbufos in rats



From Cheng (1992)

Table 1. Terbufos and its metabolites

CL No.	Chemical name	Common name
92,100	*O*,*O*-diethyl-*S*-*t*-butylthio-methylphosphorodithioate	Terbufos (parent compound)
94,301	Phosphorodithioic acid, *S*-(*t*-butylsulfinyl) methyl *O*,*O*-diethyl ester	Terbufos sulfoxide
94,320	Phosphorodithioic acid, *S*(*t*-butylsulfonyl) methyl *O*,*O*-diethyl ester	Terbufos sulfone
94,221	Phosphorothioic acid, *S*-(*t*-butylthio) methyl *O*,*O*-diethyl ester	Terbufoxon
94,302	Phosphorothioic acid, *S*-(*t*-butylsulfonyl) methyl *O*,*O*-diethyl ester	Terbufoxon sulfone
94,365	Phosphorothioic acid, *S*-(*t*-butylsulfinyl) methyl *O*,*O*-diethyl ester	Terbufoxon sulfoxide
202,474	Methane, (*t*-butylsulfinyl)(methylsulfinyl)	
99,844	Sulfoxide, (*t*-butylsulfonyl) methyl methyl	
99,843	Sulfoxide, *t*-butyl (methyl-sulfonyl) methyl	
99,875	Sulfone, *t*-butyl (methyl-sulfonyl) methyl	

From North (1973) and Cheng (1992)

202 474; lesser amounts of CL 99 843, CL 99 844 and CL 99 875 were found. The only phosphorus-containing metabolite detected was CL 94 365; no parent compound was found. During the 12–24 h interval, for all dosing regimens and for both sexes, about 49–80% of the faecal residue of radiolabel was reported to have been characterized. Of this, about 17–34% was described as non-phosphorus-containing metabolites, about 8–44% as phosphorus-containing metabolites, and about 7–22% as unknown substances (five

substances). The non-phosphorus-containing metabolites were CL 202474, CL 99843 and/or CL 99844, and CL 99875. The major phosphorus-containing entity was the parent compound (CL 92100) with lesser amounts of CL 94301 and CL 94365. The proposed metabolic pathway for terbufos in rats, on the basis of the metabolites found in excreta, is depicted in Figure 1. Sulfoxidation and desulfuration of terbufos is followed by hydrolysis of the thiolophosphorus bond (S–P), enzymatic *S*-methylation and then additional *S*-oxidation.

There were no apparent sex differences in the absorption and metabolic fate of [^{14}C]terbufos in Sprague-Dawley rats on the basis of the results of this study (Cheng, 1992). Statements of compliance with QA and GLP were provided. The protocol was generally consistent with United States Environmental Protection Agency (EPA) Subdivision F Guidelines (November 1982 and revised, 1984).

1.2 Biotransformation

In a study of the biotransformation of terbufos (purity, 91%; apparently purchased from a pesticide factory in China), livers of male Wistar rats (180–220 g) were perfused in situ with 100 µl of terbufos (0.1 mol/l, dissolved in methanol and added to the perfusate reservoir) for 1 h at a flow rate of 5 ml/liver per min. Metabolic materials collected from perfusate effluent were separated with a solid-phase extraction cartridge and were characterized and quantified by gas chromatography–infrared spectrometry (GC–IR) and gas chromatography–mass spectrometry (GC–MS). The recovery of terbufos and its metabolites was expressed as a percentage of the concentration of the parent compound entering the liver. Recovery was incomplete, totaling only about 47.13%. Substances in the effluent separated into five major peaks. These were identified as terbufos (40.8%), terbufos oxon (2.13%), and three triakylphosphorothioate metabolites: metabolite I: $(C_2H_5O)_2POSCH_3$ (hydrolysate of terbufos oxon, 0.13%); metabolite II: $(C_2H_5O)_2PSSCH_3$ (hydrolysate of terbufos, 2.65%); and metabolite III: $(C_2H_5O)_2PSSC_2H_5$ (methylate of the metabolite II, 1.42%). It was suggested that metabolite III may have formed via a detoxification reaction involving *S*-adenosyl-L-methionine methyl transferase. The potential for certain trimethyl and triethyl phosphorothioates, such as metabolites I, II and III, to cause cholinergic toxicity and/or pulmonary toxicity in rats by a non-cholinergic mechanism or mechanisms was discussed. Sulfoxide and sulfone metabolites were not detected in the effluent (Li et al., 1999).

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of terbufos is summarized in Table 2.

Terbufos is of very high acute toxicity when administered by the oral, dermal, or inhalation routes. LD_{50} values for acute oral toxicity in rodents and dogs were similar, ranging from 1.4 to 9.2 mg/kg bw. The acute dermal LD_{50} was about 1 mg/kg bw in rabbits, and the acute inhalation LC_{50} in rats ranged from 0.0012 to 0.0061 mg/l. Clinical signs observed were those typical of cholinergic toxicity and, depending on the study, route and species, included tremors, salivation, exophthalmos, prostration, decreased activity, chromodacryorrhoea, diuresis, piloerection, ataxia, urogenital staining, nasal discharge, anorexia, and laboured breathing. Deaths following acute exposures occurred within minutes to hours or up to a week after administration. With regard to dermal absorption, terbufos is rapidly penetrating after dermal or ocular application.

Table 2. Acute toxicity of terbufos

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Purity (%)	Reference
Mouse	CF1 albino	Female	Oral	Corn oil	5.0	85.8	American Cyanamid Company A72-95 (1972a)
Mouse	CF1 albino	Female	Oral	Corn oil	9.2	96.7	Morici (1972)
Mouse	CF-1 albino	Male	Oral	Corn oil	3.5	96.7	Morici (1972)
Rat	Wistar (RH albino)	Female	Oral	Corn oil	9.0[b]	96.7	Morici (1972)
Rat	SD (Crl : CD(SD)BR)	Female	Oral	Corn oil	1.4[d]	89.7	Bradley (1996)[a]
Rat	SD (Crl:CD(SD)BR)	Male	Oral	Corn oil	3.2[d]	89.7	Bradley (1996)[a]
Rat	Wistar RH albino)	Male	Oral	Corn oil	1.6[d]	85.8	American Cyanamid Company A72-95 (1972a)
Rat	Wistar (RH albino)	Male	Oral	Corn oil	4.5[b]	96.7	Morici (1972)
Rat	SD (CD)	Female	Inhalation; 4 h, whole body	Administered as a vapour	0.0012 (1.2 µg/l)	89.6	Hoffman (1987)[6]
Rat	SD (CD)	Male	Inhalation; 4 h whole body	Administered as a vapour	0.0061 (6.1 µg/l)	89.6	Hoffman (1987)[6]
Rat	Wistar (RH albino)	Male	Inhalation; 7 h	Administered as a vapour	Could not be calculated[g]	96.7	Morici (1972)
Rabbit	New Zealand white	Female	Dermal	Report stated applied as received[c]	0.93[b]	89.6	Fischer (1985)
Rabbit	New Zealand white	Male	Dermal	Report stated applied as received[c]	0.81[b]	89.6	Fischer (1985)
Rabbit	Albino	Male	Dermal	Report stated applied as received[c]	1.0	85.8	American Cyanamid Company A72-95 (1972a)
Rabbit	Albino	Male	Dermal	Report stated applied as received[c]	1.1	96.7	Morici (1972)
Dog	Beagle	Female	Oral	Report stated applied as received[e]	6.3[d]	96.7	Morici (1972)
Dog	Beagle	Male	Oral	Report stated applied as received	4.5[d]	96.7	Morici (1972)

Although reports for most of these studies (except as footnoted below) were summary in nature and did not contain GLP or QA statements, protocols appeared to be generally consistent with the intent of EPA Subdivision F Guidelines (1982 or 1984, revised)

[a] Detailed report contained QA and GLP statements, but stated there was no confirmation of the concentration of test material; the protocol was consistent with US EPA Subdivision F Guidelines (1982 or 1984, revised)

[b] Report stated that animals were not fasted

[c] Test material was a liquid

[d] Report stated that animals were fasted

[e] Test material in a gelatin capsule was administered to fasted animals

[f] Detailed report contained QA and GLP statements and protocol was consistent with US EPA Subdivision F Guidelines (1982 or 1984, revised)

[g] Ten animals were exposed for 7 h at 25°C to air that was near-saturated with product vapour at a nominal chamber concentration of 1.99 mg/l. There were two deaths, one on day 5 and the other during days 6–14 after dosing. Clinical findings described as transient irritation and discomfort were present at 0–15 min after dosing and the lung of one survivor was abscessed at necropsy. The findings of this study are inconsistent with those of Hoffman, 1987

(a) Ocular and dermal irritation

Rabbit

In a study of primary skin irritation, 0.5 ml of technical-grade terbufos (purity, 96.7%) was applied "as received" to shaved rabbit skin for 24 h under an impervious patch. The product was extremely toxic by the dermal route when administered in a single treatment; all rabbits (number not specified) died within 24 h after dosing. All animals exhibited signs of cholinesterase inhibition before death. The product (a liquid) was said to penetrate rabbit skin and mucous membranes very easily. No indications of dermal irritation or corrosion were reported (Morici, 1972).

In a second study of primary skin irritation, a single application of 0.25 ml of technical-grade terbufos (purity, 85.8%) was administered "as received" to shaved rabbit skin in a similar protocol to that of Morici (1972), with similar results. All animals died within 24 h after dosing and showed signs of cholinesterase inhibition before death. No skin irritation was reported (BASF, 1972a).

In a study of primary eye irritation, 0.1 ml of technical-grade terbufos (purity, 96.7%) was applied "as received" to the conjunctival sacs of six rabbits. The product was extremely toxic by the ocular route when administered in a single treatment; all animals died on the day of dosing and were observed to exhibit signs of cholinesterase inhibition before death. The product (a liquid) was said to penetrate rabbit skin and mucous membranes very easily. No indications of ocular irritation were reported (Morici, 1972).

In a second study of primary eye irritation, a single application of 0.1 ml of technical-grade terbufos (purity, 85.8%) was introduced "as received" into the conjunctival sacs of six rabbits in a protocol similar to that of Morici (1972), with similar results. All animals died 2–3 h after dosing and were observed to exhibit signs of cholinesterase inhibition before death. No ocular irritation was reported (BASF, 1972a). Although the reports for most of these studies were summary in nature and did not contain statements of compliance with GLP or QA, protocols appeared to be generally consistent with the intent of EPA Subdivision F Guidelines (1982 or 1984, revised).

(b) Dermal sensitization

A study of skin sensitization of technical grade terbufos was not performed owing to the severe toxicity observed in the studies of primary skin and eye irritation.

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a preliminary study, groups of 10 male and 10 female albino CF1 mice were given diets containing technical-grade terbufos (CL 92 100; purity, 96.7%) at a nominal concentration of 0, 1.0, 4.0 or 16 mg/kg (nominally equivalent to 0, 0.218, 0.911 and 3.30 mg/kg bw per day in males and 0, 0.286; 0.988 and 3.70 mg/kg bw per day in females) for 31 days. Data on analysis of concentrations of test material in the diet were not provided, so test material intake and stability in the feed could not be confirmed. Very few parameters were evaluated. Cholinesterase activity was not assessed. Six females at the highest dose were found dead between days 5 and 14 and one female at the lowest dose was found dead on

day 9. Autolysis prevented attempts to determine the cause of death. Mortality was, however, only notable in females receiving the highest dose (60%), therefore it is possible that the increase in mortality was related to treatment. Decreases in body weight and food consumption were reported in males and females at the highest dose. Weights of the two organs examined (liver and kidney) in four to five animals of each sex per group treated with terbufos were not statistically significantly different from those of respective control groups. For the parameters examined, no effects were observed in other groups. It was reported that upon gross pathological examination of four to five animals of each sex per group at study termination, no gross lesions were found that were attributed to treatment (gross pathology data were not provided). The no-observed-adverse-effect level (NOAEL) in males and females was nominally 4.0 mg/kg (nominally equivalent to 0.911 mg/kg bw per day in males and 0.988 mg/kg bw per day in females; intake of test material could not be confirmed) on the basis of decreases in body weight and food consumption in both sexes and mortality of 60% at the dose above in females only (Morici, 1972). No statements of compliance with QA or GLP were provided and the study was not performed to address a specific guideline.

Rats

In a short-term feeding study, groups of five male and five female Sprague-Dawley rats (aged 4 weeks at study initiation) were given diets containing technical-grade terbufos (CL 92 100; purity, 90.1%), prepared in a vehicle of corn oil and methylene chloride (1 : 1), at a concentration of active ingredient of 0, 0.125, 0.250, 0.500, 1.00, 3.00 or 6.00 mg/kg per day (equal to 0, 0.020, 0.039, 0.080, 0.16, 0.49, and 0.77 mg/kg bw per day in males and 0.017, 0.033, 0.066, 0.132, 0.409 and 0.750 mg/kg bw per day in females, respectively) for 14 days. The parameters evaluated were limited, including observations for mortality, morbidity and clinical signs of toxicity (data for individual animals were not provided for signs), measurement of body weight and food consumption, organ weight determinations (liver and kidney only) and a gross examination at study termination. Plasma and erythrocyte cholinesterase activity was assessed before treatment and on days, 1, 4, 7, and 14 in the control groups and in groups of males and females receiving the four lowest doses (i.e. 0.020, 0.039, 0.080 and 0.16 mg/kg bw per day). Cholinesterase inhibition was determined relative to the value for the appropriate concurrent control group. Brain cholinesterase activity was not measured, and clinical chemistry, haematological, urine and histopathological examinations were not conducted in this study.

There were two deaths; two females in the group receiving the highest dose (6.00 mg/kg) died or were sacrificed in a moribund condition on days 11 and 13 from treatment-related causes, respectively. Before death, the animals exhibited severe tremors, salivation and prostration. Clinical signs of toxicity in males and females were reported to start on day 2 and last until termination in the group receiving the highest dose (6.00 mg/kg) and included, initially, ataxia, tremors and miosis. From day 7 on, more severe tremors developed and exophthalmos and piloerection were also observed. At 3.00 mg/kg, clinical signs (slight tremors) were also noted in both sexes from day 4 until the end of the study. Signs of toxicity were considered to be treatment-related and were not observed at lower doses. Changes in body weight and/or food consumption seen at the two highest doses were also considered to be related to treatment. Statistically significant decreases in body weight and body-weight gain relative to respective control groups were observed in both sexes (being more severe in females) at 6.00 mg/kg during both weeks. Food consumption was also statistically significantly decreased in males and females at the highest dose in weeks 1 and 2. At 3.00 mg/kg, body weight was reduced (statistically significantly in males) during the first

week only in both sexes, while food consumption did not appear to be affected. Statistically significant decreases in liver and kidney absolute weights and weights relative to body weight were observed in both sexes at 6.00 mg/kg, but terminal body weights were also reduced. At gross necropsy, there were no findings that were attributed to treatment with the test material in any group.

At 1.00 mg/kg (the highest dose assessed), plasma cholinesterase activity was statistically significantly inhibited by 21–37% at all time-points during treatment in males, and by 27–38% on days 4, 7, and 14 in females. At 0.500 mg/kg, plasma cholinesterase was statistically significantly inhibited on day 4 in males (15%) and on days 4 and 7 in females (23%), but no significant inhibition was observed on day 14 in either sex. Erythrocyte cholinesterase was statistically significantly inhibited at 1.00 mg/kg (the highest dose assessed) in males by 51–61% and in females by 40–52%, on days 4, 7 and 14. At 0.500 mg/kg, a statistically significant reduction in erythrocyte cholinesterase activity was noted on these same days, ranging from 22% to 25%, in males. In females at this dose, a significant decrease in erythrocyte cholinesterase activity of only 16% was seen on day 4. This finding in females was not considered to be of toxicological relevance, although the study authors considered that the decreases in erythrocyte cholinesterase activity in both sexes at this dose were associated with administration of the test material. If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, and considering that brain cholinesterase activity was not measured in this study, the NOAEL was 0.250 mg/kg (equal to 0.0039 mg/kg bw per day) in males and 0.5 mg/kg (equal to 0.066 mg/kg bw per day) in females on the basis of statistically significant inhibition of erythrocyte cholinesterase activity (Fischer, 1978).

A statement of compliance with QA, but not with GLP, was provided. The study was preliminary in nature and was not conducted to fulfil a particular guideline.

In a preliminary study performed to aid in selection of doses for a study of subchronic neurotoxicity, groups of five male and five female albino rats (outbred) (Crl:CD®(SD)IGS BR VAF/Plus®) (aged 6 weeks at study initiation) were given diets containing technical-grade terbufos (AC92100; purity, 89.7%) (dissolved in acetone, mixed with a GRIT-O'Cobs® carrier) at a concentration (adjusted for purity) of 0 (acetone and carrier), 1.0, 5.0 or 6.0 mg/kg in males (equal to 0, 0.11, 0.55 and 0.67 mg/kg bw per day, respectively) and of 0 (acetone and carrier), 0.5, 3.0 or 4.0 mg/kg in females (equal to 0, 0.06, 0.33, and 0.43 mg/kg bw per day, respectively) for at least 21 days. Diets were made available to animals until termination on day 22. The number of parameters evaluated was limited and included observations for general condition, mortality and clinical signs of toxicity, and measurement of body weight and food consumption. Plasma, erythrocyte and brain (one-half homogenate) cholinesterase activities were measured from samples obtained and processed on day 22 at study termination and stored frozen at −70 C until analysis. Cholinesterase inhibition was determined relative to the value for the appropriate concurrent control group. Clinical chemistry, haematological, urine, organ weight, and gross and histopathological evaluations were not conducted in this study.

No animals died and no clinical signs of toxicity were observed. Statistically significant decreases in body-weight gain relative to respective control groups, considered to be treatment-related, were noted during each of the 4 weeks in males at the highest dose (6.0 mg/kg) and during weeks 1 and 2 in females at the highest dose (4.0 mg/kg). Food consumption was statistically significantly decreased only in males at the highest dose during

the first week of the study. Treatment-related decreases in blood and brain cholinesterase activities were observed in males and females at the two higher doses. In males at 5.0 mg/kg and 6.0 mg/kg, plasma cholinesterase activity was statistically significantly inhibited by 73% and 85%, respectively. In females, plasma cholinesterase activity was inhibited at 3.0 mg/kg and 4.0 mg/kg by 84% (not statistically significant) and 94% (statistically significant), respectively. Erythrocyte cholinesterase activity was statistically significantly inhibited by 98% and 99% in at 5.0 mg/kg and 6.0 mg/kg, respectively. A smaller inhibition of erythrocyte cholinesterase of about 35% was noted in males at 1.0 mg/kg. In the study report, the finding was not considered to be associated with the administration of test material, as it was not statistically significant and there were no reductions in either plasma or brain cholinesterase activity in males at this dose. It may, however, have been related to treatment as the values for cholinesterase activity for four of the five animals in this group fell below those in the control group in replicate assays. In females, at 3.0 mg/kg and 4.0 mg/kg, respectively, erythrocyte cholinesterase was inhibited by 99% to 100%. Statistically significant decreases in brain cholinesterase of 64% and 81% were observed at 5.0 mg/kg and 6.0 mg/kg in males, respectively, as were statistically significant decreases in females at 3.0 mg/kg and 4.0 mg/kg, of 68% and 84%, respectively.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect and brain (not erythrocyte) cholinesterase inhibition and clinical signs of toxicity are considered to be relevant effects for terbufos, the NOAEL was 1.0 mg/kg in males (equal to 0.11 mg/kg bw per day) and 3.0 mg/kg in females (equal to 0.06 mg/kg bw per day) on the basis of statistically significant inhibition of brain cholinesterase inhibiton at the next highest dose (Mandella, 1999). Statements of compliance with QA and GLP were provided. This study was preliminary in nature and was not conducted to fulfil a particular guideline.

In a preliminary feeding study, groups of 10 male and 10 female albino RH Wistar rats were given diets containing technical-grade terbufos (CL 92 100; purity, 96.7%) at a nominal concentration of 0, 0.125, 0.5 or 2.0 mg/kg (nominally equivalent to 0, 0.012, 0.069 and 0.299 mg/kg bw per day in males and 0, 0.012, 0.053, and 0.212 mg/kg bw per day in females) for 31 days. Data on analysis of test material levels in the diet were not provided so test material intake and stability in the feed could not be confirmed. Study parameters examined included observations for appearance, mortality, and measurement of body weight and food consumption. In five animals of each sex per group (when possible), haematological and limited clinical chemistry evaluations (glucose, urea nitrogen and glutamic-pyruvic transaminase), liver and kidney weight measurements and a gross examination were conducted. At the end of the study, blood (from fasted animals) and brain samples (one-half of the cerebrum) were taken from five animals of each sex per group for determination of cholinesterase activity. Brain samples were stored frozen until analysis, and assays were conducted on homogenates. Inhibition of cholinesterase activity was determined relative to the value for the appropriate concurrent control group.

There were many deaths in the study; four males at the highest dose died on day 3, 17, 31 or 31, one male at the intermediate dose died on day 12, and one male at the lowest dose died on day 31. One female at the highest dose was terminated in a moribund condition on day 24, two females at the intermediate dose died on day 9 or 24 and one control female died on day 31. The deaths of five unspecified animals were thought to be the result of a respiratory infection, and autolysis prevented attempts to determine the cause of death in the remainder (again, unspecified). Therefore, it was not possible to ascertain whether any of the deaths were related to treatment. There was no apparent effect of treatment on

body weight, food consumption, on measured haematological and clinical chemistry parameters, or on organ weights in any group treated with terbufos. Cholinesterase activity was statistically significantly inhibited only at the highest dose in both sexes. Statistically significant inhibition of cholinesterase activity in plasma (57%), erythrocytes (36%) and brain (28%) was noted in males at the highest dose, as was (mostly) a statistically significant inhibition in plasma (68%), erythrocytes (37%; not statistically significant), and brain (53%) in females at the highest dose. These decreases were attributed to treatment. There was also a decrease of 29% in erythrocyte cholinesterase activity in males at the intermediate dose; although not statistically significant, this was considered to be a possible result of treatment owing to the magnitude of the decrease. It was reported that upon gross pathological examination of four to five animals of each sex per group at study termination, no gross lesions were found that were attributed to treatment (gross pathology data were not provided). An overall NOAEL could not be identified in this study because insufficient information was provided about the mortality that occurred at all doses in males and at the intermediate and highest dose in females. There were a number of unspecified deaths that were judged likely to be the result of infection in the animal facility. In addition, intake of test material could not be confirmed (Morici, 1972). No statements of compliance with QA or GLP were provided. This study was not performed according to a specific guideline.

In a feeding study, groups of 20 male and 20 female Sprague-Dawley rats were given diets containing technical-grade terbufos (purity, 90.1%; prepared in corn oil and methylene chloride, 1 : 1) at a concentration (adjusted for purity) of 0 (vehicle), 0.125, 0.250, 0.500 or 1.000 mg/kg (equal to mean intakes of test substance of 0, 0.011, 0.021, 0.041, and 0.082 mg/kg bw per day in males and 0, 0.012, 0.023, 0.048 and 0.095 mg/kg bw per day in females) for 3 months. Parameters evaluated included observations for mortality and clinical signs of toxicity, assessments of body weight, food intake and food efficiency, ophthalmoscopic, haematological, and clinical chemistry evaluations, urine analysis, organ weight determinations, a macroscopic examination in all animals and a microscopic evaluation of organs and tissues in animals at the highest dose and in the control group only. The heart, liver, and kidney, any gross lesions or masses, and any other tissues, as indicated by findings at the highest dose, were examined microscopically in all animals. Plasma and erythrocyte cholinesterase activities were measured on day 1, and at weeks 1 and 2 and months 1, 2 and 3 in 10 animals of each sex per group. Brain cholinesterase activity was measured in 10 animals of each sex per group at study termination. Inhibition of cholinesterase activity was determined relative to the value for the appropriate concurrent control group.

One female at 0.5 mg/kg was mistakenly sexed as male until week 4, when the animal was put with other females in the group. All animals survived until the end of the study, except for one female and one male at 0.5 mg/kg, which died of accidental causes on days 8 and 51. No clinical signs of toxicity were observed and there were no obvious effects of treatment on body weight, food consumption, food efficiency, haematological, clinical chemistry, or urine analysis parameters at any dose. Slight statistically significant increases in liver weight to body weight ratios but not absolute weights in females at the two highest doses were not considered to be of biological relevance. Plasma cholinesterase activity was statistically significantly decreased only at the highest dose, at which activity was inhibited throughout the study in both sexes. At study termination, decreases in activity at the highest dose were 33% in males and 52% in females. Erythrocyte cholinesterase and brain cholinesterase activities were not affected by treatment. The macroscopic examination was not remarkable. Upon microscopic examination of tissues and organs, increases were observed in the incidence of mandibular lymph node hyperplasia at the highest dose in

males (20%) and females (70%) compared with that in male control animals (6.25%) and female control animals (32%). Also, the incidence of mesenteric lymph node hyperplasia was increased in females at the highest dose (50% compared with 25% in the control group), but not in males at the highest dose (25% compared with 30% in the control group). There was no clear association with treatment for these or other histopathology findings in the study. In the study report, the mesenteric lymph node lesions were considered to be related to nematodiasis. Other groups treated with terbufos were not examined for the incidence of either mandibular or mesenteric lymph node hyperplasia. Some respiratory tract lesions that occurred with similar frequency in control groups and in groups treated with terbufos were ascribed to chronic murine pneumonia (said to be a common finding in rodent colonies). If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL was ≥1.00 mg/kg (the highest dose tested) in males and females (equal to 0.082 mg/kg bw per day in males and 0.092 mg/kg bw per day in females) (Daly & Knezevich, 1979). A statement of compliance with QA, but no GLP statement, was provided. The protocol was generally consistent with US EPA Subdivision F Guidelines (November 1982 and 1984, revised).

In a study of toxicity, groups of 30 male and 30 female CD® (Sprague-Dawley derived) COBS® rats were given diets containing technical-grade terbufos (AC 92 100; purity, 89.6%) (prepared in corn oil and methylene chloride, 1 : 1) at a concentration of 0 (vehicle only), 0.125, 0.5, or 1.0 mg/kg (equal to 0, 0.007, 0.028, and 0.055 mg/kg bw per day for males and 0, 0.009, 0.036, and 0.071 mg/kg bw per day for females) for 1 year. Parameters assessed included observations for general health and mortality, clinical signs of toxicity, measurement of food consumption and body-weight changes, haematological and clinical chemistry determinations, urine analysis, organ weight measurements and ophthalmoloscopic and macro- and microscopic pathology examinations. Plasma and erythrocyte cholinesterase activities were assessed in 10 animals of each sex per group at week 6, months 3 and 6, and at study termination. Brain cholinesterase activity was determined at study termination. Inhibition of cholinesterase activity was calculated relative to values for values for concurrent controls.

There were six deaths in the study; two control animals (one male on day 306 and one female on day 271) and two animals at the intermediate dose (one male on day 351 and one female on day 262) were terminated in a moribund condition. One male at the intermediate dose was found dead on day 310 and one female at the highest dose died accidently on day 97. There was no obvious pattern in the deaths in the animals treated with terbufos that would suggest a relationship with administration of the test material. There was no clear relationship with treatment for the slight increases observed mostly during the last half of the study in the group of females at the highest dose in the incidence of excess lacrimation, chromodacryorrhoea and alopecia compared with the control and other treated groups. As similar increases were noted at lower doses in males, the findings in females were considered to be likely to be caused by random variation. There were no clear effects of treatment on body weight. Variation was noted among groups in food consumption over the course of the study, but there was no consistent pattern of findings that would clearly indicate an effect of treatment in groups of males or females treated with terbufos. There was no evidence that the results of the urine analyses or those of the ophthalmoscopic and haematological examinations were related to treatment. Statistically significant decreases, of small magnitude, in blood urea nitrogen and bilirubin at termination in females at the highest dose were not considered to be clearly of adverse significance in the absence of other findings. Clinical chemistry evaluations were otherwise not remarkable. Slight, statistically signifi-

cant reductions were observed at the highest dose in the absolute weight and weight relative to brain weight (but not relative to body weight) of male testes, and slight statistically significant decreases in the absolute weight and weight relative to brain weight (but not relative to body weight) of the kidney were noted in females at the intermediate and highest doses. These organ-weight changes were not clearly adverse in the absence of other supporting indications of toxicity. No obvious effect of treatment was indicated by the results of the gross and microscopic examinations. There was no evidence of carcinogenity.

Decreases in cholinesterase activity that could be clearly related to treatment were observed only at the highest dose in both sexes. Plasma cholinesterase activity was statistically significantly inhibited in males at the highest dose by 25% and 29%, respectively, at 6 and 12 months. Statistically significant inhibition of plasma cholinesterase activity (33–51%) was noted at all time-points in females at the highest dose. Erythrocyte cholinesterase activity was not statistically significantly inhibited at any dose or time-point in either sex. Brain cholinesterase activity in males was decreased relative to control values at the lowest, intermediate and highest dose, respectively by 4% (statistically significant), 3% (not statistically significant) and 8% (statistically significant). On the basis of the magnitude and pattern of the response, inhibition at the lowest dose was not considered to be related to treatment. In females, brain cholinesterase activity was statistically significantly inhibited only at the highest dose. Virtually no changes relative to the control group was observed at the lowest and intermediate doses. Although the decrease observed at the highest dose in males and females may have been related to treatment, because of the relatively low magnitude of the response and the absence of clinical signs at this dose, it was not considered to be toxicologically relevant.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect and inhibition of brain cholinesterase activity of 8–10% is not considered to be toxicologically relevant, the NOAEL was 1.0 mg/kg (equal to 0.055 mg/kg bw per day in males and 0.071 mg/kg bw per day in females), the highest dose tested (Daly, 1987). This study complied with QA and GLP and was consistent with US EPA Subdivision F Guidelines.

Dogs

In a short-term study to assess cholinesterase activity, groups of four male and four female beagle dogs (except at the highest dose, where two dogs of each sex were used) were given technical-grade terbufos (AC 92 100; purity, 89.6%) at a dose (adjusted for purity) of 0 (vehicle), 1.25, 2.5, 5.0, or 15.0 µg/kg bw per day, administered orally in corn oil in gelatin capsules, once daily in the morning for 29 days. Originally planned for 28 days, dosing was extended by 1 day through a protocol amendment. The number of parameters evaluated was limited and included observations for mortality, morbidity and clinical signs of toxicity, and measurement of body weight and food consumption. Plasma and erythrocyte cholinesterase activities were assessed before dosing and after 1, 2, and 4 weeks of treatment from blood samples collected before dosing on the given day. Brain cholinesterase activity was determined at study termination in samples from the cerebrum and cerebellum obtained 20–24 h after administration of the last dose. In the study report, cholinesterase inhibition in the plasma and erythrocytes was determined and statistically analysed relative to values obtained before the start of dosing. Inhibition of cholinesterase activity in the brain was determined relative to the value for the appropriate concurrent control group. Clinical chemistry, haematological, urine, organ weight and gross and microscopic histopathological evaluations were not performed in this study.

There were no deaths in the study and no clinical findings were observed that could be ascribed to treatment in any group. One instance of vomiting was observed in each of two dogs at the lowest dose only. There were no appreciable differences in body weights or food consumption among groups of either sex during the study. Statistically significant decreases in plasma cholinesterase activity ranging from 33% to 37% were noted at the highest dose in both sexes at all time-points and were ascribed to treatment. Inhibition of plasma cholinesterase activity of 20–21% was observed at 5.0 µg/kg bw per day in males after 2 and 4 weeks of treatment and in females at all time-points; these values were not statistically significantly from those for the respective groups of pre-treatment controls and were considered to be of marginal biological relevance in the study report. If inhibition of plasma cholinesterase activity was determined relative to values for the appropriate concurrent controls instead of pre-treatment values (as in the study report), there was not much difference in the magnitude of the decreases calculated by either procedure for either sex at any week or dose, except in females at 2.5 and 5.0 µg/kg bw per day. In these groups, slightly greater decreases in plasma cholinesterase activity were measured relative to values for concurrent controls at all time-points (28–30% at 5.0 µg/kg bw per day, and 18–23% at 2.5 µg/kg bw per day) than relative to pre-treatment values (20–21% at at 5.0 µg/kg bw per day, and 10–18% at 2.5 µg/kg bw per day). Under the conditions of the study, there was no effect of treatment in either sex or at any dose on erythrocyte cholinesterase activity (relative to pre-treatment values or to values for the appropriate concurrent controls) or on brain cholinesterase activity relative to the values for the appropriate concurrent controls. If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL was 15 µg/kg bw per day (the highest dose tested) in males and females (Shellenberger, 1987). Statements of compliance with QA and GLP were provided. This was a special study that was not conducted to comply with a particular guideline.

In a preliminary feeding study, groups of two male and two female beagle dogs (aged 8–12 months) were given diets containing technical-grade terbufos (CL 92 100; purity, 96.7%) (prepared in a vehicle of corn oil) at a nominal dose of 0, 0.01, 0.05, or 0.25 mg/kg bw per day administered daily for 30 consecutive days. Food consumption was estimated by visual inspection and many animals did not always consume all the food offered. In addition, data on the analysis of concentrations of test material in the diet were not provided, so intake of test material could not be determined with any degree of confidence, and stability of the test material in the feed could not be confirmed. Parameters assessed included observations for appearance, mortality and measurement of body weight and food consumption, haematological and limited clinical chemistry evaluations (plasma glucose, glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase, and urea nitrogen), liver and kidney organ weight measurements and gross examination of all animals. Plasma and erythrocyte cholinesterase activities were assessed before the start of dosing, at week 2 and at study termination in fasted animals. Brain cholinesterase activity was measured at study termination in homogenates of samples taken from the cerebrum. Cerebrum tissue was stored frozen until analysis. Inhibition of cholinesterase activity was determined relative to the values for the appropriate control group.

There were no deaths in the study and no clinical signs of toxicity were observed. On the basis of the estimated food refusal (%) during the study, received doses were as much as 20–35% lower than nominal doses in most animals at the lowest and intermediate doses, and by as much as 35–50% at the highest dose. Over the course of the study, males at the highest dose failed to gain weight and females at the highest dose and one female at the intermediate dose lost weight; these findings were statistically significant for the groups

receiving the highest dose. There was no apparent effect of treatment on organ weights, or on the haematological and clinical chemistry parameters measured. No treatment-related findings were observed during the gross examination. As values for cholinesterase activity were similar in males and females at the same dose, data were pooled for statistical analysis. By week 4, statistically significant decreases in plasma cholinesterase activity of 68% and 84%, and in erythrocyte cholinesterase activity of 35% and 80% were observed at the lowest and intermediate doses, respectively. Brain (cerebrum) cholinesterase activity was statistically significantly inhibited only at the highest dose (by 66%). An overall NOAEL could not be identified because intake of test material could not be reliably estimated (Morici, 1972). No statements of compliance with QA or GLP were provided. This study was not performed to comply with a specific guideline.

In a feeding study, groups of four male and four female beagle dogs (aged 10–14 months, i.e. somewhat older than the age recommended in the guidelines) were given terbufos (AC 92,100; purity not specified) at a nominal concentration of 0 (vehicle only), 2.5, 10.0, or 40.0 mg/l (nominally equivalent to 0, 2.5, 10, and 40 µg/kg bw per day) in corn oil, administered daily for 6 days per week, for 6 months. One ml of the test material in the vehicle was injected via a syringe on top of each dog's daily food ration (kibbled dog chow). Doses were adjusted weekly for each individual animal's body weight. A set amount of treated food (apparently totalling 3300 g per week) was offered for 1 h each day and any food not consumed in that time was removed and weighed in order to measure food consumption and estimate intake of test material. Neither dosing solutions nor treated diets were analysed to confirm content or stability of the test material. Parameters examined included mortality and clinical signs of toxicity, assessments of body weight and food intake, ophthalmoscopic, haematological, and limited clinical chemistry (alkaline phosphatase, blood urea nitrogen, fasting blood sugar, and serum glutamic-pyruvic transaminase) evaluations, urine analysis, weights of selected organs (adrenals, gonads, kidneys, heart and liver), and macroscopic and microscopic examinations. Plasma and erythrocyte cholinesterase activities were measured before the start of dosing and at weeks 0, 4, 12 and 26. Brain cholinesterase activity was assessed at week 26. Although data on cholinesterase activity were provided for individual animals, in the study report data from both sexes were combined for statistical analysis (*t*-test) for each dose and type of cholinesterase activity, as differences between the sexes were not considered to be remarkable. Data on cholinesterase activity from groups treated with terbufos were compared with data for the combined concurrent control group.

Test material intake could not be reliably determined. In addition to a lack of analytical information on the purity, dietary content and stability of the test substance, some animals did not always consume all their food. Since the solution containing the test material was placed on top of the daily food ration, it is not clear how much of the test material was ingested by animals that did not consume the daily food allotment. Female dogs had a greater tendency not to consume the entire meal. The approximate percentage of unfinished meals over the course of the study for all females at each dose was 36% in the control group, 38% at the lowest dose, 40% at the intermediate dose and 23% at the highest dose. Over the duration of the study, some females received only about 80–90% of the intended dose of terbufos. The situation was less severe in males; the approximate percentage of all unfinished meals over the duration of the study for all males at each dose was 31% for the control group, 5% at the lowest dose, <1% at the intermediate dose and 2% at the highest dose. Also, initial body weights varied more than might be desirable (20% difference between some animals of the same sex).

One male in the control group was terminated in a moribund condition and was found to have a colon obstruction and peritonitis. Clinical signs of toxicity were not observed in any animals (individual animal or summary data was not included in the report).

With regard to general condition, one male at the lowest dose, one male at the highest dose, three females in the control group, one female at the highest dose, and possibly one female at the lowest dose and and one female at the intermediate dose, had histopathological indications of bronchopneumonia. Body-weight loss (measured over the duration of the study) was noted at termination at week 26 in individual animals in all groups except females at the highest dose. The incidence of weight loss (and average body-weight change) during the study was: in males, 2 (–0.55 kg), 3 (–0.45 kg), 3 (–1.8 kg), and 3 (–0.4 kg), respectively; and in females, 1 (0.35 kg), 1 (0.075 kg), 1 (0.15 kg) and 0 (0.425 kg), in the control group and at the lowest, intermediate and highest dose, respectively. There was no obvious dose-related pattern in these findings. The greater weight loss in the group of males at the intermediate dose was due to one animal that was the heaviest animal in the group at initiation of treatment and that lost 5.7 kg in weight during the study, apparently exhibiting no clinical signs or indications of toxicity, and no unusual findings with regard to food consumption or other parameters examined (except for a slight elevation in erythrocyte sedimentation rate at week 26). The only gross or histopathopathological finding in this animal was diffuse mild leukocytosis in the liver, which the study report stated could, along with the elevated erythrocyte sedimentation rate, have been related to an infection in this dog near the end of the study. In other animals, there was no clear effect of treatment on the haematological, biochemical, urinary parameters or organ weights assessed or in the gross or histopathological examinations.

Inconsistencies and variability were noted in the data on cholinesterase activity. In the study report, differences between sexes for a given type of cholinesterase activity and at a particular dose were considered to be minimal and data were combined for both sexes for statistical analysis. Under these conditions, there were no statistically significant differences in brain or erythrocyte cholinesterase activity at the end of the study (week 26). Plasma cholinesterase activity at week 26 was statistically significantly decreased relative to values for the combined control group, by 26% at the intermediate dose and 31% at the highest dose; the study report considered these decreases to be minimal but treatment-related. The value for plasma cholinesterase activity from the control male that was terminated in a moribund condition was not included in the calculation, although it was not an outlying value compared with those for the rest of the animals in the group. Data for individual animals were provided. When plasma cholinesterase activity was calculated for each sex separately at week 26, without statistical evaluation, there was no clear effect on plasma cholinesterase activity in males relative to that of the control group, either with the inclusion of the terminated control male (no decrease at the lowest dose, decrease of 35% at the intermediate dose, decrease of 15% at the highest dose) or without it (decrease of 9% at the lowest dose, decrease of 47% at the intermediate dose, decrease of 31% at the highest dose) owing to the lack of a clear dose–response relationship at the intermediate and highest doses. In females at week 26, decreases in plasma cholinesterase activity relative to that of the control group were noted at the intermediate dose (19%) and highest dose (32%), indicating a possible, small effect of treatment, particularly at the highest dose. Erythrocyte cholinesterase activity at the highest and intermediate doses was decreased by 26% and 6%, respectively, when data for males were taken separately (as compared with reduction of 17% and 13% (neither statistically significant) at the highest and intermediate doses, respectively, when

data from both sexes were combined), suggesting a possible minimal, but certainly not clear, effect of treatment at the highest dose.

There were no clear adverse findings associated with treatment in this study, but because nominal concentrations of test material in the diet could not be confirmed, intake of test material could not be reliably estimated and underconsumption of diets containing terbufos was noted, especially in females, a NOAEL could not be identified (Morgareidge, 1973). Statements of compliance with QA and GLP were not provided. The protocol and study conduct were considered to be inadequate according to current standards.

Subsequent to the 6-month feeding study in dogs (Morgareidge, 1973), a study was conducted to attempt to address questions about the 6-days-per-week dosing regimen used in that study, and to determine whether a 7-days-per-week regimen would influence cholinesterase activity, particularly in erythrocytes. Groups of two male and two female beagle dogs (aged 9.5–12 months) were given diets containing technical-grade terbufos (purity, 88%) at a dose of 50 µg/kg bw daily, for (1) 7 days per week for 28 days; or (2) 6 days per week (basal food given on day 7) for 28 days, followed in both groups by a 28 day recovery period without treatment. Five out of the eight dogs in the study had previously been exposed to dichlorvos, another cholinesterase-inhibiting chemical, 4–7 months before their arrival in the testing facility. No other details were provided. A concurrent control group was not included in the study. The test material was prepared as a solution in corn oil, added to a fixed amount of food via a syringe at the rate of 1 ml/kg bw, and given to each dog individually in the morning. If treated food was not eaten within 1 h, it was removed and weighed. It was stated that any food treated with terbufos that was not consumed in the morning was re-administered to the animal in the afternoon (regular feed of plain dog chow), such that any remaining test substance was eaten. Erythrocyte and plasma cholinesterase activities were measured before treatment on days -7 and -6, on days 1, 3, 7, 10, 14, 18, 21 and 28, and after treatment on days 29, 31, 35, 38, 42, 49 and 56. Average cholinesterase inhibition for each group (both sexes combined) was calculated relative to the respective average value before treatment (sexes combined) at each time-point, but apparently these comparisons were not analysed statistically. Statistical analysis was performed for each time-point (both sexes combined) to determine whether there was a significant difference between the 6-day treatment regimen and the 7-day treatment regimen. Animals were observed for general condition, signs of toxicity and body-weight changes during treatment. After the first treatment phase of the study was concluded, a 28-day cross-over phase was performed in which the group of animals dosed for 7 days per week were dosed for 6 days per week for 28 days and the group of animals dosed for 6 days per week were dosed for 7 days per week for 28 days. Erythrocyte and plasma cholinesterase activities were assessed before treatment and on days 1, 3, 7, 10, 14, 18, 21, 25, and 28. There was no recovery period. Brain cholinesterase activity was not assessed in either the first phase or the cross-over phase.

Dosing solutions were analysed four times during the first phase of the study and found to contain 87.6–98.8% of the target amounts (average, about 92%). All animals survived both study phases and no clinic signs of toxicity were observed. In the first phase, initial body weights in some dogs varied considerably; a difference of about 40–50% was noted between some animals in the 7- or 6-day dosing group. Three out of four dogs in both the 7- and 6-day feeding groups lost weight during at least part of the first phase of treatment; this may have been related to treatment, but there were no control groups available

for comparison. Some weight gain was noted in most animals in both groups during the recovery period. Recorded weekly food intakes for individual animals in the first phase were fairly variable week-by-week and between individuals in dogs fed the test material for 7 days per week; it is thus not clear whether all animals in that group ate all the treated food offered. Far less variability was observed in dogs fed test material for 6 days per week, as apparent weekly maximums of 2400 g of food per dog were commonly consumed.

Owing to the small sample size and variability in the data, it was difficult to determine whether there was a sex difference in cholinesterase activity measurements. In the study report, it was assumed that there were no sex differences and group means from data from both sexes combined were compared. In the first phase of the study, on day 28, although data were apparently not analysed statistically, erythrocyte and plasma cholinesterase activities decreased by 14% and 68%, respectively, in the group fed test material for 7 days per week, and by 4% and 40%, respectively, in the group fed test-material for 6 days per week relative to values before treatment (there was no concurrent control group). The difference between the groups treated for 7 days per week and for 6 days per week was not statistically significant with regard to erythrocyte cholinesterase activity at any time-point, either during or after treatment. Differences in plasma cholinesterase activity between the groups treated for 7 days per week and for 6 days per week were statistically significant at weeks 7, 21 and 28. Based on the data provided, recovery to pre-treatment or almost pre-treatment levels was noted during the recovery phase with both dosing regimens, but progress appeared to be a little more rapid on the 6-day regimen and plasma cholinesterase activity seemed to recover more rapidly than erythrocyte cholinesterase activity on either regimen.

In the cross-over phase of the study, after 28 days of treatment, erythrocyte and plasma cholinesterase activities decreased by 18% and 55%, respectively, in the cross-over group fed test material for 7 days per week and by 28% and 52%, respectively, in the cross-over group fed test material 6 days per week, relative to values before treatment. No statistically significant differences were found between the groups on the 6- and 7-day feeding regimens at any time-point assessed. Body-weight losses and variable food intakes were noted with both groups. The study authors concluded that the decrease in cholinesterase activity noted was reversible after cessation of treatment. They also concluded that there were no cumulative adverse effects in erythrocyte or plasma cholinesterase activity on either regimen. In the first phase, however, there did appear to be a slightly larger effect on cholinesterase activity on the 7-day regimen compared with the 6-day regimen, and in the second phase a stronger effect on erythrocyte activity was observed relative to the first phase, possibly implying some carry-over influence between treatments. Owing to the small sample size, the limited protocol (no assessment of brain cholinesterase activity), the lack of a concurrent control group, the use of only one dose, questions about the intake of test material based on food consumption data, the variability in data for individual animals and other uncertainties (e.g. previous treatment of test animals with dichlorvos), this study is not suitable for the identification of a NOAEL or for performing regulatory toxicology assessments for terbufos. No statements of compliance with QA or GLP were provided and the study was not performed to comply with a particular guideline (Berger, 1977).

In a 1-year study, groups of male and female beagle dogs were fed gelatin capsules containing technical-grade terbufos (AC 92,100; purity, 89.6%) at an initial dose (not adjusted for purity) of 0 (vehicle only), 15, 60, 240 or 480 µg/kg bw per day in corn oil for 1 year. Owing to toxicity that resulted in mortality, the dose of 480 µg/kg bw per day was

reduced to 120 µg/kg bw per day 1 day before the start of week 6, and the dose of 240 µg/kg bw per day was decreased to 90 µg/kg bw per day 2 days after the beginning of week 8. Eight animals of each sex were assigned to the vehicle control group and six animals of each sex were assigned to each of the groups receiving terbufos. During weeks 3 and 4, an error occurred and the doses administered were only 5.2% of those intended. Administration of the test material continued until 20–24 h before termination, and animals were terminated during the 5 days following the 1-year period of treatment. Parameters assessed included general condition, mortality, morbidity and clinical signs of toxicity, measurement of body weight and food consumption, and clinical chemistry, haematology, urine analysis, ophthalmoscopy, organ weight, and gross and microscopic evaluations in all animals. Plasma and erythrocyte cholinesterase activity in fasted animals was assessed before treatment, at months 3 and 6 and at study termination. Brain cholinesterase activity in samples from the cerebrum and cerebellum was measured from tissues taken at study termination and stored frozen until analysis. Inhibition of cholinesterase activity was determined relative to the value for the appropriate concurrent control group.

There were three deaths attributable to treatment-related causes. One male and one female at the highest dose died during week 6, while the dose of 480 µg/kg bw per day was being administered. Clinical signs observed in these animals included vomiting, slight tremors and inactivity in the male and tremors, diarrhoea, weak hind legs and excessive salivation in the female. Decreased body weight and food consumption were noted in both dogs. One female at 240/90 µg/kg bw died during week 7, also from causes related to treatment, while receiving the dose of 240 µg/kg bw per day. Clinical findings in this animal included tremors that increased in severity, inactive behaviour, red-tinged faeces, excessive salivation, dehydration, listless behaviour, rough hair coat and decreased body weight and food consumption. One other female at the highest dose was sacrificed in a moribund condition at the start of week 31, owing to causes unrelated to treatment (prolapsed vagina).

Clinical signs of toxicity noted in surviving males at 480 µg/kg bw per day included tremors (slight to more severe) and inactivity. In males at 240 µg/kg bw per day, clinical signs of toxicity included excessive salivation, dehydration, red-tinged faeces, listlessness and inactivity. Symptoms in females were generally more severe than in males at these doses and included tremors (slight to more severe), inactivity, excess salivation, red-tinged faeces, vomiting and weak hind legs at the highest dose (480 µg/kg bw per day), and slight tremors, inactive behaviour, excessive salivation, weak hind legs, diarrhoea, and red-tinged faeces at the lowest dose (240 µg/kg bw per day). Decreases in body weight and food consumption were noted in males at both 480 µg/kg bw per day and 240 µg/kg bw per day and to a greater extent in females at these doses during the early weeks of the study before and just after the decrease in dose. During this time, body-weight decreases reached statistical significance in females at the highest dose, and statistically significant decreases in food consumption were noted for males and females at the highest dose and for females at 240 µg/kg bw per day. Shortly after the two higher doses were lowered to 120 and 90 µg/kg bw per day, respectively, and for the remainder of the study, there was no apparent effect of treatment with terbufos on body weights or food consumption at any dose.

After the two higher doses were reduced, the only clinical findings noted during the remainder of the study, for which an association with treatment could not be dismissed, were two instances of severe convulsions in one female at 120 µg/kg bw per day during weeks 46 and 47. There were no obvious effects of treatment on the ophthalmoscopic, clinical chemistry or urinary parameters assessed or on organ weights after doses of terbufos

of ≤120 µg/kg bw per day. In males, at month 3, slight statistically significant decreases were found in erythrocyte counts at 90 and 120 µg/kg bw per day, and in haemoglobin, erythrocyte volume fraction, and mean corpuscular haemoglobin concentration at 120 µg/kg bw per day. Numbers of platelets were also slightly increased (statistically significantly) in males at 90 and 120 µg/kg bw per day. Slight, statistically significant decreases in haemoglobin and erythrocyte volume fraction were noted at month 3 in females at 120 µg/kg bw per day. These perturbations were transient and had resolved by the next assessment at month 6. They may have been related to treatment at these doses or may have been residual effects from previous dosing at 240 and 480 µg/kg bw per day.

Data on cholinesterase activity reported in this study were difficult to interpret owing to variability in the values for individual animals and because of generally inconsistent or sometimes shallow dose–response relationships. Data were apparently not analysed statistically. Plasma cholinesterase activity was inhibited by about 40% or more in all groups receiving terbufos at all time-points in both sexes. At study termination (week 52), plasma cholinesterase activity was inhibited at the lowest, low intermediate, high intermediate and highest doses by 44%, 66%, 67% (shallow dose–response relationship) and 68%, respectively, in males, and by 45%, 68%, 67% (shallow dose–response relationship) and 74%, respectively, in females. Owing to the magnitude of the relative decreases, a relationship to treatment could not be excluded at any dose. At study termination (week 52), erythrocyte cholinesterase activity was lower than the control value at the lowest, lower intermediate, higher intermediate and highest doses in males by 4%, 13%, 18% and 19%, respectively. Similar patterns were found at earlier time-points. The decreases at week 52 were considered to be marginal and the changes of slightly higher magnitude at the two higher doses were considered to be of no toxicological significance. In females, inhibition of erythrocyte cholinesterase activity varied little from control values at the lowest and lower intermediate doses, and ranged from 18% to 28% at the higher intermediate dose and 27% to 35% at the highest dose, at all time-points. At study termination (week 52), erythrocyte cholinesterase activity was lower than the control value in the lowest, lower intermediate, higher intermediate and highest doses in females by 6%, 15%, 20% and 27%, respectively. The magnitude and consistency over time of the change at the highest dose was such that a relationship to treatment could not be dismissed. At week 52, cerebellum cholinesterase activity was not inhibited at any dose (values were 98% to 125% of those of the controls) in males. Cerebrum cholinesterase activity in males was lower than the control value at the lowest, low intermediate, higher intermediate and highest dose by 5%, 10%, 22%, and 22%, respectively. Despite the lack of a dose–response relationship at the two higher doses and the large variability in the data, the magnitude of the response in the brain at the two higher doses was such that a relationship to treatment could not be dismissed. For the same doses, inhibition of cholinesterase activity in the cerebellum in females was 3%, 3%, 12% and 21%, respectively. The magnitude of inhibition in the brain at the two higher doses could not be ignored as a possible effect of treatment; however, the decrease of 12% was not considered to be toxicologically relevant. At the lowest and highest doses, cholinesterase activity in the cerebrum of females was similar to that in controls (although activity fell below the mean for controls by 18% and 24% at the two intermediate doses). Owing to the lack of a dose–response relationship, a treatment-related effect was not readily supported.

Gross lesions seen in the intestinal tracts of animals that died during weeks 6 and 7 had non-neoplastic microscopic correlates. On histopathological examination, the male at 480 µg/kg bw per day that was found dead at week 6 had diffuse congestion of the

duodenum, jejunum, ileum and colon, congestion of the lungs, kidneys and liver and fibrinous thrombi in the pulmonary vessels of the lungs and in the arteries of the pancreatic mesentery. Microscopic findings in the female at the highest dose found dead at week 6 were marked haemorrhage and congestion in the mucosa and muscularis of the jejunum and areas of necrosis in the muscularis, mucosa and Peyer patches of the ileum (possibly secondary to intussusception). The female at 240 µg/kg bw per day found dead at week 7 had congestion of the muscularis and mucosa of the jejunum on microscopic examination. The study authors considered that these findings were likely to be related to treatment. Other gross and microscopic findings in the study were considered to be incidental and not related to treatment.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, and inhibition of brain (not erythrocyte) cholinesterase activity is considered to be a relevant effect for terbufos, the NOAEL was 60 µg/kg bw per day in males on the basis of a decrease (22%) in cerebral cholinesterase activity for which a relationship to treatment could not be excluded, and 90 µg/kg bw per day in females on the basis of a decrease in cerebellar cholinesterase activity of 21% and instances of severe convulsions in one female for which an association with treatment could not be dismissed (Shellenberger & Billups, 1986). Statements of compliance with QA and GLP were provided and the protocol was consistent with US EPA Subdivision F Guidelines (November 1982 and 1984, revised).

Sheep

In a feeding study, groups of three wethers (males) were given diets containing technical-grade terbufos (purity, 89.8%; in corn oil, diluted with an equal volume of methylene chloride) at a concentration (not adjusted for purity) of 0 (with vehicle only), 0.01, 0.1 or 1.0 mg/kg (equal to 0, 0.0003, 0.0023, and 0.0245 mg/kg bw per day) administered daily in two portions (half in the morning and half in the afternoon) for 42 days. Parameters assessed included mortality and clinical signs of toxicity, measurement of body-weight changes and food consumption, ophthalmoscopic examination, haematological and clinical chemistry determinations, urine analysis, and measurement of heart and respiratory rate. Necropsies were not conducted at study termination. Erythrocyte cholinesterase activity was determined before the start of treatment and on days 1, 3, 7, 14, 21 and 42. Erythrocytes were stored frozen for an unspecified period of time before analysis. Erythrocyte cholinesterase activity in groups treated with terbufos was compared with that for the appropriate concurrent controls at each time-point. Brain cholinesterase activity was not measured. The study report stated that the blood plasma of sheep had little or no cholinesterase activity and therefore was not assessed.

No deaths occurred during the study and no clinical signs of toxicity were observed. There was no obvious effect of treatment with terbufos on any parameter examined, including erythrocyte cholinesterase activity. It is not known whether storage conditions before assay had any effect on the cholinesterase activity of the erythrocytes. Brain cholinesterase activity was not assessed. The NOAEL in males (the only sex tested) was ≥1.00 mg/kg (equal to 0.0245 mg/kg bw per day) (the highest dose tested) (Garces et al., 1977). No statements of compliance with QA or GLP were provided. The study report stated that the facility in which the animals were maintained was fully accredited by the American Association for Accreditation of Laboratory Animal Care.

(b) Exposure by inhalation

In a short-term study of whole-body inhalation, groups of 10 male and 10 female Sprague-Dawley rats were given technical-grade terbufos (AC 92,100; purity, 90.1%) at a target concentration of 0, 0.005, 0.01, 0.05 or 0.10 mg/m^3 (not adjusted for purity) as a vapour for 8 h per day, for 5 days per week over a period of 3 weeks, for a total of 15 exposures, followed by a 2-week recovery period. A 2-week treatment period had originally been planned, but during week 1 of exposure, analytical chamber concentrations were only about 2–44% of target concentrations. During exposure weeks 2 and 3, when vaporization flasks were heated slightly, analytical concentrations were much closer to those targeted, however, the range of daily means varied widely suggesting chamber concentrations were not well maintained. Mean daily analytical chamber concentrations and the range of daily means for exposure weeks 2 and 3 corresponding to the control, 0.005, 0.01, 0.05 and 0.10 mg/m^3 groups were, respectively, for males 0, 0.0117 (range, 0.0003–0.0380), 0.0243 (range, 0.0066–0.05669), 0.0458 (range, 0.0098–0.0763) and 0.0946 (range, 0.0394–0.1523) mg/m^3, and for females 0, 0.0112 (range, 0.0003–0.0380), 0.0256 (range, 0.0066–0.0569), 0.0468 (range, 0.0098–0.0763), and 0.1001 (range, 0.0448–0.01523) mg/m^3. Estimated inhalation doses on a mg of active ingredient/kg bw per day basis for the control group and at thes lower intermediate, higher intermediate and highest dose over the last 2 weeks of exposure, assuming a retained dose of inhaled material of 50%, were: males, 0, 0.0030, 0.0065, 0.0122 and 0.0246 mg/kg bw per day; and females, 0, 0.0041, 0.0097, 0.0175 and 0.039 mg/kg bw per day, according to the study report. Possible contributions to exposure via the dermal and oral routes (i.e. from grooming) were not discussed. Owing to mortality, exposure of females at the highest dose was stopped early, on day 18. The study was terminated for half the animals in all groups at the end of the exposure period on day 21 and for the other half at the end of the recovery period on day 37. Parameters evaluated for both the treatment and recovery phases of the study included observations for mortality, clinical signs of toxicity, measurement of body weight and food consumption, haematological and clinical chemistry determinations (in five animals of each sex per group), urine analysis, selected organ weight measurements (adrenals, heart, kidneys, liver, and lungs) and an examination for gross pathology. Plasma and erythrocyte cholinesterase activities were measured in five animals of each sex per group before testing, on days 1, 5, 8, 13 for all groups, on day 18 for surviving females at the highest dose and five control animals, and on days 21 and 37 for all groups. Brain cholinesterase activity was measured in five animals of each sex per group on days 21 and day 37. Cholinesterase inhibition was calculated relative to the appropriate concurrent control group.

All animals survived except for one male at 0.0117 mg/m^3 that died accidently on day 8 and two females at the highest dose that died on days 17 and 19 from treatment-related causes. The only clinical findings ascribed to treatment in the study were observed during the treatment period in the group of females at the highest dose and included body tremors, body coldness, and rapid or laboured breathing. Body weights were statistically significantly depressed in females at the highest dose, as was food consumption, particularly towards the end of the period of exposure. Haematological and clinical chemistry findings, which were generally statistically significantly different from the respective control group and which may have been related to treatment, were noted primarily during week 3. These included decreased haemoglobin, erythrocyte volume fraction and erythrocyte counts and an elevated clotting time in females at the highest dose, and decreased blood glucose concentrations in males at the highest dose. The results of urine analysis were not remarkable, although there were some low level increases in ketones, bilirubin and occult blood in males at the highest

dose at the end of the recovery period. The only remarkable organ weight change was a slight increase in the adrenal : body weight ratio of males at the highest dose (0.03580) relative to the control group (0.0272) at the end of week 3. No gross necropsy findings were considered to be related to treatment.

There were some inconsistencies observed in the data on cholinesterase activity, which sometimes made interpretation difficult, but it appeared that by the end of the exposure period (day 18 for females at the highest dose and day 21 for all the other groups), the following changes in blood cholinesterase activity were related to treatment: statistically significant inhibition of erythrocyte cholinesterase activity of 28% and 33% in females at the intermediate and highest doses; reduced erythrocyte cholinesterase activity of 22% in males at the highest dose with statistically significant reductions of 33% and 24% seen at the two previous time-points, respectively (days 13 and 8); statistically significant decreases in plasma cholinesterase activity of 30% and 61% in females at the intermediate and highest doses, respectively, and of 12% and 21%, respectively, in males at the intermediate and highest doses. Blood cholinesterase activity values were not statistically significant at the end of the recovery period. On day 21, brain cholinesterase activity was statistically significantly inhibited in females at the highest dose (based on data from three animals) by about 45% and in males by about 15% decreases that were considered to be associated with treatment (although the decrease in males was not considered to be toxicologically relevant). At the end of the recovery phase, brain enzyme activity values were not significantly different from those of controls, but statistically significant inhibition of 29% was observed in males at the highest dose, possibly indicating a lack of recovery. However, this interpretation of the data was questionable because statistically significant inhibition of 35% was also noted in brain cholinesterase activity at 0.0243 mg/m^3, but not at the next highest dose of 0.048 mg/m^3.

No other remarkable findings in other parameters, except as already noted, were reported for the recovery period. If plasma cholinesterase inhibition is not considered to be an adverse effect of treatment and toxicologically significant brain (not erythrocyte) cholinesterase inhibition and clinical signs of toxicity are considered to be relevant effects for terbufos, the NOAEL was 0.0458 (range, 0.0098–0.0763) mg/m^3 (reflecting mean daily analytical chamber concentrations over the 3-week period of exposure and the range of daily means) in males and females on the basis of a statistically significant decrease and increase, respectively in blood glucose concentration and the adrenal to body weight ratio at the next highest dose in males, and mortality, clinical signs of toxicity, decreases in body weight and food consumption, and haematological changes in females at the next highest dose (Whitney, 1980). A statement of compliance with QA, but not for GLP, was provided. The protocol was not done to satisfy a particular guideline but was generally satisfactory for the intended purpose of the study.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a combined long-term study of toxicity and carcinogenicity, groups of 65 male and 65 female CD-1 mice were given technical-grade terbufos (AC 92,100; purity, 89.6%; prepared in a 1 : 1 solution of corn oil and methylene chloride) at a concentration (adjusted for purity) of 0 (vehicle), 3, 6, or 12 mg/kg equivalent to 0 (vehicle only), 0.45, 0.9 and 1.8 mg/kg bw per day for males and females for 18 months. Ten animals of each sex per group were scheduled for interim termination at 12 months (week 53) and the remaining

55 animals of each sex per group for termination at 18 months (week 80). The rationale for dose selection was not provided. Parameters assessed included observations for general health and mortality, clinical signs of toxicity, measurement of food consumption and body-weight changes, haematological determinations, organ weight measurements and macro- and microscopic pathology examinations. Cholinesterase activities were not assessed in this study.

Mortality was greater at the highest dose in both sexes relative to at the other doses at both interim and final termination. At week 53, mortality in the control group, and at the lowest, intermediate and highest dose was 3.6%, 0%, 1.8% and 12.7% for males, and 7.2%, 1.8%, 7.3% and 12.7% for females, respectively. At week 80, mortality in these same groups was 12.7%, 9%, 5.5% and 27.2% for males and 27.2%, 14.5%, 21.8% and 34.5% for females, respectively. A relationship to treatment for the decreased survival at the highest dose could not be dismissed (although the study report did not come to this conclusion). There were no clinical findings in the study that were obviously related to treatment. Body weight was statistically significantly decreased in both sexes relative to respective control groups at most weekly measurement time-points throughout the study. At week 54 and study termination, statistically significant reductions of about 8% were noted in males and of 10% and 11%, respectively, in females. At the intermediate dose, during the first 4–5 weeks of treatment, but not thereafter, statistically significant decreases in body weight of up to about 5% in males and up to about 8% in females were observed. These changes may have been related to treatment as they were not clearly associated with decreases in food consumption that might have indicated a palatability problem. Group mean body-weight gains over the entire study were reduced at the highest dose by 10% in males and 20% in females relative to respective control groups (statistical significance was not assessed) while group mean body-weight gains at the intermediate and lowest dose in both sexes exceeded those observed in animals in the control group. Statistically significant decreases in food consumption associated with treatment occurred at various times throughout the study at the highest dose in both sexes, with females somewhat more affected than males. Food consumption was not clearly affected by treatment at in the intermediate and lowest doses. There were no remarkable findings in the haematological, organ weight, or gross pathology assessments for any group treated with terbufos. With regard to non-neoplasic findings noted microscopically, slight increases in total occurrences of fatty metamorphosis in the liver were observed in test material-treated groups of males and females. For males in the control group and at the lowest, intermediate and highest doses, respectively, the incidence was 13%, 13%, 25% and 28% and for females in these same groups, the incidence was 30%, 38%, 34% and 48%, respectively. In the absence of other associated findings (e.g. organ weights or other histopathological lesions), the increased instances were considered to be of a spontaneous nature and not related to treatment (there were no historical control data available for comparison). Other microscopic findings in the study had no obvious relationship with treatment. The NOAEL for systemic toxicity was 3 mg/kg, equivalent to 0.45 mg/kg bw per day in males and females, on the basis of statistically significant decreases in body weights in both sexes during the first 4–5 weeks of the study that were not clearly associated with decreased food consumption at the next highest dose. There was no evidence of carcinogenicity (Silverman et al., 1986). Statements of compliance with QA and GLP were provided. The protocol was consistent with US EPA Subdivision F Guidelines (November 1982 and revised, 1984).

Rats

In a combined long-term study of toxicity and carcinogenicity, groups of male and female Long Evans rats were given diets containing terbufos (AC 92,100; purity not specified) at a nominal concentration of 0.25, 1.00 or initially 2.00 mg/kg (nominally to 0.0125, 0.05, and 0.1 mg/kg bw per day) for 2 years. For both sexes, the highest dose was raised to 4 mg/kg (nominally 0.2 mg/kg bw per day) at the beginning of week 6 (day 35), raised again to 8 mg/kg (nominally 0.4 mg/kg bw per day) at the beginning of week 12 (day 77), and lowered again for females only to 4 mg/kg (nominally 0.2 mg/kg bw per day) during week 16 (around day 105). Two concurrent control groups of males and females were included in the study. Diets containing terbufos were prepared by adding a premix nominally containing 100 mg/kg of feed (0.01%) of active ingredient to lab chow to achieve the desired nominal concentrations. Diets were apparently not analysed for test material content, homogeneity or stability (no supporting data or information were provided in the study report), and intake of test material was estimated based on nominal dose and food consumption data. Animals in the control group were given lab chow only. The study was initiated with 60 animals of each sex per group. A subset of this group (five animals of each sex in the control group and 10 animals of each sex in each group treated with terbufos) was terminated at 3 months. The remaining 55 animals of each sex per group in the control group and 50 animals of each sex per group in each group treated with terbufos continued into the long-term portion of the study, which terminated at 24 months. During both parts of the study, all animals were monitored for mortality, clinical signs of toxicity, body-weight changes and food consumption. Also, during the 3- and 24-month portions of the study, urine analysis, and haematological and limited clinical chemistry examinations (serum glutamic-pyruvic transaminase, alkaline phosphatase, fasting glucose and blood urea nitrogen concentrations) were performed on three control animals of each sex per group and six animals of each sex in groups treated with terbufos. At study termination, a gross examination was performed on five control animals of each sex per group and on 10 animals of each sex in groups treated with terbufos at 3 months, on all survivors at 24 months and on all animals terminated in a moribund condition or found dead.

At 3 and 24 months, selected organ weights were measured (heart, kidney, liver and thyroid) in five control animals of each sex per group and on 10 animals of each sex in each group treated with terbufos and a histopathological examination was conducted on five control animals of each sex per group and on 10 animals of each sex treated with terbufos at the highest dose. Lung, liver, kidneys and heart were evaluated microscopically in a similar number of animals at the lowest and intermediate doses at 3 and 24 months. Tissues from other animals in the 24-month study were stored. Subsequent to the release of the original study report, tissues from all rats that were not previously processed were examined microscopically and the results of the 24 month exposure study were re-evaluated on this basis. Ophthalmoscopic examinations were performed only at 24-months. In three animals of each sex per control group and six animals of each sex in each group treated with terbufos, plasma and erythrocyte cholinesterase activities were determined for both sexes at 3, 6, 12 and 18 months, and brain cholinesterase activity was determined at 3 and 24 months. Inhibition of cholinesterase activity was assessed relative to values for the appropriate concurrent control groups.

There did not appear to be any deaths in the first 3 months of the study. At the intermediate dose, one animal, which was mis-sexed as a male, was placed with other females of that group, resulting in 59 males and 61 females. When the animals terminated at 3

months were not included in the calculations, mortality compared with that in the combined concurrent control groups was statistical significantly increased at the highest dose over the first 12 months of the study in males (28% in treated compared with 6.4% in the combined controls) and in females (28% compared with 0% in combined controls) and was statistically significantly increased over the 24 months at the intermediate and highest doses in males (57.1% and 62%, respectively, compared with 38.2% in combined controls) and in females at the highest dose (60% compared with 32.7%, respectively, in combined controls). Although a relationship of mortality rate and treatment could not be dismissed, the pathology report indicated illness existed among the test animals such as endemic bronchopneumonia (associated with bacterial infection) and pulmonary disease (said to be associated with the inhalation of food particles) and suggested that these conditions could have compromised animal well-being to a certain extent in some animals and/or contributed to the demise of others.

Neither individual nor summary data were provided in the study report for clinical findings or clinical signs of toxicity. According to the discussion in the study report, signs consistent with inhibition of cholinesterase activity (muscle tremors, excessive salivation, hyperactivity, hyperpnoea and tachycardia) were first noted in females at the highest dose during administration of the diet containing terbufos at 8 mg/kg (weeks 12–15). After the dose was lowered, the signs reportedly decreased in incidence in females at the highest dose and were not observed from months 18 until the end of the study. Some females at the intermediate dose reportedly exhibited some of the clinical signs (not specified) during months 5 and 6. Muscle tremors were reported in eight males at the highest dose during study months 22–24. Starting at around the time when females at the highest dose were placed on the die containing terbufos at 8 mg/kg, exophthalmos was noted in this group. Eventually, the condition manifested itself in all other groups of females including the controls and was said to persist until about month 15. The etiology of this condition was not clear. Ophthalmoscopic examination at the end of the study revealed an increase in corneal scarring and cataracts in males and females, but particularly in females, at the highest dose. Statistically significant decreases in body weight and food consumption compared with respective control values were observed in males and females at the highest dose throughout much or most of the 2-year study. At 24 months, there was no obvious effect of treatment on haematological, clinical chemistry or urine parameters examined. The significance, at 3 months, of small magnitude perturbations in concentrations of glucose and blood urea nitrogen in females at the highest dose was difficult to judge due to the changing doses. Small magnitude perturbations at the highest dose in relative or absolute weights at 3 months (kidney, heart, liver) and at 24 months (liver, kidney, and heart) may have been related to decreases in terminal body weights noted in this group.

Despite some variability in the magnitude and consistency of response among the various time-points measured, patterns of cholinesterase inhibition were noted for which an association with treatment could not be dismissed. Erythrocyte cholinesterase activity was statistically significantly inhibited at months 3, 6, 12, 18 and 24 at the highest dose in males (42–70%) and in females (35–80%). At the intermediate dose, statistically significant inhibition of erythrocyte cholinesterase activity was observed in males at months 6, 18 and 24 (32–40%) and in females at months 3 and 24 (43–46%). Brain cholinesterase activity was inhibited in males only at the highest dose at both 3 and 24 months (62–63%) and in females at the highest dose at 3 months (25%) and 24 months (58%) and marginally at the intermediate dose, 10% at 3 months (not statistically significant) and statistically significantly by 12% at 24 months. Inhibition of brain cholinesterase activity in females at the inter-

mediate dose was not considered to be toxicologically relevant. Plasma cholinesterase was statistically significantly inhibited in females at the highest dose at months 3, 6, 18 and 24 by 54 to 70% and marginally in males (statistically significant inhibition of about 40% only at months 12 and 18). Plasma cholinesterase data showed a remarkable degree of variability with time and dose.

Because an insufficient number of animals had originally been evaluated for pathology, a re-evaluation of the 24-month exposure period was conducted. Apparently, most of the tissues and masses from animals not previously processed for microscopic examination were available for examination. The total number of rats whose tissues were re-evaluated histopathologically out of the original numbers started on test (60) were for (males/females): control group I, 54/55; control group II, 53/55; at the lowest dose, 50/50; at the intermediate dose, 44/50; and at the highest dose, 47/49. Animals terminated after 3 months were not included.

A new gross examination could not be re-conducted, but the previous report was said to have been used, as far as was possible, to make correlations with regard to gross and microscopic examinations.

With regard to the microscopic examination, the re-evaluation report for the 24-month treatment period discussed a number of non-neoplastic findings in both males and females. Inflammatory lesions in the lung were associated with two conditions. One was the endemic bronchial pneumonia (thought to be related to bacterial infection) found in increased incidence in the group at the highest dose. There was also a higher incidence at the highest dose of a second type of pneumonia (granulomatous) attributed to the inhalation of food particles containing plant fibres which were said to act as foreign bodies but for which treatment with test material may have been a contributing or pre-disposing factor. It was suggested that an increased incidence of oesophageal distension in animals at the highest dose may have been related to an effect of treatment on muscle contractility, but the pathology report suggested possible relationship of this finding to the bacterial infection and sequelae of the bronchopneumonia. A higher incidence at the highest dose of gastric mucosal ulceration and/or erosion was also of uncertain etiology, although a relationship to treatment could not be dismissed.

The report concluded that there was no evidence that the test material had an effect on tumorigenesis.

The study was inadequate to assess chronic toxicity owing mainly to outstanding questions involving the etiology and/or relationship to treatment and/or dose of certain non-neoplastic findings (including ocular, lung and stomach lesions), and also uncertainty associated with the variability in some of the cholinesterase measurements for which a relationship to treatment could not be dismissed, and lack of sufficient documentation of clinical signs of toxicity. Therefore, a NOAEL for chronic (systemic) toxicity was not identified.

Despite some variability and inconsistencies, there were apparent response patterns noted in the data on cholinesterase activity for which an association with treatment could not be dismissed. If inhibition of plasma cholinesterase activity is not considered to be an adverse effect and toxicologically significant inhibition of brain (but not erythrocyte) cholinesterase activity is considered to be a relevant effect for terbufos, the NOAEL for cholinesterase inhibition was 1.00 mg/kg in males and females (nominally 0.05 mg/kg bw

per day) on the basis of statistically significantly decreased cholinesterase at the next highest dose.

With regard to the carcinogenicity phase of the study, information presented in the pathology re-evaluation report for the 24-month period of exposure was considered to be adequate to support the conclusion that there was no evidence of a carcinogenic response in the study, provided that the nominal intake of test material intake could be justified (Rapp, 1974). No statements of compliance with QA or GLP were provided.

2.4 Genotoxicity

The results of assays for genotoxicity with terbufos are summarized in Table 3.

Most of the tests for mutagenicity with terbufos in vitro and in vivo gave negative results. However, in one acceptably performed study of dominant lethal mutation in vivo, results were inconclusive. In a paper from the open scientific literature (Gentile et al., 1982), positive results were reported in an acceptably performed assay for mitotic gene conversion in yeast cells (*ade* locus) with technical-grade terbufos in the presence or absence of a metabolic activation system, and also with a commercial grade of terbufos, without metabolic activation. However, insufficient purity and analytical data were provided in the paper for the materials tested.

Although the results of an assay for unscheduled DNA repair synthesis in cells in primary culture were negative, only male Fischer 344 rat hepatocytes were used; an optimal protocol would also have included assessment of hepatocytes from female rats.

2.5 Reproductive toxicity

(a) Multigeneration study

Rats

In a two-generation study of reproductive toxicity, four groups of 25 male and 25 female Sprague-Dawley (COBS CD) rats, F_0 generation or F_1 generation parental animals, were given diets containing technical-grade terbufos (AC 92,100; purity, 89.6%; dissolved in a 1 : 1 solution of methylene chloride and corn oil) from the pre-mating period until termination of the adults. Terbufos was administered at a dietary concentration of 0 (vehicle only), 0.5, 1.0, or 2.5 mg/kg (equal to group mean intakes for the F_0 generation during the pre-mating period of 0, 0.035, 0.07, and 0.18 mg/kg bw per day in males, and 0, 0.04, 0.085, and 0.22 mg/kg bw per day in females, and for the F_1 generation during the pre-mating period of 0, 0.0372, 0.0742, and 0.1943 mg/kg bw per day in males, and 0, 0.04, 0.089, and 0.24 mg/kg bw per day in females. An additional group of F_0 generation animals was given terbufos at a dietary concentration of 5.0 mg/kg (equal to group mean intakes of 0.42 mg/kg bw per day in males and females); this group was, however, terminated early in week 6 of the pre-mating period because of toxicity in the females.

According to a standard protocol, the F_0 and F_1 generations were mated twice to produce F_{1a} and F_{1b}, and F_{2a} and F_{2b} litters, respectively. Dosing commenced in the F_0 generation at 63 days before mating when parental animals were aged about 7 weeks and continued for about 191 days in males and 210 days in females (during the lactation period for the F_{1b} litters). Initiation of dosing for F_1 generation parental animals (from F_{1b} litters) commenced after weaning of the F_{1b} litters and continued for about 205 days in males and

Table 3. Results of studies of genotoxicity with terbufos

End-point	Test object	Concentration/dose	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *E. coli* WP2 *uvrA*$^-$	50–5000 µg/plate, 1000 µg/disc in DMSO, ±S9[h]	89.6	Negative	Allen (1985)[a,b]
Point mutation	Chinese hamster ovary cells (CHO-K_1-BH_4), *Hprt* locus	10–100 µg/ml & in DMSO, ±S9[c,i]	87.8	Negative[d]	Allen & Johnson, (1983)[a,b]
Chromosomal aberration	Chinese hamster ovary cells	2.5–100 nl/ml in DMSO, ±S9[e,i]	87.8[f] (1983)[a,b]	Negative	Thilager (1983)
Unscheduled DNA synthesis	Primary rat (male, Fischer 344) hepatocytes	0.33–33.33 µg/well in DMSO[g,i]	87.8[f]	Negative	Godek (1983)[b,n]
Mitotic gene conversion	*S. cerevisiae* strain D4 (*ade* and *trp* loci)	Apparently 0.33–33 µg/tube in DMSO, ±S9	Technical and commercial grades (purities not specified; no analytical information)	Technical-grade: positive at the *ade* locus (±S9); Commercial-grade: weakly positive at the *ade* locus (−S9)[o]	Gentile et al. (1982)
In vivo					
Dominant lethal mutation (10 mating cycles)	Crl : CD(SD)BR rats (10 male rats per group)	0, 0.1, 0.2, or 0.4 mg/kg bw per day[h,j,k] in corn oil by gavage daily for 5 days at the start of the first mating cycle[l]	89.6	Inconclusive[l]	MacKenzie (1986)[a,b]
Chromosomal aberration	Sprague-Dawley rats (20 males, 20 females per group), bone-marrow cells	Single intraperitoneal doses of 0, 0.2,0.6, 1.5 (females only)[m] or 1.8 mg/kg bw in corn oil[i]	89.6	Negative	Putnam (1986)[a,b]

DMSO, dimethyl sulfoxide

[a] Consistent with US EPA Subdivision F guidelines (1984,revised)

[b] Statements of compliance with GLP and QA were provided

[c] Cytotoxicity observed at 75 and 100 µg/ml in main study and at higher concentrations in the dose-finding study

[d] Slightly higher mutation frequencies (+S9) observed relative to the concurrent solvent control at 25 and 50 µg/ml were generally in the range of values for historical solvent controls and were attributed to unusually low mutation frequencies in concurrent controls . A repeat assay (+S9) gave negative results at concentrations of up to and including 50 µg/ml

[e] Cytotoxicity was observed at 100 nl/ml in main study and at higher concentrations in dose-finding study

[f] Purity information obtained from the sponsor

[g] Cytotoxicity observed at higher concentrations (100 µg/well and above)

[h] Dose calculations corrected for % active ingredient

[i] No correction of dose for % active ingredient

[j] No clinical signs of toxicity or effects on body weight were reported at the highest dose, but this dose was approximately one-tenth of the reported LD_{50}

[k] After treatment, each male was paired with two non-treated virgin females, 5 days per week for 10 weeks. Females were evaluated for number of implants, viable and nonviable fetuses and number of corpora lutea about 15 days after the mating period mid-point. Fertility index and implantation efficiency were calculated. The positive control used, triethylenemelamine, was administered intraperitoneally to 10 males for 5 days at a dose of 0.05 mg/5 ml saline/kg bw before the first mating cycle

[l] At mating 9, the number of viable implants at 0.4 mg/kg bw per day was reduced slightly compared with the concurrent vehicle control group (statistically significant) and with respect to the positive control group (e.g. 12 compared with 14 and 13, respectively). At mating 7, implant efficiencies at the lowest, intermediate and highest doses (95%, 93%, and 89%, respectively) were lower (with a difference that was statistically significant at the lowest and highest doses) than that of the concurrent vehicle control group (98%) and the positive control group (97%). A slightly lower implantation efficiency (not statistically significant) relative to all other groups continued at the highest dose through the remaining three mating cycles numbers 8–10; the study report concluded that the NOAEL in males treated for 5 days was 0.2 mg/kg bw per day

[m] Evaluations of structural chromosomal aberration were conducted in five animals per group (including replacement animals) at 12, 24, and 36 h after dosing. Clinical signs of toxicity and mortality were observed after an intraperitoneal dose of 1.8 mg/kg bw in males and females and 1.5 mg/kg bw in females. Excessive mortality was observed in females at both of these doses, indicating an increased sensitivity of females to the test material. Protocol was consistent with US EPA Subdivision F guidelines (1984, revised), but only hepatocytes from males were used; hepatocytes from females should also have been assayed

[o] Results given only for the dose said to give highest recombinogenic activity. Assay protocol appeared to be generally acceptable, but no rationale for dose selection was presented. Commercial-grade terbufos gave negative results in *Zea mays* in a field plot assay for plant mutation in situ (technical grade not tested)

220 days in females. F_1 and F_2 litters were reduced to eight pups (equal numbers of each sex, if possible) on day 4 of lactation. F_{1a}, F_{2a} and F_{2b} litters were sacrificed at or after weaning on day 21 of lactation. Pups were examined externally for abnormalities and gross malformations. One F_{1b} and F_{2b} pup of each sex per litter, as well as any dead or stillborn pups, were necropsied and any abnormal tissues found were preserved.

Assessments performed for parental animals included observations for general condition, mortality and clinical signs of toxicity, measurement of body weight and food consumption, ophthalmoscopic examinations, evaluation of reproductive performance, including mating and fertility and pregnancy rate, duration of gestation and data on parturition, litter size and survival until day 21 of lactation, and macroscopic examination. Organ weight measurements were not made. At termination of each generation, histopathological evaluation was made of the following tissues: testes, epididymides, prostate, seminal vesicles, ovaries, uterus, vagina, pituitary and any gross lesions; this was performed only for animals receiving the highest dietary concentration (2.5 mg/kg) and for control F_0 and F_{1b} adults that had been selected for mating. Offspring parameters examined included assessment of general appearance, viability, litter sex ratio, and body weight (on days 1, 4, 7, 14, and 21 of lactation). Inhibition of plasma, erythrocyte and brain cholinesterase activities was calculated relative to the values for the appropriate concurrent control group for adult F_0 males and females (week 27) and F_{1b} adults, and for males and females at termination. Cholinesterase activity was not assessed in adult animals receiving terbufos at a dietary concentration of 5.0 mg/kg, nor in pups at any dose.

Six out of 25 females at the highest dietary concentration tested (5.0 mg/kg) died from treatment-related causes after 22–34 days. Clinical signs of toxicity, including tremors, a generally poor condition, emaciation, and unkempt appearance, were also noted in females in this group. There were no deaths among males at 5 mg/kg and few, if any, clinical signs were observed in males in this group during the same period (two males had excess salivation at pre-mating week 4). During certain periods before termination of all animals at 5.0 mg/kg at pre-mating week 6, statistically significant decreases in body weight were reported in both sexes, as were decreases in food consumption.

In the study of four groups, with 2.5 mg/kg as the highest dose, parental mortality was very low and of comparable incidence in groups treated with terbufos and respective control groups in the F_0 and F_1 generation. With the possible exception of soft stools in F_0 females at the highest dose during week 28 (12 occurrences compared with 2, 3 and 6 occurrences in the control group, and at the lowest and intermediate doses, respectively), there were no clinical findings that could definitively be associated with treatment with terbufos in either the F_0 or F_1 parents. Animals with symptoms considered in the study report to be consistent with sialodacryoadenitis viral infection (including dry eyes in females at the lowest and intermediate doses) were noticed during weeks 26 to 28 (towards the end of the F_0 generation). The study report indicated that the outcome of the study was considered not to have been affected by the presence of the virus at this stage.

There was no apparent effect of treatment on male or female body weight of parents in either the F_0 or F_1 generations, except in lactating females at 2.5 mg/kg. Over the duration of the lactation period (days 0–20) for F_{1a}, F_{1b}, and F_{2b} litters, females at the highest dose exhibited statistically significant body-weight loss relative to that in the respective control groups. Food consumption did not appear to be adversely affected in parental

animals of either generation. The results of ophthalmoscopic examinations were not remarkable.

In F_0 adults, statistically significant inhibition of plasma cholinesterase activity was observed in males at the highest dose (46%) and females at the intermediate and highest doses (61% and 94%, respectively). Statistically significant reductions were noted in erythrocyte cholinesterase activity in males at the highest dose (11%) and in females at the intermediate and highest doses (7% and 15%, respectively). Brain cholinesterase activity was statistically significantly inhibited in males at 1.0 and 2.5 mg/kg (8% and 29%, respectively) and in females at 0.5, 1.0, and 2.5 mg/kg (7%, 22%, and 66%, respectively).

In F_1 adults, statistically significant inhibition of plasma cholinesterase activity was observed in males (by 20% and 53%, respectively), and in females (by 50% and 87%, respectively) at the intermediate and highest doses. Erythrocyte cholinesterase activity was statistically significantly reduced only at the highest dose in males (by 15%) and in females (by 13%). Brain cholinesterase activity was statistically significantly inhibited at the intermediate and highest doses in males (by 8% and 34%, respectively) and in females (by 21% and 59%, respectively).

Decreases in brain cholinesterase activity of 7–8% in F_0 and F_1 adults at the lowest and intermediate doses, although possibly treatment-related, were not considered to be toxicologically significant.

At 2.5 mg/kg, a treatment-related reduction in male fertility and the number of pregnant females was observed for the production of F_{1b} litters and F_{2b} litters compared with values for these parameters in the respective control groups. In the control group, and at the lowest, intermediate and highest dose, pregnancy rates were 92%, 88%, 96% and 80% in the first generation (F_{1b}) and 86%, 92%, 82% and 63% in the second generation (F_{2b}), respectively. The corresponding male fertility index (number impregnating per number mated) for F_{1b} litters was 91%, 87%, 96% and 79% and for F_{2b} litters, 94%, 95%, 94% and 61%, respectively.

At the highest dose, there were some, generally slight, changes in several F_{1b} and F_{2a} and F_{2b} neonatal parameters (such as in mean numbers of pups, live pups or dead pups per litter and total numbers of live or dead pups per litter). When these parameters were looked at overall, they were suggestive of possible subtle treatment related decreases in pup viability, number and/or survival at 2.5 mg/kg. However, they lacked statistical significance or persistence; the only statistically significant change, a decrease in the number of live offspring in F_{1a} litters relative to that in the control group (9.8 compared with 12.8) before reduction of litter sizes on lactation day 4, was not found at later time-points).

A more concrete case for an effect of treatment with terbufos on offspring concerned pup body weights. On days 14 and 21 of lactation, decreases (about 15–17%) in the mean weight of viable pups relative to those of pups in the the respective control group, were observed in F_{1a}, F_{1b}, F_{2a} (smaller decreases of 7–9%) and F_{2b} litters and were considered to be related to treatment. Decreases were statistically significant in F_{1a} litters, F_{1b} litters (a decrease of 11% was also noted on day 7), and F_{2b} litters (a decrease of 16 % was also noted on day 7).

Other parental reproductive or neonatal parameters examined were not obviously affected by treatment with terbufos. There were no obvious treatment-related findings upon gross examination of pups or adults, or upon histopathological evaluation of the adult tissues and organs selected for analysis.

The NOAEL for reproductive effects was 1.0 mg/kg for males and females (equal to 0.07–0.074 mg/kg bw per day in males and 0.0854–0.089 mg/kg bw per day in females) on the basis of decreases in male fertility and pregnancy rate, respectively. The NOAEL for parental toxicity was 2.5 mg/kg in males (equal to 0.18–0.19 mg/kg bw per day) on the basis of statistically significant decreases in body weight and decreases in food consumption at the next highest dose and 1.0 mg/kg in females (equal to 0.085–0.089 mg/kg bw per day) on the basis of statistically significant body-weight loss during lactation and an apparent increase in soft stools at the next highest dose. The NOAEL for offspring toxicity was 1.0 mg/kg (equal to 0.07–0.074 mg/kg bw per day in males and 0.085–0.089 mg/kg bw per day in females) on the basis of statistically significant decreases in the mean body weight of viable pups on days 14 and 21 of lactation in F_0 and F_1 litters.

If plasma cholinesterase inhibition is not considered to be an adverse effect and toxicologically significant brain (not erythrocyte) cholinesterase inhibition is considered to be a relevant effect for terbufos, the NOAEL for cholinesterase inhibition was 1.0 mg/kg (equal to 0.07–0.074 mg/kg bw per day) in parental males and 0.5 mg/kg (equal to 0.04–0.044 mg/kg bw per day) in parental females on the basis of statistically significant decreases in brain cholinesterase activity in the F_0 and F_1 generations at the next highest doses (Schroeder, 1989). Statements of compliance with QA and GLP were provided. The protocol was consistent with US EPA Subdivision F guidelines (1982 and 1984, revised).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of Charles River albino rats were given technical-grade terbufos (AC 92,100; purity not specified) at a dose of 0 (vehicle only; 20 rats), 0.075 (20 rats) or 0.150 (21 rats) mg/kg bw per day daily on days 6–15 of gestation, inclusive, by gavage in corn oil. The study was terminated on day 20 of gestation. The basis for dose selection was not discussed. Adult animals were observed for mortality, unusual reactions, clinical signs of toxicity, and body-weight changes. Other parameters assessed were group mean numbers of corpora lutea, implantation sites, resorption sites, and viable fetuses, as well as number of fetuses, group mean fetal body weights, sex ratio and the incidence of females with one or more resorption sites. Fetuses were examined externally for effects of treatment and, where possible, approximately equal numbers of each sex were evaluated for skeletal and internal developmental abnormalities using the Hurley method of staining with alizarin, or the Wilson and Warkany technique, respectively. Cholinesterase activity was not measured.

There were no deaths or unusual reactions in adult animals. No treatment-related effects were noted in any parameter. This study had many deficiencies, including the following: the purity of the test material and the concentration of the test material in dosing solutions could not be confirmed; it was not clear if randomization techniques were used in assigning animals to treatment groups; no data for individual adult animals or fetuses were provided in the study report; the number of dead fetuses was not indicated; maternal or fetal data were generally reported only as group means; it was not clear how fetuses were

selected for examinations for developmental abnormality; fetal abnormalities were not assessed on a litter basis; the number of fetuses examined for abnormalities did not match the number of fetuses reported; complete necropsies of adult animals were not conducted. Also, animals were shipped to the laboratory on day 1 of gestation and were allowed no acclimation period. NOAELs for maternal and developmental toxicity could not be identified owing to the many deficiencies in study design and data reporting (Haley, 1972). No statements of compliance with GLP or QA were provided, the protocol was not consistent with EPA or OECD guidelines and was unacceptable by current standards; the study was conducted in 1972 (before the establishment of guidelines for GLP and EPA guidelines).

In a preliminary study of developmental toxicity, groups of five female Charles River COBS® CD® rats were given technical-grade terbufos (AC 92,100; purity, 87.8%) at a dose (adjusted for purity) of 0 (vehicle only), 0.4, 0.8, 1.4, 3, or 6 mg/kg bw per day once daily by gavage in corn oil during days 6–15 of gestation, inclusive. These doses were actually twice the amounts intended owing to an unintentional protocol error such that the volume administered was 10 ml/kg instead of 5 ml/kg. Owing to excessive toxicity and mortality in all groups treated with terbufos, the study was terminated prematurely (before day 20 of gestation) and two additional groups of five females were subsequently given the test material at a dose of 0.05 or 0.2 mg/kg bw per day administered in a volume of 5 ml/kg. This second phase was terminated as planned on day 20 of gestation. For all groups in both phases, planned evaluations in adult females included observations for general condition and clinical signs of toxicity, mortality and moribundity, body-weight changes, abortions, number of corpora lutea, and terminal necropsy. Litter and fetal assessments included number of viable and dead fetuses and fetal position in the uterus, number of implantation sites, early and late resorptions, early implant loss in uteri with no gross evidence of implantation, and postimplantation loss. No examinations were conducted of fetuses for external, visceral or skeletal abnormalities, body weight or sex ratio in either phase and cholinesterase activity was not measured.

During the first phase of the study, all animals died or were sacrificed in a moribund condition as a result of treatment at doses of ≥0.4 mg/kg bw per day. The cause of death was cardiorespiratory arrest or cerebral haemorrhage. Findings at necropsy included hepatic congestion, renal and pulmonic hyperaemia, gastric congestion and loss of epithelium, intestinal congestion and diarrhoea, and in some animals at doses of 1.4 mg/kg per day and above, haemorrhagic intestines. Deaths at 0.4 mg/kg bw per day occurred between days 10 and 16 of gestation. At higher doses, deaths or early terminations occurred on days 7–9 of gestation. Severe body-weight losses were observed in all groups treated with the test material. Clinical signs of toxicity, described as primarily yellow urogenital matting and dried red matter around the eyes and nares were noted primarily in animals given doses of 0.4 and 0.8 mg/kg per day, as survival was longest in these groups. Pregnancy rates in animals in the vehicle control group and groups treated with terbufos ranged from 80% to 100%. No abortions were reported. Uterine assessments were incomplete owing to mortality but, relative to groups receiving the highest dose, an increase in early litter resorptions was noted at necropsy at doses of 0.4 mg/kg bw per day and, to a lesser extent, at 0.8 mg/kg bw per day; this effect may have been related to maternal toxicity and day of gestation. At 0.4 mg/kg bw per day and 0.8 mg/kg bw per day, respectively, early resorptions were observed in five out of five gravid females (maternal deaths on days 10–15 of gestation in this group) and two out of four gravid females (maternal deaths on days 8 or 9 of gestation in this group), while none were reported in pregnant animals at doses of ≥1.4 mg/kg bw per day (maternal deaths on day 7 of gestation in these groups).

In the second phase of the study, the results provided indicated that doses could have, at least sometimes, been 10–15% below nominal levels. No mortality or clinical signs of toxicity were reported at 0.2 or 0.05 mg/kg bw per day. Groups treated with terbufos gained slightly less weight than did the vehicle control group during dosing (days 6–16 of gestation) and overall gestation (days 0–20 of gestation), but a statistical analysis of the data was not performed. Pregnancy rates were 100%, 80% and 100% in the vehicle control group and groups receiving the lowest and intermediate doses, respectively. A delivery early in the study on day 10 of gestation by one female at the lowest dose was attributed to an error in the detection of mating, such that the day of parturition was assumed to be 22 on the basis of pup size and development. The results of necropsy of this and other animals in the second phase were not remarkable. There were no other premature deliveries nor were there any abortions. Litter incidence (relative to that in the vehicle control group) of viable fetuses, implantation sites and corpora lutea were similar for the three dams available for evaluation at the lowest dose and the five available at the highest dose. There were no dead fetuses or late resorptions in any group. A slightly higher postimplantation rate of 1.7 per litter at 0.05 mg/kg bw per day, associated with early resorptions, was noted compared with a value of 1.0 per litter in the control group, but the value for the group receiving the lowest dose was based on an evaluation of three animals only and the rate at 0.2 mg/kg bw per day (0.6 per litter) was lower than that for the control group. On the basis of the parameters assessed in phases one and two, the NOAEL for maternal toxicity was 0.2 mg/kg bw per day, as doses administered above this were excessively toxic. A NOAEL for developmental toxicity could not be identified owing to the small number of animals evaluated and the limited study design and assessments made (Rodwell, 1984). Statements of compliance with QA and GLP were provided. Protocol deficiencies were noted, but the study was not performed to meet a specific guideline, being a preliminary study.

In a study of developmental toxicity, groups of 25 Charles River COBS CD rats were given technical-grade terbufos (AC 92,100; purity, 87.8%) at a dose of 0 (vehicle only), 0.05, 0.10, or 0.20 mg/kg bw per day by gavage in corn oil once daily on days 6–15 of gestation, inclusive. Doses were adjusted for test material purity. The study was terminated on day 20 of gestation. Selection of doses for this main study was based on the results of a preliminary study of developmental toxicity in the same strain of rats; treatment-related mortality and clinical signs of toxicity had been reported after administration of test material at daily doses of ≥0.4 mg/kg bw per day by gavage on days 6–15 of gestation, inclusive (Rodwell, 1984). In the main study, evaluations in adult females included observations for general condition and clinical signs of toxicity, mortality and moribundity, body-weight changes, abortions, number of corpora lutea, and terminal necropsy. Litter and fetal assessments included number of viable and dead fetuses, number of implantation sites, early and late resorptions, early implant loss in uteri with no gross evidence of implantation, postimplantation loss, and fetal weight, sex, sex ratio, and uterine location. All fetuses were examined for external abnormalities. Approximately one-half of the fetuses from each litter were examined for soft tissue findings using the Wilson sectioning method, while the remainder were examined for skeletal defects using a modification of the Dawson alizarin red S staining technique and low power magnification. Cholinesterase activity was not measured.

In adult animals, no mortalities or treatment-related clinical signs of toxicity were observed during the study. Slightly lower body-weight gain was noted in groups treated with terbufos at the intermediate and highest doses, relative to the control group, during dosing (days 12–16 of gestation and during days 6–16 of gestation), and after dosing (days 16–20

of gestation). During days 12–16 of gestation, body-weight gains at the intermediate and highest doses were 7% and 10% less than that of the control group, respectively, while during days 6–16 of gestation, body-weight gains in these groups were 7% less than that of the control group. After dosing, body-weight gains at the intermediate and highest doses were 5% less than that of the control group. In the study report, the relatively lower body-weight gains at the intermediate and highest doses were considered to be related to administration of the test material. These decreases in body weight were not considered to be toxicologically significant, being of similar magnitude at the intermediate and highest doses, relatively small, and not statistically significant. In addition, there was no indication of an increase in body-weight gain after cessation of dosing.

Pregnancy rates were similar among all groups, ranging from 96% to 100%, and there were no abortions or premature deliveries.

There were no statistically significant differences in the number of corpora lutea or implantation sites in groups treated with terbufos relative to values for these parameters in the concurrent control group. Late resorptions, although not statistically significantly increased, were observed only at 0.2 mg/kg bw per day. All late resorptions (eight) and the only dead fetus reported in the study were found in the litter of one dam at the highest dose, along with one early resorption and one fetus with no remarkable findings out of a total of 11 conceptuses. At necropsy of the dam (which survived the study), abnormalities described collectively in the study report as "severe pathology" but not treatment-related, were found on gross examination of the liver (described as pale and soft with accentuation of lobular markings), spleen (described as congested enlarged and indurated) and kidney (described as pale, bilateral). Follow-up histopathology was not performed. Weight gain (50 g) in this animal during dosing (days 6–16 of gestation) was not dramatically different from the mean for the control group (57 g) or the mean for the group receiving the highest dose (53 g); however, it was the only animal at the highest dose to lose weight (−1 g) after cessation of dosing (mean weight gain at the highest dose after cessation of dosing was 60 g). The relationship between litter/fetal findings and the pathology observed in this animal is not clear.

Early resorptions at the intermediate and highest doses of 1.2 and 1.0 per litter, respectively, were slightly elevated (not statistically significantly) relative to values for the concurrent control group (0.8 per litter) and the group receiving the lowest dose (0.5 per litter).

Fetal body weights were comparable in all groups. The number of viable fetuses at the intermediate and highest doses (13.7 and 13.6 per litter, respectively) were slightly lower, although not statistically significantly so, than those in the concurrent control group and the group receiving the lowest dose (15.0 and14.8 per litter, respectively), and were within the range of data for historical controls for the laboratory and rodent strain used (mean, 13.9 per litter; range, 11.9–15.4). These decreases in the number of viable fetuses were attributed to slight increases (not statistically significant) in postimplantation loss at the intermediate and highest doses (1.2 and 1.3 per litter, respectively) compared with those in the respective concurrent control group and group receiving the lowest dose (0.8 and 0.5 per litter, respectively). At the intermediate dose, the increased postimplantation loss (1.2 per litter) was just inside the range for historical controls (mean, 0.6 per litter; range, 0.1–1.2) and was associated with losses due to early resorption (no early resorption data for historical controls were provided). At the highest dose, postimplantation loss (1.3 per litter) was just outside the range for historical controls and was associated with both early and late resorptions and the one fetal death. If litter/fetal data from the dam with the reported macro-

scopic pathology at necropsy were not considered, the changes in litter or fetal parameters at the highest dose relative to those in the control group appeared to be eliminated.

There was a statistically significant difference in the group mean fetal sex ratio in all groups treated with terbufos relative to the value for concurrent controls. This was attributed in the study report to a skewed sex ratio in the concurrent control group (male : female, about 1 : 1.4) relative to that based on the historical control data provided (calculated as male : female 1 : 1.006), although group means and ranges for historical controls were not provided in the study report. External, visceral and skeletal fetal examinations did not reveal any morphological findings associated with treatment.

The study authors did not consider any of the findings on fetal viability, resorptions, or postimplantation loss at the intermediate and highest doses to be biologically meaningful, because none of the changes were statistically significant relative to the control group. Available data for historical controls generally supported this contention at the intermediate dose and, in the case of fetal viability, at the highest dose. In addition, there was no apparent effect on postimplantation loss, fetal viability or resorptions (early or late) at the highest dose, if litter/fetal data from the female with reported pathology at the highest dose are eliminated from consideration. Although no clear maternal or developmental toxicity was considered to have occurred in this main study, mortality was seen at a dose of 0.4 mg/kg bw per day in a preliminary study (Rodwell, 1984), at just twice the highest dose used in the main study (0.2 mg/kg bw per day), thus providing support for dose selection in the main study.

The NOAEL for maternal toxicity and developmental effects was 0.2 mg/kg bw per day, the highest dose tested (Rodwell, 1985). Statements of compliance with GLP and QA were provided and the protocol was consistent with US EPA Subdivision F (1982) guidelines.

Rabbits

In a study of developmental toxicity, groups of 17 New Zealand white (Hra:(NZW)SPF) rabbits were given technical-grade terbufos (AC 92,100; purity, 89.6%) at a dose (adjusted for purity) of 0 (vehicle only), 0.05, 0.10, 0.25 or 0.50 mg/kg bw per day by gavage in corn oil, once daily during days 7–19 of gestation. The study was terminated on day 29 of gestation. Data on dose selection were not provided. Upon analysis, the range of received concentrations of test material was 87–98% of target concentrations. Evaluations in adult females included observations for general condition and clinical signs of toxicity, mortality and moribundity, body-weight changes, food consumption, abortions, premature delivery, uterine weights, number of corpora lutea, and terminal necropsy. Litter and fetal assessments included number of viable and dead fetuses, number of implantation sites, early and late resorptions, postimplantation loss, and fetal weight, sex, sex ratio and uterine location. All fetuses carried to study termination were examined for external, visceral and skeletal abnormalities using standard techniques (including the use of alizarin red S staining). Cholinesterase activity was not measured.

There were no adult deaths and no premature deliveries. Two animals aborted, one in the group receiving the highest dose on day 21 of gestation (9 conceptuses) and the other in the group receiving a dose of 0.10 mg/kg bw per day (6 conceptuses) on day 22 of gestation. Litters were not available for analysis, presumably due to cannibalization. The

study report did not attribute this litter loss to treatment, as there were no signs of toxicity or apparent gross lesions at necropsy. There was some decrease in body weight and food consumption in both animals in the days immediately before abortion. A possible relationship of abortion at the highest dose to treatment could not be discounted, as other maternal treatment-related findings occurred at that dose.

The only clinical related to treatment was a statistically significant increase in the incidence of does with soft or liquid faeces at a dose of 0.5 mg/kg bw per day. This effect, which did not occur in the vehicle control group, was observed in four rabbits on 1 or 2 days and was associated with some decrease in body weight and food consumption, but no gross lesions at necropsy. Three rabbits (one not pregnant) had occurrences during the latter part of or just outside of the dosing period during days 16–20 of gestation. Another pregnant animal had one occurrence on day 29 of gestation. Instances of soft or liquid faeces associated with transient decreases in body weight and food consumption at other doses were not considered to be related to treatment, as there was no statistically significant increase and the incidence was low (e.g. found once, on day 18, in one animal at 0.05 mg/kg per day and twice, on days 24 and 25, in one animal at 0.10 mg/kg bw per day).

Maternal body-weight gain at the two higher doses (+ 0.16 and +0.16 kg, respectively) was decreased relative to that in the vehicle control group and at the two lower doses (+0.26, +0.23 and +0.23 kg, respectively) during the time interval encompassing the dosing period (days 7–20 of gestation). Between days 16 and 20 of gestation, there was some loss of body weight in animals at the highest dose, which was not seen in other groups. Although none of the body-weight findings at the two higher doses was statistically significant, in the study report they were considered to be related to treatment. Slight decreases in food consumption relative to that of controls were only noted at the two higher doses and then only during the period after cessation of dosing (days 20–29 of gestation); the study report suggested that this could have been related to a delayed effect of treatment. The present reviewer considered that his interpretation was possible but questionable. At necropsy, two animals at the highest dose (not those with soft or liquid faeces) were found to have reddened areas in the fundic area of the stomach that were considered to be treatment-related in the study report. The results of necropsy in other animals were not remarkable.

The incidence of pregnancy in the control group and at 0.05, 0.10, 0.25 and 0.50 mg/kg bw per day was 94%, 71%, 82%, 76% and 88%, respectively. There was no apparent effect of treatment with the test material on the number of corpora lutea, implantations, or live and dead fetuses (all fetuses were reported to be alive at study termination), or on litter size, sex ratio, or uterine weights (not including data from the two females that aborted). Although incidences of early or late resorptions were comparable between groups, at the highest dose there was an increase in the incidence of does with any resorption site. For the control group and at 0.05, 0.10, 0.25 and 0.50 mg/kg bw per day, the total number (and percentage) of does with resorptions of any type was 3 (18.8%), 5 (41.7%), 5 (38.5%), 4 (30.8%) and 10 (71.4%), respectively. The increase at the highest dose was not statistically significant and was not considered to be treatment-related in the study report. However, the incidence was higher than that in the concurrent control and was outside of the range for historical controls (data provided on a percentage basis, e.g. mean, 129 (39.2%) with a range of 0 (0%) to 10 (64.3%) based on data from 329 control groups from 21 studies conducted at the test facility between 1986 and 1988; current study conducted in 1988), such that a relationship to treatment could not be dismissed. In addition, a slight decrease in the group mean fetal body-weight at the highest dose (42.48 g) relative to that of the

concurrent control (44.8 g) was considered by the study authors to be an effect of treatment, although the difference was not statistically significant. Similar values were obtained when male and female fetal body weights at the highest dose were separately compared with those for the appropriate concurrent control group. No fetal external, visceral or skeletal findings were considered to be related to treatment with the test material.

With regard to maternal toxicity, although the study report suggested that there was a treatment-related effect at the intermediate dose, the evidence cited to support this contention (a relatively small body-weight decrease during the dosing period, which was not dose-dependent and was not statistically significant in a species in which body-weight variability is common; and possibly a slight decrease in food consumption after dosing) is of questionable toxicological significance in the absence of other findings. The NOAEL for maternal toxicity was 0.25 mg/kg bw per day on the basis of an increased number of does with soft or liquid faeces, maternal body-weight loss during days 16–20 of gestation, occurrence of reddened areas in the fundic region of the stomach at necropsy, and one abortion at the next highest dose. The NOAEL was 0.25 mg/kg bw per day on the basis of decreased fetal body weights and an increase in the incidence of does with any resorption sites at the next highest dose (Hoberman, 1988a, 1988b). Statements of compliance with GLP and QA were provided and the protocol was consistent with EPA Subdivision F Guidelines (1982 or 1984, revised).

2.6 Special studies

(a) Neurotoxicity

(i) Acute delayed neurotoxicity

Technical-grade terbufos (AC 92,100; purity, 96.7%) was tested for acute delayed neurotoxicity potential in sex-link hens (aged approximately 1 year). Confirmation of the content of test material in the dosing solution was not provided in the study report. In a preliminary study to aid in dose selection for the main study, the acute oral LD_{50} in this strain of hen for technical-grade terbufos in corn oil was estimated by probit analysis to be 40 mg/kg bw (95% confidence interval (CI), 31.8–48.2 mg/kg bw). The test material was administered by gavage as single doses at 10 (one animal), 20 (one animal), 28.3 (three animals), 40 (four animals), 56.6 (four animals) or 80 mg/kg bw (two animals). The incidence of mortality in these groups respectively, was 0/1, 0/1, 1/3, 1/4, 4/4 and 2/2. There was some variability in the time to death. Mortality was observed at 22 h after dosing at 28.3 mg/kg bw, at 4.5 h after dosing at 40 mg/kg bw, at 1.5–22 h after dosing at 56.6 mg/kg bw and within 2.5 h of dosing at 80 mg/kg bw. The only clinical findings reported were observed in some animals at 28.3 and 40 mg/kg bw and consisted of wobbly gait or unsteady gait and stance at 20–24 h after dosing, and resolved by 70–72 h after treatment.

The main study consisted of two phases. In phase one, 10 hens received the test material in corn oil as a single dose of 40 mg/kg bw by gavage. Included in this group of 10 were the three surviving animals that had received a dose of 40 mg/kg bw in the preliminary study for determination of acute LD_{50}. Other groups of hens received corn oil alone (four animals) or tri-ortho-cresyl phosphate (TOCP) at 500 mg/kg bw in corn oil as the positive control (10 animals). There was no pretreatment with atropine. Hens were observed for 21 days after dosing for mortality, signs of neurotoxicity and locomotor abnormalities.

All animals surviving study phase one were entered into study phase two. This inadvertently included any animals exhibiting effects consistent with delayed neurotoxicity (e.g. animals treated with TOCP) that were originally intended for sacrifice on day 22 after dosing in phase one. In phase two (which appeared to commence about 26 days after phase one dosing), the seven terbufos-treated hens surviving phase one received a second single dose of the test material by gavage in corn oil, while the surviving three or seven hens in the vehicle and positive control groups, respectively, were similarly dosed a second time with either corn oil only or 500 mg/kg bw of TOCP. Animals treated with TOCP were pretreated with atropine. After a 21-day observation period, animals treated with TOCP (seven) and vehicle control group animals (three) were terminated on day 22 after dosing. Surviving hens (six) treated with TOCP were terminated three days after dosing in phase two (i.e. 29 days after dosing in phase one). The original study report indicated that the spinal cords of only the three animals in the vehicle control group and the six animals treated with TOCP (animals considered to display signs of (delayed) neurotoxicity) were subjected to histopathological examination. Procedures used in preparation for the examination were only described briefly in the study report. It was stated that spinal cords were fixed in situ (perfusion was not indicated) in buffered formalin for 2 days before sectioning. Although brain, spinal cord (cervical, thoracic and lumbosacral sections), and sciatic nerve tissue were taken, only lumbosacral spinal cord sections (stained with haematoxylin and eosin) were examined microscopically. The tibial nerve was not examined. The hens were not subjected to a period of forced motor activity and there was no evaluation of cholinesterase or neuropathy target esterase activities in either study phase one or two.

During phase one of the main study, one out of four control animals died on day 19 and exhibited no signs or symptoms before death. Three out of 10 animals treated with terbufos and not pretreated with atropine died; two on day 0 and one on day 12 after dosing. No clinical symptoms were reported for the animals that died on day 0. The animal that died on day 12 exhibited two instances of wobbly gait and/or wobbly stance of low severity on days 1 and 7 after dosing. On day 1 after dosing, three additional animals treated with terbufos displayed single occurrences of low severity wobbly gait and/or wobbly stance. The deaths and clinical observations in the animals receiving terbufos were attributed to cholinergic toxicity. None of the animals treated with terbufos or the vehicle control animals dying during phase one were examined histopathologically. Based on the deaths in the terbufos animals in the preliminary study and in phase one of the main study, the acute oral LD_{50} in hens was re-estimated to be 43.5 mg/kg bw.

Animals treated with TOCP in phase one exhibited no signs of acute toxicity but did have symptoms of locomotor impairment (wobbly gait and/or wobbly stance) that started on day 11 after dosing in all animals and grew progressively worse, such that by day 21 after dosing most animals fell while walking, could only stand or walk in a squatted position or could not walk or stand at all. Three of the most severely affected hens died 26 days after dosing in phase one and were not examined histopathologically.

After dosing in phase two, signs of acute toxicity were observed in all seven animals treated with terbufos (and pretreated with atropine). The findings, which were attributed to cholinergic toxicity, lasted up to 72 h and were generally of low severity (wobbly gait and/or wobbly stance) except in the case of one animal that could not walk or stand one day after dosing only. There were no observational findings in these animals after day 3 after dosing. According to the study report, these animals were not examined histopathologically after

termination on day 22 after dosing in phase two because they showed no indications of delayed neuropathy.

Of the seven hens that inadvertently received a second dose of TOCP, three (which already had severe symptoms of locomotor impairment subsequent to phase one) died on day 2 or 3. The remaining hens in this group were terminated on day 3 after dosing in phase two and the spinal cord of six of the seven hens was subjected to histopathological examination. Minimal (two hens), slight (two hens), and moderate (two hens) degrees of demyelination of spinal cord fibre tracts (white matter) were found. One animal had swollen axis cylinders (white matter) and another had gliosis, both effects being of low severity. There were no observational or histopathological findings in the three control animals carried over into phase two.

Subsequently, the spinal cords of the seven animals treated with terbufos in phase two were examined histopathologically; it is not clear how the tissues of these animals were prepared for examination. Two animals had a perivascular lymphocytic cell infiltrate of low severity in the spinal cord, which was not considered to be of any concern. No demyelination or other adverse changes were apparent in the tissues examined. There was no evidence to suggest that terbufos caused delayed neuropathy in hens under the conditions of the study (Smith, 1972, 1973). No statements of compliance with QA or GLP were provided. The protocol was generally consistent with EPA Subdivision F Guidelines (1982 and 1984, revised).

(b) Acute neurotoxicity

In an study of acute oral neurotoxicity, groups of 20 male and 20 female Sprague-Dawley-derived (outbred) Crl:CD®(SD)IGS BR VAF/Plus® rats (fasted before dosing) were given technical-grade terbufos (AC 92,100; purity, 89.7%) as a single dose (not adjusted for purity) of 0 (vehicle only) 0.15, 0.30, or 0.90 mg/kg bw by gavage in corn oil. The study was terminated on day 15, following an observation period after dosing.

The selection of doses administered in the definitive study was based on the results of a pilot study to determine time of peak effect in males and females of the same strain of rat. In this study, groups of five males and five females were given single doses of the test material at (0 (vehicle only), 0.025, 0.05, 0.15, 0.40, 1.2 or 1.6 mg/kg bw in males and 0 (vehicle only), 0.025, 0.05, 0.15, 0.30, 0.90 and 1.2 mg/kg bw in females by gavage in corn oil. Five additional females were treated with terbufos at the highest dose, in case replacements were needed. All animals were monitored for clinical signs of toxicity, and at about 6 h after dosing (designated as the time of peak effect), plasma, erythrocyte and brain cholinesterase activities were measured in five animals of each sex per group. In the pilot study, one female died from causes attributed to treatment with the test material within 5 h of treatment with a dose of 1.2 mg/kg bw. Clinical signs of toxicity consistent with inhibition of cholinesterase activity were noted at the two higher doses in males and females about 6 h after dosing; females were said to have been more severely affected. At 1.6 mg/kg bw in males, excessive salivation, irregular gait and tremors were observed in one male and irregular gait was observed in two males at 1.2 mg/kg bw group. Seven females at 1.2 mg/kg bw group exhibited irregular gait and tremors. Other findings in this group were excessive salivation and lacrimation, moist rales and ventral surface and anogenital yellow staining in two animals, and decreased activity in one animal. At a dose of 0.9 mg/kg bw, the only clinical finding observed was ano-genital staining in one female. No clinical signs were

observed in other groups. Miosis was not reported for any animals in the study. Plasma cholinesterase activity was statistically significantly inhibited in males by 45%, 90% and 88% at doses of 0.40, 1.2, and 1.6 mg/kg bw per day, respectively, and in females by 17%, 87% and 96% at 0.3, 0.9, and 1.2 mg/kg bw, respectively. Erythrocyte cholinesterase activity was inhibited at the two higher doses in both sexes: in males, by 92–94% (statistically significant); and in females, by 88–93% (not statistically significant but considered to be treatment-related). Brain cholinesterease activity was statistically significantly inhibited by 57% and 52% at 1.2 and 1.6 mg/kg bw, respectively, in males. A statistically significant decrease in brain cholinesterase activity of 67% was observed in females at 1.2 mg/kg bw, and a decrease of 41% at 0.9 mg/kg bw was considered to be treatment-related, if not statistically significant. In the pilot study, no treatment-related effects were reported in either sex at doses of <0.40 mg/kg bw in males and <0.30 mg/kg bw in females.

Before the start of the definitive study, survival was assessed in an additional group of 10 females given the test material at a dose of 0.90 mg/kg bw by gavage. The only data reported for this study were the findings for animals manifesting clinical signs of toxicity during daily observations. Signs of toxicity (first observed on the day of treatment and apparently disappearing by day 5 after dosing) were reported for six of the 10 animals and included bilateral tremors in fore and hind paws, slight to moderate anogenital staining, decreased faecal volume, fasciculation, extreme lacrimation and excessive salivation. No miosis was observed.

In the definitive study, all animals were assessed for physical condition, mortality, clinical signs of toxicity, body-weight changes and food consumption. Neurobehavioural evaluations (motor activity and functional observational battery) were conducted on 10 animals of each sex per dose pre-test, at approximately 6 h after dosing (time of peak effect) and on days 8 and 15 (study termination day). Blood was collected for plasma and erythrocyte cholinesterase activity measurements from 10 animals of each sex per dose at about 6 h after dosing, and on day 8, and in five animals of each sex per dose on day 15. Brain cholinesterase activity in one-half brain homogenates was also assessed in the same number of animals at about 6 h after dosing and on day 15. Cholinesterase inhibition was determined relative to the appropriate concurrent control group value. All animals received macroscopic examinations and selected tissues from the central and peripheral nervous system were evaluated histopathologically in five perfused animals of each sex per group.

In the definitive study, the only death attributed to treatment occurred in females at the highest dose, 5–6 h after dosing. There were no accompanying clinical findings reported for this animal, which was replaced by another female for the remainder of the study. Clinical signs of toxicity, some commencing on the day of treatment, were noted in the daily physical examinations in one surviving female at the highest dose; these included moderate to extreme ano-genital staining, lethargy, decreased faecal volume and food consumption, and oral/buccal staining. No clinical findings were reported after day 5 during daily physical observations in this female. One male at the intermediate dose exhibited a red ocular exudate on day 9, but this may have been related to orbital sinus bleeding for cholinesterase measurements. Slight statistically significant decreases in body weights observed in females at the highest dose may have been largely related to decreases noted in the one animal exhibiting clinical signs of toxicity. There were no significant changes noted in male body weights or food consumption in either sex, relative to values for the respective control groups.

There were no statistically significant differences noted in the mean value for motor activity for the group, relative to values for the respective control group, on days 1, 8 and 15. The study report mentioned that the one female at the highest dose that had clinical signs of toxicity during the daily physical examinations also exhibited decreased motor activity on days 1 and 8, but not on day 15.

Treatment-related abnormalities in the functional observational battery, attributed to cholinergic toxicity, were apparent in males and females at the intermediate and highest dose and only during the peak time assessment at day 1. Miosis, present in three males at the intermediate dose, six males at the highest dose and one female at the intermediate dose, was the only finding at these doses. A wider spectrum of findings was present in females at the highest dose. In addition to miosis in all 10 rats, females in this group exhibited tremors (seven animals), and muscle fasciculations (four animals) and, to a lesser extent, ataxic gait and slightly impaired locomotion, tip-toe gait, moderate lacrimation and soiled coat. In one animal, very low arousal state, flattened posture (in home cage), no approach response, severe lacrimation and slight salivation, no open field movement, soiled coat, and markedly decreased forelimb and hindlimb grip strength were reported. At the next two time-points (days 8 and 15), no abnormal findings were evident in any group.

On day 1, around the time of peak effect, plasma cholinesterase activity was statistically significantly decreased in males by 31% and 69%, and in females by 20% and 90% at the intermediate and highest doses, respectively. There was no statistically significant inhibition at any dose in either sex on days 8 or 15. Erythrocyte cholinesterase activity was statistically significantly inhibited on day 1 only in males (67%) and females (90%) at the highest dose. Values continued to be decreased in females at the highest dose by about 45% on days 8 and 15. Brain cholinesterase activity was statistically significantly inhibited on day 1 only at the highest dose by 21% in males, and by 51% in females. Statistically significant depression of brain cholinesterase activity (of 13%) continued to be observed on day 15 in females at the highest dose.

Macroscopic and microscopic examinations did not reveal any findings related to treatment. Trauma related to retro-orbital sinus bleeding was considered to account for the minimal to slight focal degeneration of optic nerve fibres accompanied by minimal to slight gliosis observed in two females at the highest dose and minimal degeneration of a single sciatic nerve fibre in only one male at the highest dose was considered to be incidental in nature. It should be noted that miosis was observed during the functional observational battery in both sexes at 0.3 mg/kg bw, with no significant decrease in concurrently measured brain and erythrocyte cholinesterase activity. Only marginal (but statistically significant) concurrently measured plasma cholinesterase activity was observed at this dose in both sexes. If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL was 0.15 mg/kg bw in males and females on the basis of miosis in the functional observational battery in both sexes at the next highest dose (Mandella, 1998). Statements of compliance with QA and GLP were provided and the study protocol was consistent with EPA Subdivision F Guidelines (1984, revised and March 1991).

(c) Neurotoxicity after repeated doses

In a study of neurotoxicity, groups of 20 male and 20 female Sprague-Dawley derived (outbred) Crl:CD®(SD)IGS BR VAF/Plus® rats were given diets containing technical-grade terbufos (AC 92,100; purity, 89.7%; dissolved in acetone and mixed with a GRIT-

O'Cobs® carrier) at a dose (adjusted for purity) of 0 (diet mixed with acetone and carrier), 0.5, 0.8, or 5.0 mg/kg in males (equal to 0, 0.036, 0.059 and 0.369 mg/kg bw per day) and of 0 (diet mixed with acetone and carrier), 0.5, 0.8 or 3.0 mg/kg in females (equal to 0, 0.042, 0.064, and 0.251 mg/kg bw per day) daily for approximately 13 weeks. Animals evaluated for potential neurobehavioural changes were treated for at least 13 weeks and animals in which cholinesterase activities were measured were treated for at least 85 days. Selection of doses was based on a 22-day preliminary study of feeding in the same strain of rat (Mandella, 1999), in which treatment-related findings were noted at doses of ≥5 mg/kg (0.55 mg/kg bw per day) in males and ≥3 mg/kg (0.33 mg/kg bw per day) in females. In the 13-week study, all animals were assessed for physical condition, mortality, clinical signs of toxicity, body-weight changes, and food consumption and received ophthalmoscopic examinations. Neurobehavioural evaluations (motor activity and functional observational battery) were conducted on 10 animals of each sex per group, before treatment and at weeks 4, 8, and 13. In animals designated for cholinesterase activity determinations (9–10 animals of each sex per group), blood samples for assessment of plasma, erythrocyte and brain (one-half homogenate) cholinesterase activities were obtained at weeks 4, 8, and 13. In animals designated for neurobehavioural examinations, blood and brain samples for measurement of cholinesterase activities were obtained from five animals of each sex per group during weeks 13 or 14. Cholinesterase inhibition was determined relative to values for the appropriate concurrent control group. At study termination (week 13 or 14), macroscopic examinations were performed on all animals and selected tissues from the central and peripheral nervous system were evaluated histopathologically in five animals of each sex per group.

One male at the lowest dose died in week 5 due to accidental causes. Otherwise, there were no deaths in the study. There were no findings during physical examinations that were considered to be treatment-related and miosis was not observed in any animal. The results of ophthalmoscopic examinations were not remarkable. Over the 13-week period, males at the highest dose gained only about 90% of the weight gained by animals in the control group; a possible effect of treatment could not be dismissed. Weight gains in males at the lowest and intermediate doses during the same period were slightly higher than those of animals in the control group. Females at the highest dose gained about 13% more weight over the duration of the study than did animals in the control group, but this was not considered to be an adverse effect. There were no changes in food consumption in either sex that could definitively be ascribed to treatment and no obvious effect of treatment with the test material on motor activity or on functional observational battery parameters. In the macroscopic and microscopic examinations, there were no findings that were attributable to treatment.

At the highest dose, plasma cholinesterase activity in animals designated for cholinesterase measurements or neurobehavioural evaluations was statistically significantly inhibited at all time-points by 70–74% in males and by 91–92% in females. In the same groups, erythrocyte cholinesterase activity was virtually completely inhibited at all time-points (decreases of 97–100% in males and 100% in females). At the intermediate dose, erythrocyte cholinesterase activity was statistically significantly inhibited in males in the group of animals designated for cholinesterase measurement at week 8 and 13 by 48% and 37%, respectively, and by 35% at week 13 in the group designated for neurobehavioural evaluations. In both groups of females at the intermediate dose, inhibition of 33% (statistically significant) and 27% (not statistically significant) was observed at week 13. At study termination, brain cholinesterase activity was decreased only at the highest dose in animals

Table 4. Acute toxicity of metabolites and degradation products of terbufos in female mice

Metabolite	Strain	Route	Vehicle	LD_{50} (mg/kg bw)	Purity (%)	Reference
Terbufoxon sulfoxide[a]	CF1 albino	Oral	Corn oil	1.1	Not stated	American Cyanamid Company A72-35 (1972d)
Terbufos sulfoxide[b]	CF1 albino	Oral	Corn oil	3.4	Not stated	American Cyanamid Company A72-37 (1972b)
Terbufoxon sulfone[c]	CF1 albino	Oral	Corn oil	3.4	Not stated	American Cyanamid Company A72-38 (1972e)
Terbufos sulfone[d]	CF1 albino	Oral	Corn oil	14[i]	Not stated	American Cyanamid Company A72-34 (1972c)
Terbufoxon[e]	CF1 albino	Oral	Corn oil	2.2	Not stated	American Cyanamid Company A72-36 (1972f)
CL 202,135[f]	CF1 albino	Oral	Corn oil	3670[g]	Not stated	American Cyanamid Company A73-21 (1973a)
CL 202,474[h]	CF1 albino	Oral	Corn oil	>2500[j]	Not stated	American Cyanamid Company A73-122 (1973b)

Although reports for these studies were not detailed and statements of compliance with GLP or QA were not provided, protocols appeared to be generally consistent with EPA Subdivision F Guidelines (1982 or 1984, revised)

[a] Phosphorothioic acid, *S*-(*t*-butylsulfinyl) methyl *O*,*O*-diethyl ester (CL 94,365)

[b] Phosphorodithioic acid, *S*-(*t*-butylsulfinyl) methyl *O*,*O*-diethyl ester (CL 94,301)

[c] Phosphorothioic acid, *S*-(*t*-butylsulfonyl) methyl *O*,*O*-diethyl ester (CL 94,302)

[d] Phosphorodithioic acid, *S*-(*t*-butylsulfonyl) methyl *O*,*O*-diethyl ester (CL 94,320)

[e] Phosphorothioic acid, *S*-(*t*-butylthio) methyl *O*,*O*-diethyl ester (CL 94,221)

[f] Methane, bis(*t*-butylsulfonyl) (CL 202,135)

[g] Report stated that acute oral LD_{50} was calculated assuming a mortality of 9 out of 10 animals at a dose of 10 000 mg/kg of feed

[h] Methane, (*t*-butylsulfinyl)(methylsulfinyl)

[i] Report stated that acute oral LD_{50} ws calculated assuming a mortality of five out of five animals at a dose of 50 mg/kg of feed

[j] Report stated that animals were not fasted

designated for cholinesterase measurement and neurobehavioural evaluation, by 58% and 55%, respectively, in males and by 71% and 68%, respectively in females.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect and statistically significant inhibition of erythrocyte cholinesterase activity of 33–43% is not considered to be a relevant effect for terbufos, the NOAEL was 0.8 mg/kg (equal to 0.059 mg/kg bw per day in males and 0.064 mg/kg bw per day in females) on the basis of statistically significant inhibition of brain and erythrocyte (almost completely inhibited) cholinesterase activity at the next highest dose (Mandella, 1999). Statements of compliance with QA and GLP were provided. The protocol was consistent with EPA Subdivision F Guidelines (November, 1984, revised, and March 1991).

(d) Studies on metabolites

In a study of short-tem toxicity, groups of four male beagle dogs were given gelatin capsules containing technical-grade terbufos (AC 92,100; purity, 89.6%) or one of its metabolites, terbufos sulfoxide (CL 94,301; purity, 90.0%) and terbufos sulfone (CL 94,320; purity, 92.0%), in corn oil, administered orally once daily in the morning for at least 14 days. A similarly treated control group of six animals received gelatin capsules containing corn oil only. The animals used were described as having physical impairments but not of sufficient magnitude to jeopardize study integrity. Doses administered (adjusted for purity) were: terbufos, 2.5 or 250 µg/kg bw per day; terbufos sulfoxide, 5, 15, 625, or 250 µg/kg bw per day; or terbufos sulfone, 15, 62.5, 250, and 1000 µg/kg bw per day. A limited number of parameters were assessed, including mortality, morbidity and clinical signs of toxicity, measurement of body weight and food consumption, and a gross examination at the end of the study. Plasma and erythrocyte cholinesterase activity in fasted

animals was assessed twice before treatment, and on days 4, 8, 11, and 15. Brain cholinesterase activity (in homogenates of cerebellum and cerebrum) was measured from tissues taken at study termination (day 15 or 16) and stored frozen until analysis. Inhibition of cholinesterase activity was determined relative to the value for the appropriate concurrent control group. Overall, the data on brain cholinesterase activity, especially those from the cerebellum, were considered to be unreliable owing to inconsistencies, variability in the data and poor dose–response relationships. Clinical chemistry, haematological, urine analysis, organ weight and histopathological evaluations were not conducted in this study.

There were no deaths in the control group. The only clinical observations reported were occurrence of soft faeces (four animals) and one occurrence of alopecia. There were no other adverse findings.

There were no deaths in the groups of animals treated with terbufos. Clinical findings attributed to treatment at the highest dose (250 µg/kg bw per day) were instances of emesis and lacrimation, and an increased incidence of soft or soft sanguineous-looking faeces (seven occurrences) compared with the control group; treatment-related decreases in body weight and food consumption were also noted in some animals. At the highest dose, plasma cholinesterase activity was statistically significantly inhibited by 57–76% at all time-points during the study, and erythrocyte cholinesterase activity was statistically significantly inhibited by 42–79% on days 8, 11 and 15, and cerebellar and cerebral cholinesterase activities were statistically significantly inhibited by 48% and 37%, respectively. At the lowest dose, statistically significant decreases in plasma cholinesterase activity were noted on days 11 and 15, but they were of low magnitude (23–25%) and not clearly related to treatment. There were no obvious effects of treatment on erythrocyte or cerebral cholinesterase activities at the lowest dose. Cerebellar cholinesterase activity at the lowest dose was statistically significantly inhibited by 42%. As a finding of significant brain cholinesterase inhibition at this dose was inconsistent with other studies of repeated dosing with terbufos, it was considered highly unlikely to be related to treatment. A finding of dark mesenteric lymph node(s) at gross necropsy in one animal in the group treated with the lowest dose of terbufos was not clearly related to treatment.

In the groups of animals treated with terbufos sulfoxide, there were no mortalities. At the highest dose (250 µg/kg bw per day), clinical findings ascribed to treatment included ataxia, salivation, languid behaviour, salivation, miosis, no faeces, emesis, and an increased incidence relative to control of soft faeces (11 occurrences). Decreases in body weight and food consumption attributed to treatment were noted in some animals at the highest dose.

At all time-points during the study, plasma and erythrocyte cholinesterase activities were statistically significantly inhibited by 60–71% and 30–93%, respectively, at the highest dose. In groups given terbufos sulfoxide at 62.5 and 15 µg/kg bw per day, there was no clear treatment-related effect on body weights, food consumption or clinical findings. Single occurrences of emesis or sanguineous-looking emesis were noted in each of these groups, as was one instance of no faeces at 15 µg/kg bw per day. Occurrences of soft stool in the control group, and at the lowest, lower intermediate, higher intermediate and highest dose were 4, 3, 13, 6, and 11, respectively. Increases relative to the control group at the two intermediate doses did not appear to be part of an obvious pattern that might be definitively atributed to treatment. Although plasma cholinesterase activity was statistically significantly inhibited at all time-points at both intermediate doses, erythrocyte enzyme activity was not statistically significantly inhibited at any time-point at either dose. Inhibition of brain

cholinesterase activity was found at the two higher doses. Cerebral cholinesterase activity was statistically significantly inhibited only at the highest dose (250 µg/kg bw per day) by 45%. At the higher intermediate and highest doses, cerebellar cholinesterase activity was statistically significantly inhibited by 52% and 31% (not statistically significant), respectively. At the lowest dose (5.0 mg/kg bw per day), there were no obvious effects of treatment on the parameters assessed. A statistically significant decrease in plasma cholinesterase activity that was noted only on day 15 was of low magnitude (19%). Findings at gross necropsy that were reported to be related to treatment were observed in one dog at the highest dose. They were described as a dark red area in the mucosa of the jejunum mucosa, associated with dark red mesenteric lymph node(s). This animal exhibited a number of clinical signs during the study and had decreases in plasma, erythrocyte cerebellar and cerebral cholinesterase activities. Dark mesenteric lymph node(s) were also reported in one other animal at the highest dose.

In groups of animals treated with terbufos sulfone, there were three treatment-related deaths preceded by clinical signs of toxicity. Two animals were found dead on day 9 and 14 and the third was terminated in a moribund condition on day 15. Clinical findings associated with treatment in this group included tremor, languid behaviour, prostration, ataxia, emesis, salivation, dyspnoea, cold-to-touch, squint eye, miosis, no faeces, an increase in soft faeces relative to the control group, prolapsed rectum, sanguineous-appearing material in cage. Also at this dose, decreases in body weight and food consumption attributed to treatment were noted in some animals. In groups given terbufos sulfone at 250, 62.5 or 15 µg/kg bw per day, there was no clear treatment-related effect on body weights, food consumption or clinical findings. Two occurrences of lacrimation and two occurrences of soft faeces were reported at 250 µg/kg bw per day, two instances of soft or sanguineous-looking faeces and one instance of emesis were found at 62.5 µg/kg bw per day and four occurrences of soft faeces were found at the lowest dose. Plasma cholinesterase activity was statistically significantly inhibited at all time-points: by 67–77% at the highest dose, by 39–56% at 250 µg/kg bw per day, and by 24–37% at 62.5 µg/kg bw per day. At the lowest dose (15 µg/kg bw per day), statistically significant inhibition of plasma cholinesterase activity (of only 20%) was seen only on day 8 but not on day 15 and thus was not clearly related to treatment. Statistically significant decreases in erythrocyte cholinesterase activity were observed only at the highest dose where inhibition was noted at all time-points ranging from 57% to 93%. Inhibition of brain cholinesterase activity was found at the two higher doses (statistically significantly only at the highest dose, by 62%). Cerebellar cholinesterase activity was inhibited by 23% (not statistically significant) at 250 µg/kg bw per day and by 46% (not statistically significant) at the highest dose.

At gross necropsy, findings related to treatment were observed in the group receiving the highest dose: dark, red or dark red areas, intussusceptions, and/or a prolapsed anus with associated redness in the mesenteric lymph nodes were observed in the gastrointestinal tract of the animals that died. Some other gross findings at this dose were ascribed to the poor condition of the animals.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL for terbufos was 2.5 µg/kg bw per day in males on the basis of clinical findings and statistically significant inhibition of erythrocyte cholinesterase activity.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL for terbufos sulfoxide was 15.0 µg/kg bw per day in males on the basis of

statistically significant inhibition of brain cholinesterase (cerebellum) at the dose above, for which a relationship to treatment could not be dismissed.

If inhibition of plasma cholinesterase activity is not considered to be an adverse effect, the NOAEL for terbufos sulfone was 62.5 µg/kg bw per day on the basis of inhibition of brain cholinesterase (cerebellar) activity at the dose above, for which a relationship to treatment could not be dismissed (Bailey, 1988). Statements of compliance with QA and GLP were provided. This study was not conducted to fulfil a particular guideline.

3. Observations in humans

There have been a number of reports of occupational and non-occupational poisoning incidents associated with exposure to terbufos. With regard to possible effects from terbufos manufacturing facilities, no "reportable incidents" have been noted and no other information was available.

Comments

In rats, absorption of single doses of [^{14}C]terbufos was rapid and fairly complete. Most of the radiolabel was excreted within 24–48 h. Most (about 70–80%) of the administered dose was excreted in the urine. Terbufos was extensively metabolized and little radioactivity was found in the tissues. There were no significant sex-specific differences in the toxicokinetics of terbufos.

Sulfoxidation and desulfuration of terbufos is followed by hydrolysis of the thiolophosphorus bond (S–P), enzymatic *S*-methylation and then additional *S*-oxidation. On the basis of a 14-day study of repeated dosing, terbufos showed little potential for bioaccumulation.

By analogy with other phosphorodithioate compounds, it is likely that terbufos is metabolically activated to terbufos oxon and other oxons, which cause inhibition of acetylcholinesterase activity.

Terbufos is of very high acute toxicity when administered by oral, dermal, and inhalation routes. Acute oral LD_{50} values in rodents and dogs were similar, ranging from 1.4 to 9.2 mg/kg bw. Clinical signs observed were those typical of cholinergic toxicity.

The acute dermal LD_{50} for terbufos was about 1 mg/kg bw in rabbits; a single application of undiluted terbufos to the shaved skin (0.25–0.5 ml) or into the conjunctival sac (0.1 ml) killed all animals within 24 h. The acute LC_{50} for terbufos administered by inhalation ranged from 0.0012 to 0.0061 mg/l in rats.

In studies in rats and dogs, the critical effects of repeated doses of terbufos were inhibition of brain cholinesterase activity and associated clinical signs. NOAELs for inhibition of brain cholinesterase activity in these studies ranged from about 0.04 to 0.11 mg/kg bw per day, and LOAELs ranged from about 0.085 to 0.55 mg/kg bw per day. Inhibition of brain cholinesterase activity of 7–12%, in the absence of clinical signs, was not considered to be toxicologically relevant. NOAELs and LOAELs for inhibition of erythrocyte cholinesterasese activity were not substantially different from those for inhibition of brain cholinesterase activity.

In a 1-year study in dogs given terbufos in capsules, the NOAEL was 0.060 mg/kg bw per day on the basis of inhibition of brain acetylcholinesterase activity at 0.090 mg/kg bw per day. The NOAELs for inhibition of brain acetylcholinesterase activity in other short-term studies in dogs were consistent with that of the 1-year study.

In an 18-month study in mice fed with terbufos, there was no evidence of carcinogenicity at doses considered relevant for risk assessment. Cholinesterase activities were not measured. The NOAEL was 3 mg/kg (equivalent to 0.45 mg/kg bw per day) on the basis of significant decreases in body weights at the next highest dose of 6 mg/kg (equivalent to 0.9 mg/kg bw per day).

A 2-year study of toxicity and carcinogenicity in rats had limitations that included outstanding questions involving the etiology and/or relationship to treatment of certain non-neoplastic findings, confounding by non-treatment related illness in test animals and the lack of supporting data to adequately quantify dietary intake, stability, and homogeneity. However, on the basis of inhibition of brain cholinesterase activity in animals receiving the highest dose, this study was considered to be adequate for testing for carcinogenicity. No increase in tumour incidence was observed after a histopathological re-evaluation of tissues for the assessment of carcinogenic potential. The NOAEL was 1 mg/kg (equivalent to 0.05 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity at 4 mg/kg (equivalent to 0.2 mg/kg bw per day).

This conclusion was supported by a subsequent 1-year study of toxicity in rats; no significant systemic or neoplastic effects were observed. The NOAEL was 1 mg/kg (equal to 0.055 mg/kg bw per day) on the basis of the absence of significant inhibition of brain acetylcholinesterase activity at all doses.

The Meeting concluded that terbufos was not carcinogenic in mice or rats.

The genotoxic potential of terbufos was assessed in an adequate range of in vitro and in vivo tests. On the basis of the overall weight of evidence from the studies of genotoxicity, the Meeting concluded that terbufos is unlikely to pose a genotoxic risk to humans.

In view of the lack of significant genotoxicity and the absence of carcinogenicity observed, the Meeting concluded that terbufos is unlikely to pose a carcinogenic risk to humans.

In a study of reproductive toxicity in rats, mortality and clinical signs of toxicity in females, some occurrences of excess salivation in males and decreases in body weight and food consumption in both sexes were observed at 5 mg/kg (equal to 0.42 mg/kg bw per day), a treatment that was terminated prematurely. At 2.5 mg/kg, an increase in soft stools and body-weight loss was noted in lactating females. Also observed were decreases in pregnancy rate, male fertility and significant decreases in the mean body weight of viable pups on days 14 and 21 of lactation in F_0 and F_1 litters. The NOAEL for effects on reproduction and offspring was 1.0 mg/kg (equal to 0.086 mg/kg bw per day). Inhibition of brain cholinesterase activity was observed in both sexes, with a NOAEL of 0.5 mg/kg (equal to 0.043 mg/kg bw per day).

In a study of developmental toxicity in rats, the NOAEL for maternal and developmental effects was 0.2 mg/kg bw per day, the highest dose tested. Mortality was seen at a dose of 0.4 mg/kg bw per day in a preliminary study.

In a study of developmental toxicity in rabbits, the NOAEL for maternal and developmental effects was 0.25 mg/kg bw per day on the basis of clinical and systemic findings in does, an increased number of does with resorption sites and decreased fetal body weights at the next highest dose of 0.50 mg/kg bw per day.

The potential of terbufos to induce delayed polyneuropathy in hens when given as a single dose by gavage was assessed. The activity of neuropathy target esterase was not measured in this study. No significant changes in spinal cord and peripheral nerves were apparent in the group treated with terbufos. The Meeting concluded that at doses relevant to dietary exposure in humans, there was no concern for the induction of delayed polyneuropathy by terbufos.

In a study of neurotoxicity in which terbufos was given to rats as a single dose by gavage, mortality, clinical signs of toxicity, including miosis, and inhibition of brain and erythrocyte cholinesterase activities were noted at the highest dose tested of 0.90 mg/kg bw. The only finding at the intermediate dose (0.3 mg/kg bw) was miosis, which was observed in the absence of inhibition of concurrently measured brain and erythrocyte cholinesterase activities. No neurohistopathological lesions were found at any dose. The NOAEL was 0.15 mg/kg bw on the basis of findings of miosis in both sexes at the next highest dose of 0.30 mg/kg bw.

A 13-week study of neurotoxicity was conducted in rats. Other than a slight decrease in body weight and inhibition of brain and erythrocyte cholinesterase activities at the highest dose of 3.0 mg/kg (equal to 0.25 mg/kg bw per day), no effects (including miosis) were observed. The NOAEL was 0.8 mg/kg (equal to 0.059 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity at 0.25 mg/kg bw per day.

The acute oral toxicity of a number of metabolites of terbufos was evaluated in female mice. LD_{50}s were as follows: 1.1 mg/kg bw (terbufoxon sulfoxide), 3.4 mg/kg bw (terbufos sulfoxide), 3.4 mg/kg bw (terbufoxon sulfone), 14 mg/kg bw (terbufos sulfone), 2.2 mg/kg bw (terbufoxon), 3670 mg/kg bw (methane, bis (*tert*-butylsulfonyl) and >2500 mg/kg bw (methane, (*tert*-butylsulfinyl)(methylsulfinyl)).

In a comparative 14-day study in dogs, terbufos given in capsules was found to be more toxic than either terbufos sulfoxide or terbufos sulfone.

There have been a number of reports of occupational and non-occupational poisoning incidents associated with exposure to terbufos. No information was available regarding possible effects from terbufos manufacturing facilities.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.0006 mg/kg bw based on an overall NOAEL of 0.06 mg/kg bw per day and a safety factor of 100 for inhibition of brain cholinesterase activity, in the 1-year study of toxicity, the 13-week study of neurotoxicity and the two-generation study of reproduction in rats, and the 1-year study in dogs.

The Meeting established an acute reference dose (RfD) of 0.002 mg/kg bw based on a NOAEL of 0.15 mg/kg bw per day for miosis in the study of neurotoxicity in rats given a single dose of terbufos, and a 100-fold safety factor. Since only in this study was miosis observed in the absence of inhibition of cholinesterase activity, it might be possible to refine the acute RfD after better characterization of this effect.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	3 mg/kg of feed, equivalent to 0.45 mg/kg bw per day	6 mg/kg of feed, equivalent to 0.90 mg/kg bw per day
		Carcinogenicity	12 mg/kg of feed, equivalent to 1.8 mg/kg bw per day[d]	—
Rat	2-year study of toxicity and carcinogenicity[a]	Carcinogenicity	4 mg/kg of feed, nominally equivalent to 0.2 mg/kg bw per day[d]	—
	1-year study of toxicity[a]	Toxicity	1 mg/kg of feed, equal to 0.055 mg/kg bw per day[d]	—
	13-week study of neurotoxicity[a]	Toxicity	0.8 mg/kg of feed, equal to 0.059 mg/kg bw per day	3.0 mg/kg of feed, equal to 0.25 mg/kg bw per day
	Single-dose study of neurotoxicity[b]	Toxicity	0.15 mg/kg bw per day	0.30 mg/kg bw per day
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	0.5 mg/kg of feed, equal to 0.043 mg/kg bw per day	1.0 mg/kg of feed, equal to 0.086 mg/kg bw per day
		Offspring toxicity	1.0 mg/kg of feed, equal to 0.086 mg/kg bw per day	2.5 mg/kg of feed, equal to 0.21 mg/kg bw per day
	Study of developmental toxicity[b]	Maternal toxicity	0.20 mg/kg bw per day[d]	—
		Embryo- and fetotoxicity	0.20 mg/kg bw per day[d]	—
Rabbit	Study of developmental	Maternal toxicity	0.25 mg/kg bw per day	0.50 mg/kg bw per day
		Embryo- and fetotoxicity	0.25 mg/kg bw per day	0.50 mg/kg bw per day
Dog	1-year study of toxicity[c]	Toxicity	0.06 mg/kg bw per day	0.09 mg/kg bw per day

[a] Diet
[b] Gavage
[c] Capsule
[d] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.0006 mg/kg bw

Estimate of acute reference dose

0.002 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

- A study of delayed neurotoxicity with neuropathy target esterase measurements (known to be ongoing)
- Further observations in humans
- Characterization of miosis

Summary of critical end-points for terbufos

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and fairly complete
Dermal absorption	No specific study; rapidly penetrating following dermal or ocular application
Distribution	Relatively rapid and fairly complete
Potential for accumulation	Little
Rate and extent of excretion	Relatively rapid and complete; most eliminated in 24–48 h; elimination in urine predominates
Metabolism in animals	Sulfoxidation and desulfuration of terbufos is followed by hydrolysis of the thiolophosphorus bond (S-P), enzymatic *S*-methylation and then additional *S*-oxidation
Toxicologically significant compounds	Terbufos Terbufos oxon Terbufos sulfoxide Terbufos sulfone Terbuoxon sulfoxide Terbuoxon sulfone
Acute toxicity	
Rat, LD_{50}, oral	1.4–9.0 mg/kg bw
Rabbit, LD_{50}, dermal	0.81–0.93 mg/kg bw
Rat, LC_{50}, inhalation	Vapour, 4-h whole body exposure: 0.0012–0.0061 mg/l
Dermal irritation	Could not be determined due to lethality, rabbit
Ocular irritation	Could not be determined due to lethality, rabbit
Skin sensitization	Not determined owing to potential for severe toxicity
Short-term studies of toxicity	
Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant oral NOAEL	0.059 mg/kg bw per day (13-week study of neurotoxicity in rats)
Lowest relevant dermal NOAEL	Data not available
Lowest relevant inhalation NOAEC	No appropriate data available
Genotoxicity	Unlikely to be genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant NOAEL	0.055 mg/kg bw per day (1-year study in rats)
Carcinogenicity	No evidence of carcinogenicity; Unlikely to pose a risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Decreases in male fertility and female pregnancy rate
Lowest relevant reproductive NOAEL	0.086 mg/kg bw per day (rats)
Developmental target/critical effect	Not teratogenic; Reduced fetal body weight
Lowest relevant developmental NOAEL	0.25 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	
Target/critical effect	Miosis
Relevant NOAEL	0.15 mg/kg bw (rats)
13-week study of neurotoxicity	
Target/critical effect	Inhibition of brain cholinesterase activity
Relevant NOAEL	0.059 mg/kg bw per day (rats)
Delayed neuropathy	No evidence to suggest toxicity at dietary exposures
Medical data	There have been a number of reports of occupational and non-occupational poisoning incidents associated with exposure to terbufos. No information was available regarding possible effects from terbufos manufacturing facilities.

Summary	Value	Study	Safety factor
ADI	0–0.0006 mg/kg bw	Rats and dogs, overall NOAEL for studies of repeated doses	100
Acute RfD	0.002 mg/kg bw	Rat, study of acute neurotoxicity	100

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