Food and Agriculture Organization of the United Nations

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Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 85th Meeting 2017

Ethion

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Ethion

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Identity

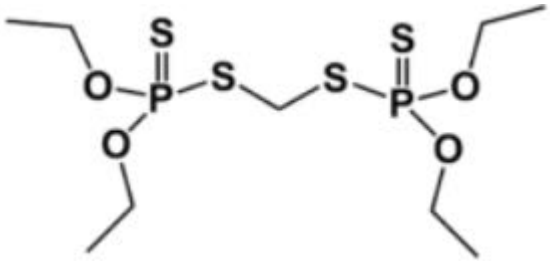
International Non-proprietary Name (INN): Ethion

Synonyms: Diethion; [(Dethoxyphosphinothioylthio) methylthio]-diethoxy-thioxophosphorane

IUPAC name: O,O,O′,O′-Tetraethyl S,S′-methylene bis(phosphorodithioate)

Chemical Abstract Service (CAS) No.: 563-12-2

Structural formula:



Molecular formula: $C_9H_{22}O_4P_2S_4$

Molecular weight: 384.48 g/mol

Other information on identity and properties

Pure active ingredient: Ethion

Appearance: Colourless to amber-coloured, odourless liquid.

Melting point: −12.2 °C (10.0 °F; 260.9 K)

Solubility (at 20 °C):

Water: 0.0001 %

Vapour pressure: 0.0000015 mmHg (20 °C)

Background

Ethion was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1968, 1972, 1986 and 1990 (JMPR, 1990). Ethion was included for review by the 85th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at the request of the Twenty-third Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF; FAO/WHO, 2016), to be evaluated using any relevant published data as well as sponsor-submitted residue depletion data. The request was specifically in relation to setting MRLs in edible tissues of cattle.

Residues in food and their evaluation

Conditions of use

Ethion is an organophosphate insecticide used in the prevention of vector-borne diseases carried by the cattle tick, *Boophilus microplus.* It can be formulated into immersion bath treatments, pour-ons, sprays and ear-tags, often in combination with cypermethrin (a pyrethroid insecticide), for administration to cattle (both beef and dairy, depending on the product).

Immersion bath treatments are marketed in the form of a concentrate solution containing (e.g.) 40 % ethion (and 10 % cypermethrin), which are then diluted with water before treatment to a suitable concentration (400 ppm ethion). The animals are then treated as a herd, by being corralled through the bath one-by-one.

Pour-ons also come in solutions, containing (e.g.) 150 g/l ethion and 50 g/l cypermethrin. Recommended doses are 5 ml for animals weighing 100 – 200 kg, 10 ml for animals weighing 200 - 400 kg and 20 ml for animals that weigh >400 kg (3.75 – 7.5 mg/kg).

Ear-tags can contain 36 – 40 g ethion per ear-tag and these are left on the animals for a period of time (e.g. 120 days) until removal. Some products recommend using one ear-tag per animal, some recommend using two.

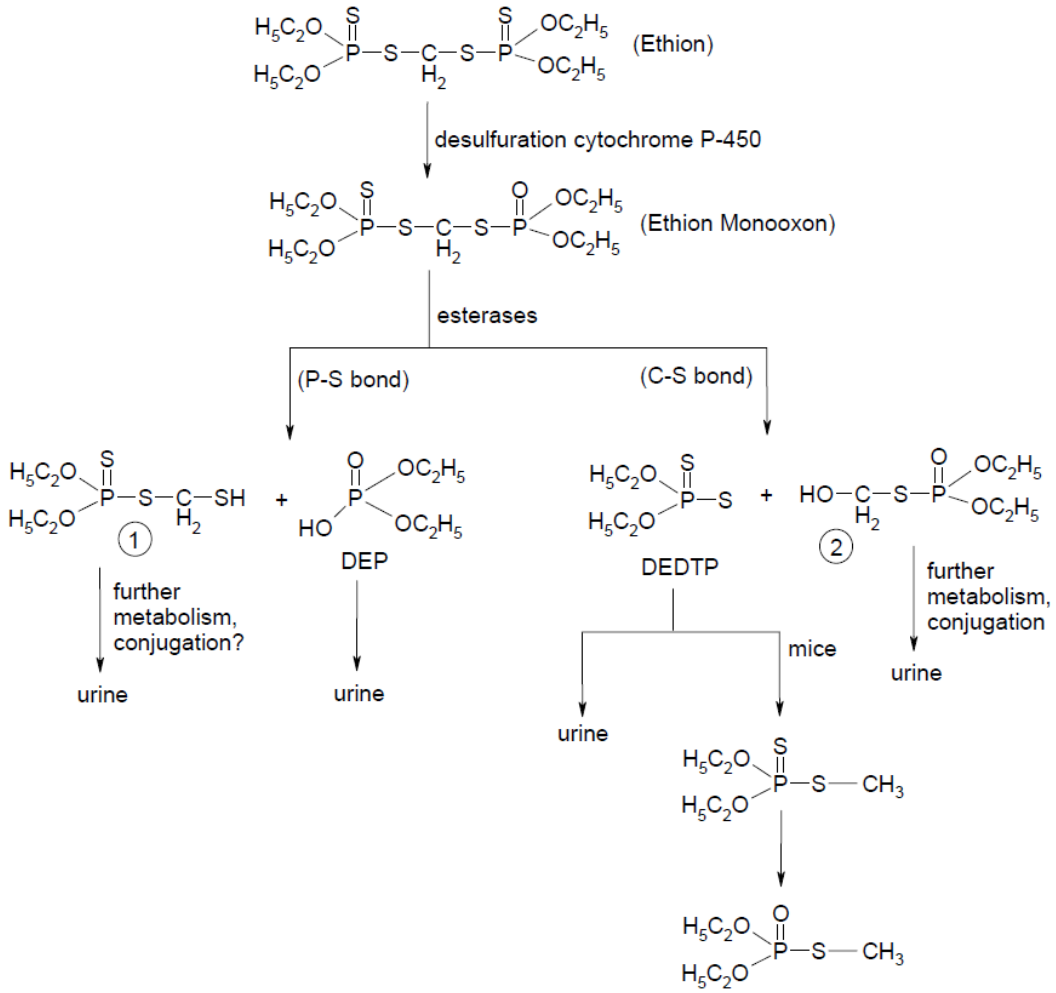
Withdrawal periods for the approved ethion formulations vary from 15 – 45 days, depending on the specific formulation and jurisdiction.

Pharmacokinetics and metabolism

Ethion is a small, lipid-soluble molecule that can be absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. Absorption appears to be rapid by the oral and dermal routes (depending on species); the time course of absorption is inferred from the onset of clinical signs within 1 hour after accidental ingestion of ethion in a 6-month-old boy (Comstock *et.al*., 1967) and deaths within 3–6 h in dermally exposed Sherman rats (Gaines 1969). Ethion is desulfurated by cytochrome P450 enzymes in the liver to its active form, ethion monoxon, which causes toxicity due to its potent inhibition of neural acetylcholinesterase.

Ethion and its oxon form can be detoxified by the action of esterases in the blood and liver, producing diethyl phosphate, diethyl thiophosphate, diethyl dithiophosphate, and other metabolites that have not been characterised.

Figure 1. Proposed Mammalian Metabolic Pathways for Ethion (ATSDR, 2000)



DEP = diethylphosphate; DEDTP = diethyldithiophosphate; 1 = O,O-diethyl-S-mercaptomethyldithiophosphate; 2 = O,O-diethyl-S-hydroxymethylphosphate

Pharmacokinetics in laboratory animals

Oral administration

Seven days after rats received a single gavage dose of radiolabelled ethion, less than 1 % of the radiolabel was detected in the body (blood, brain, heart, pancreas, leg muscle, lungs, adipose, spleen, bone, skin, hair, kidney, liver, gonads [uterus and ovaries for females, testes, seminal vesicle, and prostate for males]) (Selim 1985a, referenced in ATSDR, 2000). Total residues ranged from 0.21 to 0.34 % of the original dose for females; and 0.18–0.28 % for males. Similar results were obtained in a study where the radioactive dose was given after 14 consecutive daily doses of unlabelled ethion (Selim 1985a).

Elimination from the body is mainly through excretion of water-soluble metabolites in the urine. Conjugation may occur; this is inferred from experiments where [^{14}C-methylene]ethion was administered orally to rats and the radioactivity in urine analysed (Selim 1985b). Samples were extracted with ethyl acetate; the aqueous and organic phases were analysed by high-performance liquid chromatography. More than 99 % of the urine radioactivity was in the aqueous phase. Another sample was acidified (presumably to hydrolyse conjugates) and also extracted with ethyl acetate.

Acidification converted about 30 % of the radioactivity in the aqueous phase to an organosoluble form, which may indicate that some of the products of ethion metabolism are present in urine as conjugates. Four to six radiolabelled metabolites were detected by HPLC, none migrated with standards for ethion, ethion monoxon, or ethion dioxon. None of the metabolites were specifically identified.

Pharmacokinetics in Food-producing Animals

No data are available for cattle. One paper (Mosha *et.al*., 1990a) was provided that investigated the distribution and elimination of ethion in laying hens and their eggs, another two papers were provided that looked into the distribution into goat tissues and milk (Mosha *et.al*., 1990b and Mosha *et.al*., 1991).

Laying Hens

Ten Rhode Island Red laying hens, aged 24-26 weeks and weighing between 1.5-2.1 kg were used in the study (Mosha *et.al*., 1990a). The birds were housed in two cages and were provided with water and feed *ad libitum*. (^{14}C-methylene)ethion with a specific activity of 2 µCi/mg was dissolved in glycerol formal and administered orally to each bird at the dose of 5 mg/kg.

Eggs were collected from the cages in the morning and afternoon. They were individually separated into egg white and yolk, each of which was liquidised. Faeces were collected daily and oven-dried.

Two hens were killed on each of days 1, 3, 7, 15 and 21 after dosing and samples of liver, kidney, skeletal muscle (pectoral muscle), heart, brain and abdominal fat were collected. Blood was collected from each of the two birds at the time of slaughter and on days 5 and 10 from the other hens as pooled samples. All tissues and plasma separated from blood were stored at -20 °C.

The concentration of unchanged ethion in egg-white, yolk and plasma was measured by gas chromatography ("cold" residues), and the concentration of ^{14}C-ethion (ethion and metabolites expressed as ethion equivalents) was determined in tissues, plasma, egg white, yolk and faeces via liquid scintillation counting.

Ethion was extracted from the plasma or egg-white by an equal volume of hexane containing parathion as internal standard. The hexane extract from each egg white was concentrated five times and then analysed using gas chromatography with a nitrogen phosphorus detector (GC-NPD). Recovery rate for ethion in plasma was 94 ± 5 % and 90 ± 2 % in egg-white. The recovery rate from the egg yolk was 69 ± 4 %. The detection limit for ethion was 3 µg/kg.

Only traces of unchanged ethion were detected in plasma, egg-white and yolk. In plasma unchanged ethion (28 µg/kg) was detected on day 1 only, while in egg white 1 µg/kg was detected on both days 1 and 2. In egg yolk, unchanged ethion was detectable on days 2 and 3 at 13 and 4 µg/kg, respectively. The concentration of ^{14}C-ethion in egg white on day 1 was about 4-5 times lower than in plasma.

The total amount of ^{14}C-ethion excreted in faeces from the hens was 69 % of the dose. Most of this (58 %) was recovered within 24 h.

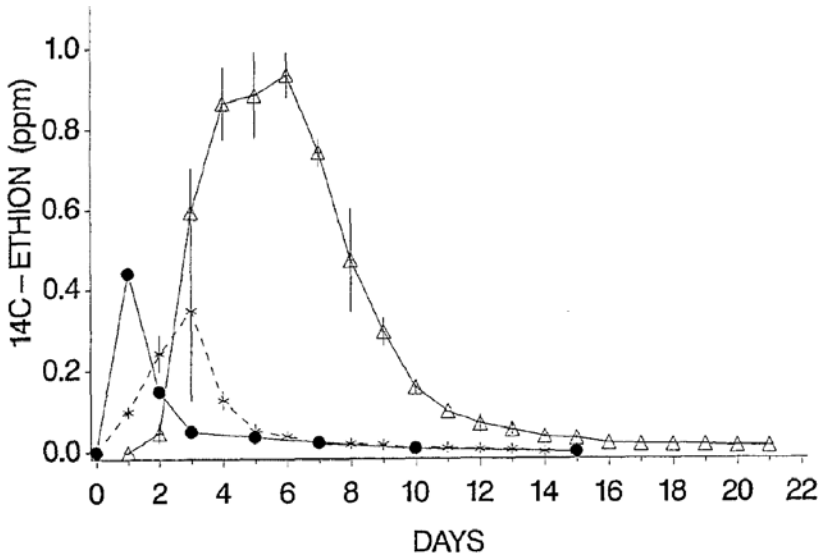
The Committee highlighted that ethion residues in laying hens are highest in liver and kidney (Table 1). This contrasts with the results from topically-applied ethion in cattle studies, where the highest concentrations are found in fat (peri-renal and subcutaneous). As the study in hens used radiolabelled ethion, and measured ethion equivalents, it may indicate that it is metabolites of

ethion that are being measured in the liver and kidney of the hens. The results for eggs indicate that ethion and its metabolites are preferentially found in more fatty matrices (i.e. the yolk, as opposed to the egg white; Figure 2).

Table 1. Concentration of ^{14}C-ethion in tissues of laying hens

Timepoint (days)	Animal ID	Level ^{14}C-ethion (µg/kg)						
		Plasma	Liver	Kidney	Muscle	Fat	Heart	Brain
1	1	390	2300	2020	80	170	300	300
	2	490	3000	2700	160	150	440	320
3	3	50	300	310	50	120	130	160
	4	50	370	420	50	70	170	140
7	5	30	130	130	60	40	110	80
	6	20	120	140	40	60	100	70
15	7	<LOD	80	60	60	90	80	70
	8	<LOD	80	60	40	80	70	60
21	9	<LOD	20	20	20	20	30	30
	10	<LOD	30	20	30	20	30	20
LOD =		**3**	**3**	**3**	**3**	**3**	**3**	**3**

Figure 2. Average concentration of ^{14}C-ethion in plasma, egg white and yolk (from Mosha 1990a).



Legend: black circle = plasma; asterisk = egg white; triangle = yolk.

Goats.

Toxicokinetic parameters and cumulative excretion were studied in goats after intravenous, oral and dermal administration of unlabelled and ^{14}C-ethion (Mosha, 1990b). All goats weighed between 41 and 56 kg and were fed hay and concentrate and given water ad libitum. In the intravenous experiment, six female adult goats (four lactating) were administered 2 mg/kg ^{14}C-ethion by IV infusion. The oral ethion experiment used five lactating goats (dose of 10 mg/kg, three were dosed with ^{14}C-ethion while two received unlabelled ethion). The dermal experiment used four goats (3 lactating) and administered unlabelled ethion (17 % solution) on the skin at the back at 100 mg/kg. The areas of application were clipped before the diluted emulsifiable concentrate was administered. The application site was about 600-700 cm^2.

Blood samples were obtained just before and at 2 (intravenously only), 5, 10, 15, 20, 30, 45, 60 and 90 min. and at 2, 3, 4, 6, 8, 10, 12, 15, 24, 30 and 48 h, and then once per day until day 14 after exposure. All plasma samples were stored at – 20 °C until analysed.

The udder was emptied of milk just before administration, and at 1, 4, 8, 12, 15, 24, 30 and 48 h and thereafter once daily until day 14 post exposure. All milk samples were stored at -20 °C until analysed.

Urine was collected only in those experiments where the radiolabelled drug was administered, as pilot experiments showed that urine did not contain unchanged ethion. The urine was collected quantitatively during the first 48 h after dosing by means of a balloon catheter. After 48 h, all urine was collected from a metabolic cage in which the animal was placed for the 14 days of the experiment. All urine samples were stored at -20 °C before analysis.

In experiments with ^{14}C-ethion, faeces were collected quantitatively throughout the 14 days and after drying ground into a fine powder.

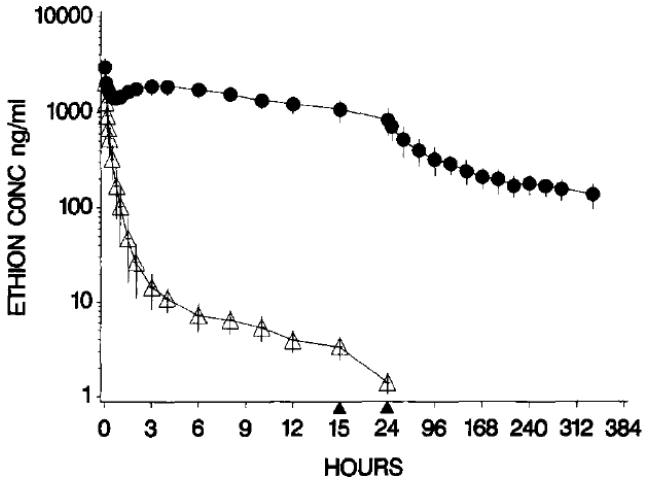
The quantification of unchanged ethion in plasma and milk was done by gas chromatography. Ethion was extracted from the plasma by an equal volume of hexane containing parathion as an internal

standard. Some hexane extracts were concentrated so that ethion in low levels could be quantified. The hexane extracts were analysed on a gas chromatograph with a nitrogen phosphorus detector (NPD). Recovery rate for ethion in plasma was 94 ± 5 %. The recovery rate from milk was 101 ± 4 %. Detection limit for ethion was 3 µg/kg.

The concentration of ^{14}C-ethion (ethion and metabolites) in plasma, milk, urine and faeces was determined by liquid scintillation counting. Tissue and faecal samples for scintillation counting were processed in triplicate.

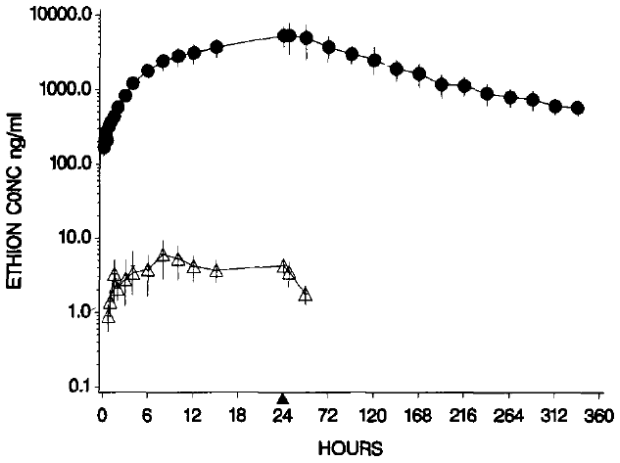
The results IV injection studies (Figure 3) showed an effective half-life ($t_{1/2}$) for unchanged ethion of 2 h, a total body clearance (CL_t) of 3.2 L/kg/h and a volume of distribution (Vd_{ss}) of 9.4 L/kg. Plasma levels of ^{14}C-ethion (ethion + metabolites) were much higher than those of unchanged ethion, and persisted at high levels for more than 14 days. The AUC for ^{14}C-ethion after IV administration was 111 ± 29 µg.h/ml. Cumulative excretion of ^{14}C-ethion was 78 % of the dose with 66 % in urine, 8 % in faeces and 4 % in milk. The much higher and more persistent plasma concentrations of ^{14}C-ethion compared to unchanged ethion (AUC 150 times higher) indicate that elimination of unchanged ethion is mainly due to metabolism. The increase in radioactivity in plasma after an initial decline in the first hour may be attributed to reabsorption from the gastrointestinal tract during enterohepatic circulation.

Figure 3. Mean plasma concentrations vs time for unchanged and ^{14}C-ethion after 2 mg/kg IV administration in goats (from Mosha 1990b).



Legend: Solid circles = total ^{14}C-ethion equivalents, open triangles = unchanged ethion

Figure 4. Mean plasma concentrations vs time for unchanged and ^{14}C-ethion after 10 mg/kg oral administration in goats (from Mosha 1990b).

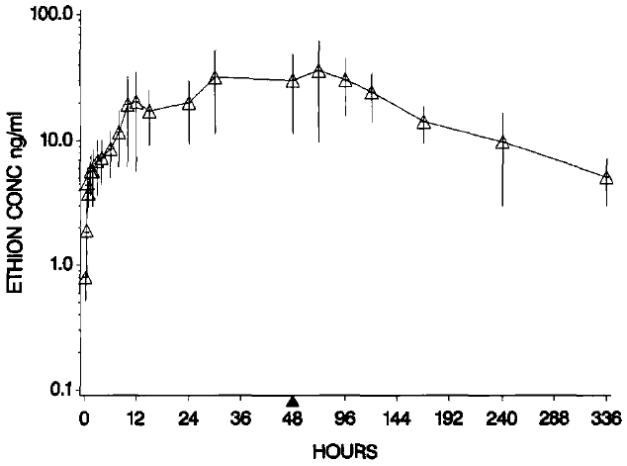


Legend: Solid circles = total ^{14}C-ethion equivalents, open triangles = unchanged ethion

After oral administration (Figure 4), the unchanged ethion levels in plasma were very low and hardly detectable after 48 h. The ^{14}C-ethion concentrations in plasma were more than 100 times higher than unchanged ethion, and persisted in plasma beyond the 14 days sampling period. Less than 5 % of the orally-administered ethion was absorbed unchanged. However, the AUC for ^{14}C-ethion was 5000 times the AUC for unchanged ethion, and proportional to the ^{14}C-ethion AUC obtained after intravenous administration. It is therefore likely that most ^{14}C from orally-administered ^{14}C-ethion is absorbed in goats (though not remaining as parent ethion). The plasma radioactivity had peak values after approximately 30 h. This prolonged absorption of ^{14}C-ethion is probably due to delayed absorption from the rumen, as oral administration of ^{14}C-ethion to rats resulted in a t_{max} of 6 h (JMPR, 1986). Cumulative excretion was 80 % of the dose; with 64 % in urine, 14 % in faeces and 1.7 % in milk.

After dermal administration (Figure 5), unchanged ethion concentrations were higher than after oral application. Dermally-applied ethion concentrations persisted beyond the 14 days sampling period, indicating a prolonged absorption phase. This prolonged absorption period corresponds with the assumption that ethion stays in the epidermis of the skin for a very long time. Approximately 20 % of the dose was absorbed during the 14 days observation period. Only 0.05 % of the dose was excreted unchanged in milk.

Figure 5. The concentration-time profile for unchanged ethion in goat plasma after dermal application of 100 mg/kg (n = 4) (from Mosha 1990b).



The combined recovery of ^{14}C-ethion in urine and milk amounted to 70 and 66 % of the dose after intravenous and oral administration, respectively, indicating an almost complete absorption of ^{14}C-ethion after oral administration. The proportions of the dose excreted in milk were quite small, especially after oral and dermal application. However, the long absorption period after dermal application is responsible for ethion residues found in milk up to five weeks after application. The remaining approximately 20 % ^{14}C-ethion not accounted for probably remains in the tissues where the ^{14}C might have entered the carbon pool to be incorporated in tissue components. The very long terminal elimination phase for ^{14}C-ethion supports this assumption. Collection of exhaled air from three of the goats administered ^{14}C-ethion IV showed that only insignificant amounts of ^{14}C are excreted by this route.

The Committee noted that the results from this study in goats are highly relevant to the current assessment in cattle. This study demonstrates that ethion undergoes extensive metabolism in goats, and that the majority of the ethion-derived residues in cattle are therefore predicted to be metabolites rather than parent ethion.

Ethion residues in goat milk were evaluated in Mosha, 1991 using the same dosage regimen as Mosha, 1990b. Intravenous ethion injection resulted in ^{14}C residues persisting much longer than unchanged ethion in milk. After oral administration, ethion was almost completely metabolised before absorption, leading to very low levels of parent ethion but high levels of ^{14}C in milk. Dermal application of ethion resulted in limited but very prolonged absorption, and detectable residue of ethion in milk for up to 5 weeks.

The Committee noted that this study confirmed the persistence of ethion metabolites in milk (relative to parent ethion). This study confirms that ethion metabolites persist much longer than the parent compound, and that these metabolites were transferred into milk.

Metabolism in Laboratory Animals

Rats

Previously available data (ATSDR, 2000) were reviewed. No new data were available to the Committee.

Metabolism in Food Producing Animals

No data was provided to the Committee, or available from the published literature, to allow characterisation of the metabolic pathway for ethion in cattle.

Comparative Metabolism

Since the data are not available for cattle, no comparison with other species can be made at this time.

Tissue residue depletion studies

Radiolabelled residue depletion studies

No radiolabelled residues depletion studies were available for review.

Residue depletion studies with non-radiolabelled drug

Cattle

Bath (immersion) treatments

A non-GLP/GCP study was conducted to determine the withdrawal period of an immersion bath product containing 40 % ethion and 10 % cypermethrin in cattle (Bringas *et.al*.,). The product was administered as per product label (1 L product diluted in 1000 L water to give a 400 ppm solution of ethion).

The animals used (n = 26) had had no contact with ethion prior to commencement of the study. The animals were split into groups based on gender and weight, to ensure each group had an equal number of males and females and that each group had a similar mean weight. After treatment (a single immersion bath), animals were slaughtered at 15, 29, 43, 57, 69 and 92 days post treatment.

At slaughter, samples of liver, kidney lumbar muscle and perirenal fat were taken for analysis. Samples were processed using a QuEChERs clean-up procedure, and analysed using LC-MS/MS.

Table 2. Ethion concentrations in cattle tissues following single immersion bath treatment (Bringas *et.al*.,).

Withdrawal time (days)	Amount of ethion found (µg/kg)			
	Muscle	**Kidney**	**Liver**	**Fat**
15 (control – no treatment)	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	39
15 (treated)	4	<LLOQ	2.8	>ULOQ
	8	<LOD	2.5	>ULOQ
	12	<LLOQ	3.8	>ULOQ
	4	<LOD	2.9	>ULOQ
29	<LOD	5	<LOD	>ULOQ
	<LLOQ	8	<LOD	>ULOQ
	<LOD	9	<LOD	>ULOQ
	<LOD	5	<LOD	>ULOQ
43	<LOD	<LOD	<LOD	12
	<LOD	<LOD	<LOD	82
	<LOD	<LOD	<LOD	12
	<LOD	<LOD	<LOD	28
57	<LOD	<LOD	<LOD	37
	<LOD	<LOD	<LOD	4.5
	<LOD	<LOD	<LLOQ	7.6
	<LLOQ	<LLOQ	<LLOQ	20
69	<LOD	<LOD	<LOD	46
	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	8
	<LOD	<LOD	<LOD	<LLOQ
92	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD
	<LOD	<LOD	<LOD	<LOD
LOD =	**1.0**	**2.0**	**2.0**	**2.0**
LLOQ =	**2.0**	**4.0**	**4.0**	**5.0**
ULOQ =	**100**	**100**	**100**	**100**
Validation range = 5 – 100 µg/kg (Determined using a 6-point calibration curve, in each matrix).				

The Committee noted that ethion residues were most persistent in the fat samples. However, since the results outside the calibration range of the method are unquantifiable, no meaningful depletion curve can be produced. Ethion metabolites were not measured in this study.

Results from another non-GLP/GCP ethion immersion bath study were provided (Anon (2)). Cattle were exposed to ethion via bath treatment containing 40 % ethion and 10 % cypermethrin. The immersion bath was prepared according to label directions (mixing 1 L of ethion/cypermethrin concentrated solution in 1000 L of water), resulting in ethion concentrations of 400 ppm (mg/L). Cattle were treated twice, 9 days apart. The actual ethion dose for each animal could not be determined due to the nature of the dosing regimen (immersion bath). Four groups of 4 animals (1 male and 3 female) each were slaughtered at 5, 10, 15 and 20 days after the final treatment. Samples of 300 g of muscle (loin), kidney, liver and subcutaneous and per-renal fat were taken from each animal. The analytical method used (multi-residue for OPs) was GC-FPD (flame photometric detector) and was applied to all tissues (fat, liver, muscle and kidney).

Table 3. Ethion concentrations in cattle tissues following 2nd immersion bath treatment (Anon-2).

Slaughter time post 2nd dose (d)	Muscle (µg/kg)	kidney (µg/kg)	Liver (µg/kg)	Perirenal Fat (µg/kg)	Subcutaneous Fat (µg/kg)
5	ND	ND	ND	1107	742
	ND	ND	ND	1072	1090
	ND	ND	ND	979	1039
	<LOQ	ND	ND	782	1125
10	ND	ND	ND	710	697
	<LOQ	<LOQ	ND	306	585
	ND	187	ND	607	489
	ND	<LOQ	ND	444	732
15	ND	ND	ND	695	793
	ND	ND	ND	540	341
	ND	ND	ND	24	No sample
	ND	ND	ND	116	557
20	ND	ND	ND	106	192
	ND	ND	ND	178	186
	ND	ND	ND	111	171
	ND	ND	ND	63	227
LOD* =	**6.5**	**6.5**	**6.5**	**6.5**	**6.5**
LOQ* =	**19.5**	**19.5**	**19.5**	**19.5**	**19.5**

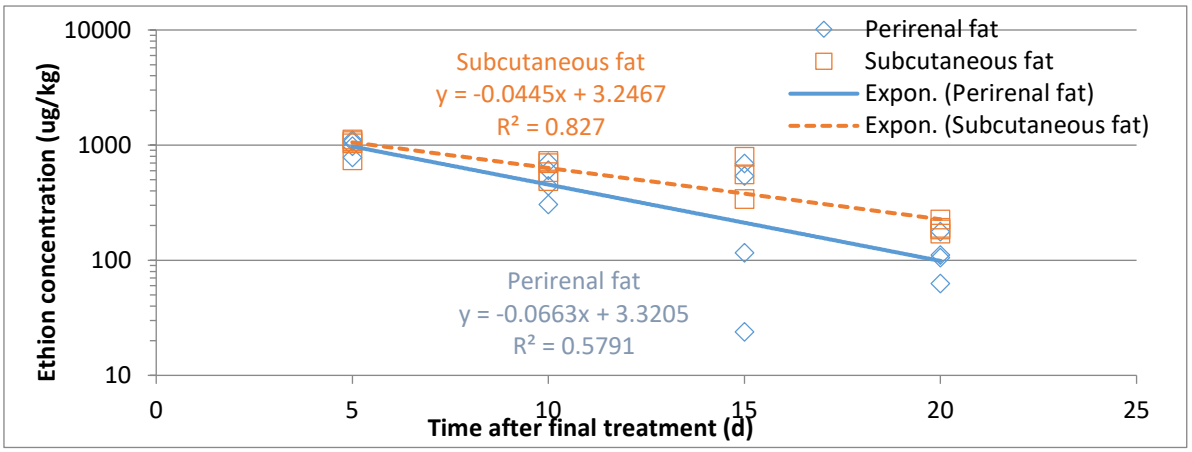
ND = not detectable (results < LOD).

* See discussion below

The Committee noted that in the study report, the LOD and LOQ for the analytical method used were stated as being 6.5 µg/kg and 19.5 µg/kg, respectively, for all tissue matrices (kidney, liver, fat, muscle). However, in the accompanying method validation study, only data for liver and fat matrices were included, and the LOD was reported to be 3.4 µg/kg for liver and 8.0 µg/kg for fat, and the LOQs were reported as 10.1 µg/kg for liver and 23.9 µg/kg for fat.

Figure 6 shows the depletion profile in the two types of fat over the duration of the study (20 days post final treatment). Residues were more persistent in subcutaneous fat than in peri-renal fat over the time period studied. As the final sampling group was slaughtered at 20 days, and ethion residues persist for significantly longer duration in fat, the applicability of this data for establishing MRLs is minimal. The data from the other tissues were unable to be analysed statistically, since the results were mostly below the LOD of the analytical method used.

Figure 6. Ethion residue depletion in fat samples following 2nd immersion bath treatment (Anon-2)



Data from a third non-GLP/GCP ethion bath immersion study in cattle were provided (Gérez García *et.al.*, 2017). The products used were immersion bath treatments containing 40 mg/L ethion and 10 mg/L cypermethrin in bath final formulation. Cattle were treated 3 times, with 21 days in between each treatment.

Study animals weighed between 364 and 516 kg and were evenly split between males and females. Animals in the first treatment group were slaughtered at the following timepoints after the final treatment: 13, 34, 70, 90, 105 and 117 days. The animals in a 2nd treatment group were slaughtered 117 days after their final treatment.

After slaughter, samples were taken of muscle (two types, loin and semi-membranous), fat (initially only perirenal fat, but subcutaneous fat was also taken from day 70 onwards), kidney and liver. Each sample weighed around 250 g.

Ethion concentrations in tissues were analysed in a first laboratory (Lab A) using GC-ECD, GC-MS and LC-MS/MS. The reported LOD was 1 µg/kg for all tissues and the LOQs were reported as 5 µg/kg for fat (HPLC) and muscle, 10 µg/kg for fat (GC-MS), liver and kidney. All samples were analysed in triplicate and quantified utilising calibration curves in matrix. A blank was performed for each matrix, as well as at least two residue recoveries for the concurrent validation, which functioned as quality control for each batch. The values above were adjusted by concurrent recovery and lipid content (in the fat samples).

Table 4. Ethion concentrations in cattle tissues following 3rd immersion bath treatment (Lab A results, Gérez García *et.al.*, 2017)

Slaughter time post 3rd dose (days)	Sex (m/f)	Residues found (µg/kg)				
		Fat		Muscle		Kidney
		Perirenal	Sub-cutaneous	Semi-membranous	Loin	
13	M	1431	NS	NS	32	<10
	F	810	NS	NS	<5	13
	F	1600	NS	NS	23	11
	M	728	NS	NS	<5	<10
34	F	184	70	NS	55	8
	F	797	460	NS	62	6
	M	337	217	NS	47	20
	M	563	392	NS	43	11

70	F	27	32	<LOD	<LOD	7
	F	77	623	<LOD	16	8
	M	272	220	<LOQ	<LOQ	14
	M	29	20	<LOD	<LOQ	8
90	M	13	20	<LOD	<LOD	12
	M	<LOQ	54	<LOQ	<LOQ	<LOQ
	F	<LOQ	72	<LOD	<LOD	<LOQ
	F	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
105	F	<LOQ	<LOQ	<LOD	<LOD	<LOD
	F	<LOQ	<LOQ	<LOD	<LOD	<LOD
	M	<LOQ	<LOQ	<LOD	<LOD	<LOD
	M	12	41	<LOQ	<LOQ	<LOD
	LOD	**1**	**1**	**1**	**1**	**1**
	LOQ	**5**	**5**	**5**	**5**	**10**

Notes: All liver results from all timepoints were <LOD (1 µg/kg).
Results from all samples from timepoint 117 days (groups 1 & 2) were <LOD in all tissues (1 µg/kg).
NS = no sample analysed.

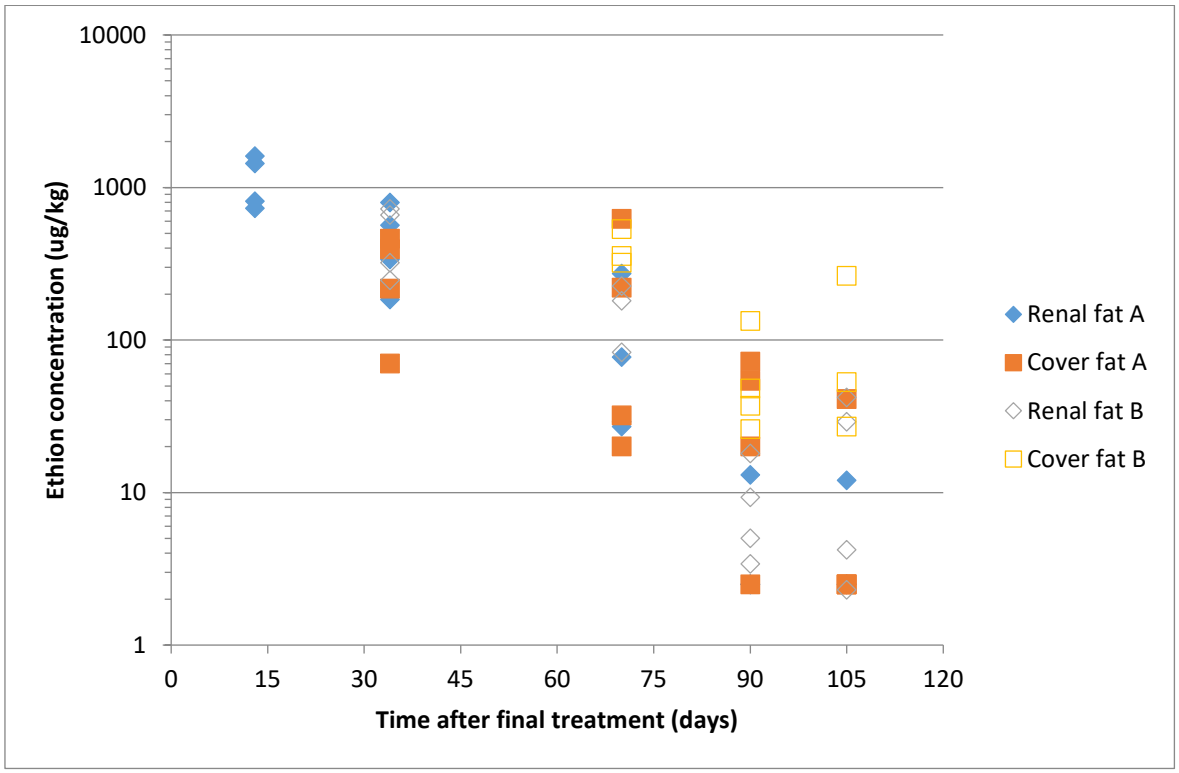
In addition, select tissue samples were re-analysed at a second laboratory (Lab B), but only muscle and fat samples from slaughter day 34 onwards. Samples were analysed by LC-MS/MS at the second laboratory, with an LOQ of 1 µg/kg and an LOD of 0.5 µg/kg.

Table 5. Ethion concentrations in select cattle tissues following 3rd immersion bath treatment (Lab B results, Gérez García *et.al*., 2017)

Slaughter time post 3rd dose (days)	**Sex (m/f)**	**Residues found (µg/kg)**					
		Fat		**muscle**			
		Perirenal	**Sub-cutaneous**	**Semi-membranous**		**Loin**	
				Lean	**15 % fat**	**Lean**	**15 % fat**
34	F	320	NS	NS	NS	1.1	NS
	F	246	NS	NS	NS	2.0	NS
	M	658	NS	NS	NS	3.6	NS
	M	722	NS	NS	NS	2.5	NS
70	F	83	321	1	NS	1.2	21
	F	180	532	<LOQ	9	1.1	33
	M	225	355	1.1	13	1.3	15
	M	NS	NS	<LOQ	NS	10	NS
90	M	18	37	<LOD	<LOQ	<LOD	1.5
	M	9.3	48	<LOD	2.0	<LOD	1.9
	F	5.0	133	<LOD	<LOQ	<LOD	<LOQ
	F	3.4	26	<LOD	<LOD	<LOD	<LOD
105	F	2.3	27	<LOD	<LOD	<LOD	<LOD
	F	4.2	53	<LOD	<LOD	<LOD	1.2
	M	29	NS	<LOD	NS	<LOD	NS
	M	42	263	<LOD	4.8	<LOD	1.7
	LOD	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
	LOQ	**1**	**1**	**1**	**1**	**1**	**1**

Notes: Results from all samples from timepoint 117 days (groups 1 & 2) were <LOD in all tissues (0.5 µg/kg), except one SC fat sample from Group 2 (reported as 3 µg/kg). NS = no sample analysed.

Figure 7. Ethion residue depletion in fat samples following 3rd immersion bath treatment (Gérez García *et.al.*, 2017)



The Committee noted that the residue concentrations determined in the same tissue sample by the two different laboratories were often highly discrepant. This may be due to differences in analytical methodology (though both assays were purportedly validated), or due to non-homogenous ethion concentrations in different regions of the same tissue sample. However, it is noted that the results were consistent in terms of order of magnitude. It is clear that ethion residues are more persistent in fat than any other tissue sampled, particularly demonstrated by the comparison between lean muscle samples and those containing 15 % fat.

When comparing the results from all three submitted ethion immersion bath studies, the Committee highlighted the following:

- All treatments consisted of almost total immersion of the cattle in the insecticide solution;
- All products used were of the same quantitative composition with respect to the active substances (40 % ethion and 10 % cypermethrin, diluted in water to give 400 ppm solutions of ethion at point of administration);
- Each of these studies used a different treatment protocol. In one study, the animals were treated once, in another study, they were treated twice, with a 9-day interval between

treatments, and in the third study, the animals were treated three times, with 21-day intervals between treatments.

- In none of the studies was it possible to determine the exact dose received by each animal.
- In all of the studies, ethion parent molecule was the marker residue (i.e. no metabolites were investigated).
- All studies demonstrated that ethion is most persistent in fat of cattle, and that it persists in cover (subcutaneous) fat for longer than in peri-renal fat.
- Overall, the studies provided were well-conducted and well-reported. All the studies reviewed were designed to calculate a suitable withdrawal period for the products investigated, and were not primarily designed for the derivation of MRLs.

Ear-tag treatments

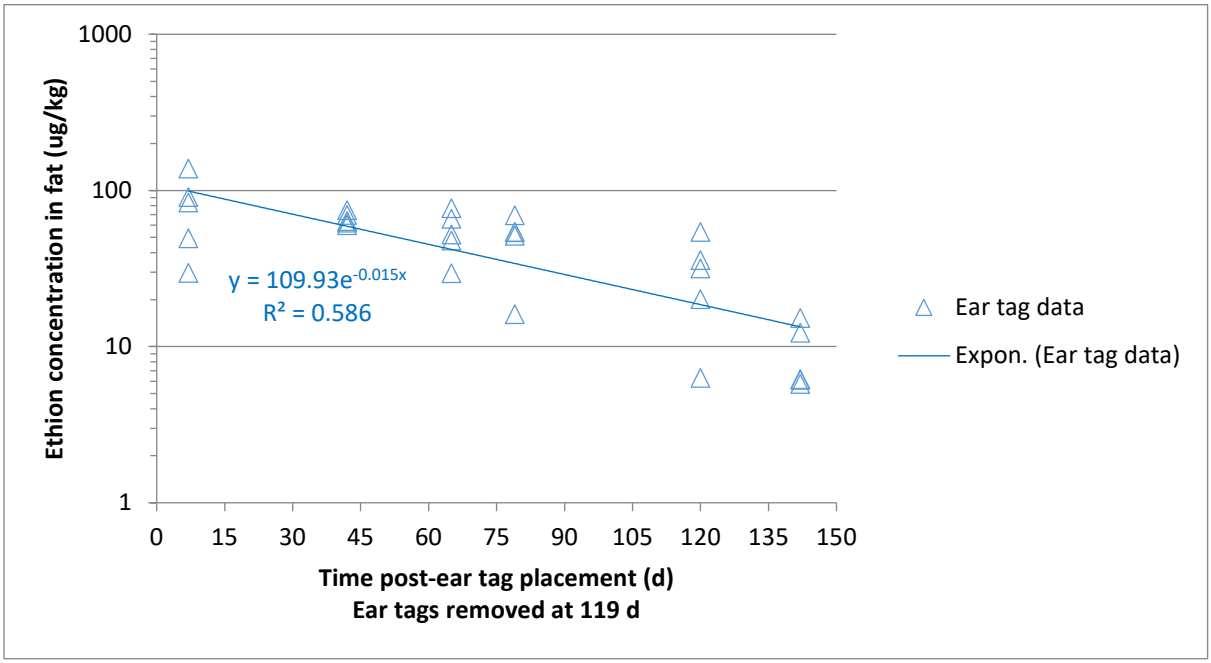
A non-GLP (claimed to be GCP) study in 30 cattle was performed with application of one ear tag (containing 40 g ethion per 100 g ear tag) per animal (Prazeres Gonçalves *et.al*., 2012). The ear tags remained in place for 119 days. Each group was slaughtered on the following days after application: D +7, D +42, D +65, D +79 (during treatment), D +120 and D +142 (1 and 23 days, respectively, after removal of the ear tags on D +119). Samples of fat, muscle, kidney and liver were taken from each animal for analysis (anatomic location of muscle and fat samples not provided). The samples were processed and stored below -20 °C until analysis via UPLC-MS/MS. Concurrent QC sample data were provided, with the majority of results within -15 to +15 %

No raw data was provided to the Committee.

Table 6. Ethion concentrations in cattle tissues following ear-tag administration (Prazeres Gonçalves *et.al*., 2012).

Time (days post ear tag application)	Mean residues (n = 2) (µg/kg)			
	Fat	**Muscle**	**Kidney**	**Liver**
7 Tag in ear	49.45	<LOQ	<LOQ	<LOD
	137.92	<LOQ	<LOQ	<LOD
	29.67	<LOD	<LOQ	<LOD
	83.73	<LOQ	<LOQ	<LOD
	90.89	<LOQ	<LOQ	<LOD
42 Tag in ear	74.78	<LOQ	<LOQ	<LOD
	62.23	<LOD	<LOQ	<LOD
	59.98	<LOQ	<LOQ	<LOD
	63.77	<LOD	<LOQ	<LOD
	69.78	<LOD	<LOQ	<LOD
65 Tag in ear	29.59	<LOD	<LOQ	<LOD
	47.72	<LOD	<LOQ	<LOD
	65.97	<LOD	<LOQ	<LOD
	76.86	<LOD	<LOQ	<LOD
	52.27	<LOD	<LOQ	<LOD
79 Tag in ear	54.22	<LOD	<LOQ	<LOQ
	69.36	<LOQ	<LOQ	<LOD
	53.95	<LOQ	8.2	<LOQ
	16.12	<LOQ	<LOQ	<LOD
	51.29	6.04	<LOQ	<LOD
119 (+1 d after tag removed)	54.33	14.46	<LOQ	<LOD
	31.70	<LOQ	<LOD	<LOD
	20.16	<LOQ	<LOD	<LOD
	35.85	5.53	<LOD	<LOD
	6.32	<LOD	<LOD	<LOD
142 (+23 d after tag removed)	12.28	<LOD	<LOD	<LOD
	5.77	<LOD	<LOQ	<LOD
	6.2	<LOD	<LOD	<LOD
	15.24	<LOD	<LOD	<LOD
	6.12	<LOD	<LOQ	<LOD
LOD =	**0.82**	**0.82**	**0.74**	**1.01**
LOQ =	**5.0**	**5.0**	**5.0**	**5.0**

Figure 8. Ethion residue depletion in fat samples following ethion ear tag placement (Prazeres Gonçalves *et.al*., 2012)



The Committee recognised that as with the immersion bath treatments, the residues of ethion persisted longest in fat after ear-tag treatment. Due to the prolonged nature of ethion exposure after ear-tag treatment, the residues persisted longer than those from immersion baths. It should be noted that the depletion of residues was investigated after completion of treatment for the immersion baths, whereas depletion was monitored throughout treatment (and for up to 23 days post-removal) for the ear-tags.

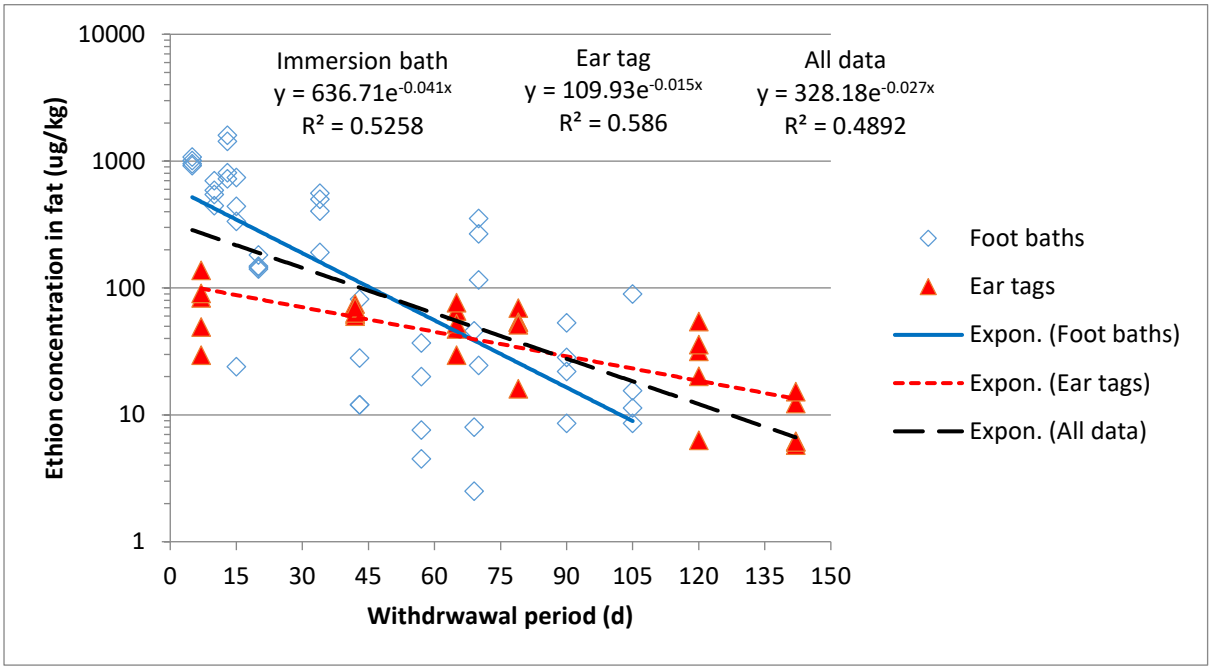
Overall comment on residues depletion studies in cattle tissues:

A brief summary comparison of the various ethion residue depletion studies submitted for evaluation by the Committee are presented in Table 7. Regarding the possibility of combining the data from the various residues depletion studies submitted to the Committee, it was deemed to be inappropriate due to the variety of ethion formulations, dose regimens, sample times, and analytical methodologies used in the different studies. Some rudimentary combination was attempted, however, in order to characterise the differences between treatments (Figure 9). Average results for perirenal/subcutaneous fat, or average of multiple lab results for the same fat sample, are reported.

Table 7. Summary of available ethion residue depletion studies submitted for evaluation by the Committee.

No. of cattle	Ethion dose	Tissues collected	Sampling Times	Assay used	LOD & LOQ (µg/kg)	Issues	Reference
26	400 ppm bath (single administration)	liver, kidney lumbar muscle and perirenal fat	15 – 92 d post dose	LC-MS/MS	1-2, 5	ULOQ only 100 µg/kg – early fat samples above this range. Cannot use to model residue depletion in fat.	Bringas *et.al.*,
16	400 ppm bath (2x, 9 d apart)	muscle, kidney, liver and subcutaneous and per-renal fat	5 – 20 d post final dose	GC-FPD	6.5, 19.5	1. LOD/LOQ are different in validation report 2. Sampling duration too short to get true residue depletion curve in fat	Anon(2)
34	400 ppm bath (3x, 21 d apart)	Muscle (2x), kidney, liver, fat (2x)	13 – 105 d post final dose	GC-ECD, GC-MS and LC-MS/MS	1, 5 – 10 (1st lab) 0.5, 1 (2nd lab)	1. Products used were not specified 2. Samples analysed at 2 labs with very different results 3. Analytical method validation data for LC-MS/MS were not provided	Gérez García *et.al.*, 2017
30	40g ethion/100g ear tag	Muscle, kidney, liver, fat, milk	7 – 142 d after ear tag applied. (last 2 samples were 1& 23 d post removal)	UHPLC-MS/MS	0.74 – 1, 5	1. Study not GLP (claims GCP)	Prazeres Gonçalves *et.al.*, 2012

Figure 9. Combined ethion residue depletion results in fat of cattle following multiple routes of ethion administration.



Statistical evaluation of ethion residue depletion data

The Committee also performed a 95/95 upper tolerance limit (95/95 UTL; upper limit of the one-sided 95 % confidence interval over the 95th percentile of residue concentrations) for all individual ethion residue depletion data sets, in order to assess the usability of such data as part of a future MRL assessment. Results from the most robust data sets (Gérez García *et.al.*, 2017, Prazeres Gonçalves *et.al.*, 2012) are shown in Figures 10 and 11. For the 3x immersion bath 95/95 UTL data in Figure 10, the 95/95 UTLs were derived for both the combined ethion fat residue data (average concentration of both anatomic fat samples from both labs), as well as the most conservative individual data (cover fat concentrations from Lab B).

Figure 10. 95/95 Upper Tolerance Limits of ethion residues in fat after 3x immersion bath (Gérez García *et.al.*, 2017).

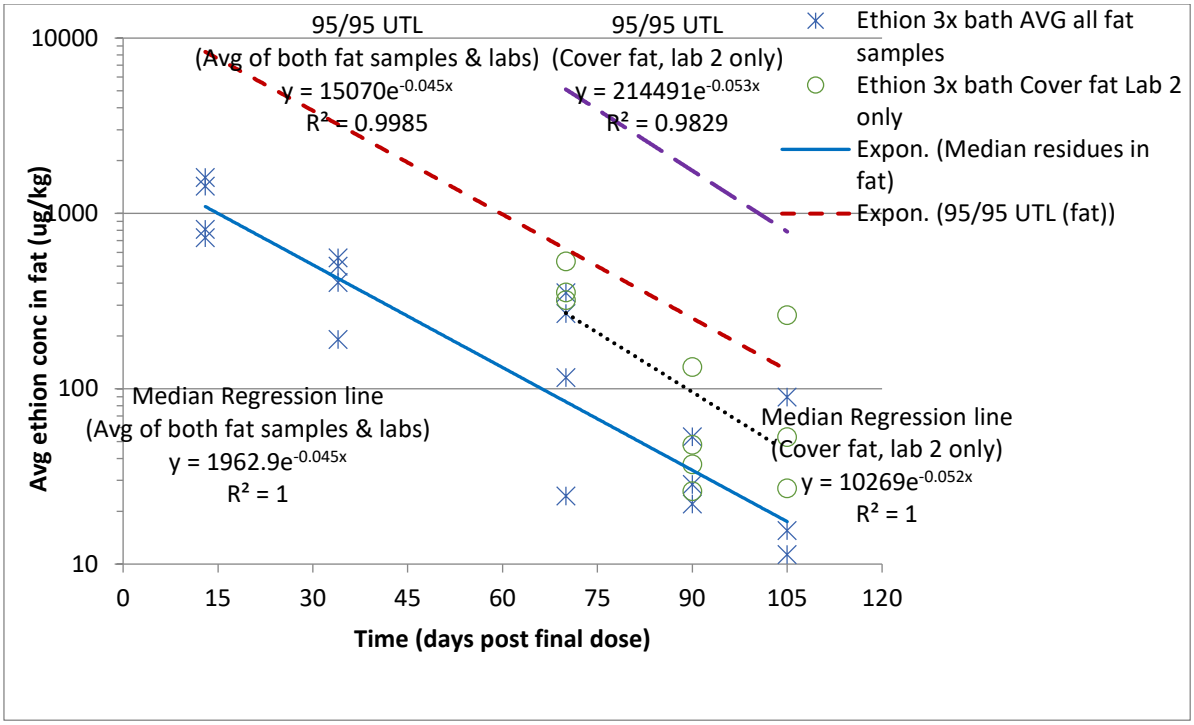
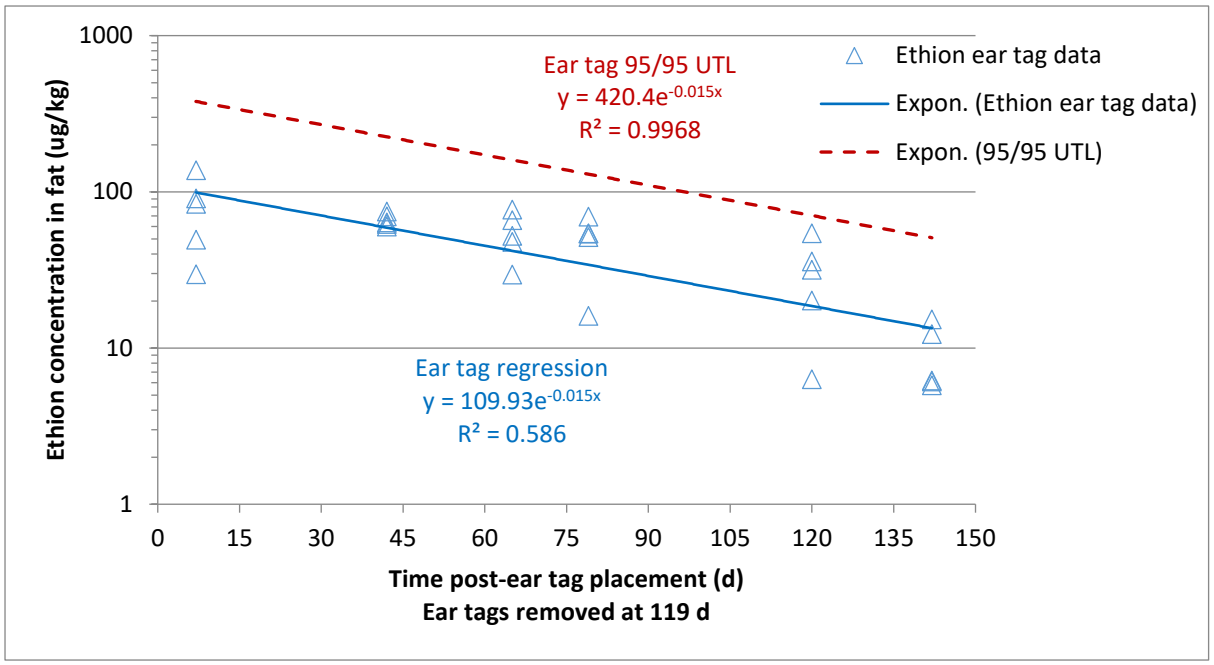


Figure 11. 95/95 Upper Tolerance Limits of ethion residues in fat after ear tag administration (Prazeres Gonçalves *et.al.*, 2012)



Methods of analysis for residues in tissues

Analytical determination of Ethion by GC-FPD, (Bianchini *et.al*., 2013).

Conducted in accordance with Technique T-CRG-003 Rev.: 07 Analytical determination of organophosphorus pesticides in animal fat and vegetable oil by GC-FPD.

This is a multi-residue analytical technique designed for the detection of many organophosphate insecticides in a single sample of tissue. The tissue is shaken, or the fat is dissolved, in a mixture of hexane-methylene chloride. The analytes are then separated using a gel permeation column where the collected fraction is evaporated to dryness and taken up in a known volume of isooctane. Then it is injected into a gaseous gas chromatograph with flame photometric detector (FPD).

Sample preparation (fat):

Approximately 30 g fat is melted in a microwave for 5 minutes. The molten fat is transferred to a haemolysis tube and stored in a refrigerator until analysis.

Sample preparation (other tissues):

If the matrix is muscle, liver or kidney, the connective tissue and fat is removed as much as possible. The tissue is then cut into small pieces and chopped finely so as to obtain a homogeneous mixture. The samples are then transferred to small plastic bottles (approximately 30 g) and stored in a freezer until analysis.

Extraction:

Two grams of sample is added to 8 ml of hexane:methylene chloride (65:35) solvent and this is vortexed. The eluate is then concentrated by heating and evaporating off the solvent. This concentrate is then added to 1 ml isooctane and injected into the GC-FPD.

Quantification:

The quantification is performed by direct comparison with the injection of a solution containing the standard analytes of interest.

In the case that an unknown sample is positive, a peak with retention time corresponding to the peak of the analyte in the standard (+/- 1 % in seconds) is obtained.

Validation of the method

The method was validated only for the matrices liver and fat, but is applied to all tissues (fat, liver, muscle and kidney).

Table 8: Validation data for GC-FPD, (Bianchini *et.al*., 2013)

Tissue:	**Fat**	**Liver**

Intraday accuracy (recovery %)	23.88 µg/kg: 100 47.76 µg/kg: 92 95.52 µg/kg: 102 143.28 µg/kg: 99 191.04 µg/kg: 105	10.11 µg/kg: 89 20.22 µg/kg: 97
Intraday precision (% CV)	23.88 µg/kg: 9.5 47.76 µg/kg: 9.7 95.52 µg/kg: 9.2 143.28 µg/kg: 7.4 191.04 µg/kg: 5.7	10.11 µg/kg: 8.4 20.22 µg/kg: 9.5
Interday precision	23.88 µg/kg: 7.6 47.76 µg/kg: 7.0 95.52 µg/kg: 6.0 143.28 µg/kg: 8.2 191.04 µg/kg: 3.9	10.11 µg/kg: 8.0 20.22 µg/kg: 6.1
LOQ / LOD (µg/kg)	23.9 / 8.0	10.1 / 3.4
Analytical range	8 – 181 µg/kg	3.4 - 191 µg/kg
Linearity (r^2)	0.9993*	
Specificity/selectivity	No interference	
Ruggedness testing	Not affected by the type of matrix used	
Stability: **Freeze-thaw** **Room temperature** **Extract** **Stock solution**	Not studied	

* it has not been specified from which tissue the linearity r^2 value is from.

Report: Determination of depletion curves of ectoparasiticides in cattle. (Gérez García *et.al*.,).

Description of the method

Fat

For both perirenal fat and cover (SC) fat, cryogenic grinding with liquid nitrogen was performed. Extract the analyte from the fat using toluene first and then acetonitrile with shaking. Centrifuge the sample, then cool to -40 °C. Centrifuge again and take the supernatant and add PSA, C18 and anhydrous $MgSO_4$. Vortex the mixture and then centrifuge again. Take the supernatant and evaporate to dryness for analysis. For GC analysis, redissolve in AcOEt and add the internal standard (bromophos methyl) and inject this solution into the analyser. For LC analysis, redissolve in acetonitrile and put in the freezer overnight. Filter the solution and inject into the LC apparatus.

Kidney

Add homogenised kidney sample to AcOEt and shake in a vortex. Add $MgSO_4$ and NaCl and shake vigorously. Centrifuge the sample and then cool to -40 °C. Centrifuge again and add d-SPE salts (C18, alumina and MgSO4). Shake vigorously and remove the supernatant. Evaporate to dryness and add this to a solution of the internal standard in AcOEt, ready for the autosampler.

Muscle

Muscle samples were prepared according to Souza *et.al.*, Development of a straightforward and cheap ethyl acetate based method for the simultaneous determination of pesticides and veterinary drugs residues in bovine liver and muscle. Chromatographia (2016) 79:1101–1112, (not supplied).

Liver

No description of the method was given.

Analytical method validation

Validation parameters were evaluated according to the European commission directorate general for health and food safety, 'Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed' (SANTE/11945/2015).

Fat

Accuracy

Table 9. Accuracy data for GC-ECD and GC-MS (fat) (Gérez García *et.al.*,)

Nominal spiked level (µg/kg) in fat	Recovery (%)	RSD (%)
100	81	2
250	85	3
500	101	4
1000	84	3

Table 10. Accuracy data for LC-MS/MS (fat) (Gérez García *et.al.*,)

Nominal spiked level (µg/kg)	Recovery (%)	RSD (%)
5	62	6
10	56	10
100	63	/

Table 11. Precision (fat) (Gérez García *et.al*.,)

Nominal spiked level (µg/kg)	Repeatability (%)	Reproducibility (%)
100	2	5
250	3	4
500	5	7
1000	3	5

Method linearity and matrix effect (fat)

Figure 12. Calibration curve in solvent (GC-ECD) (Gérez García *et.al*.,)

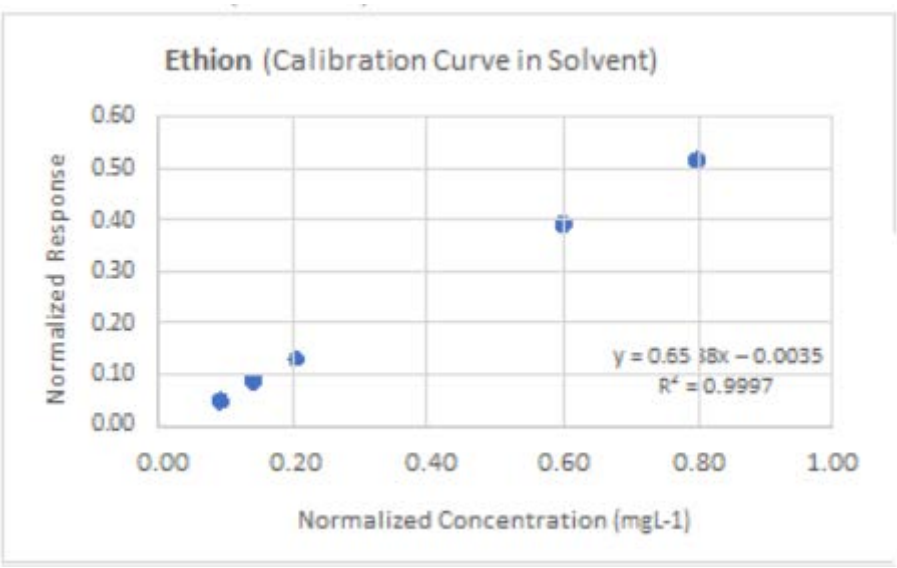


Figure 13. Calibration curve in matrix (fat) (Gérez García *et.al*.,)

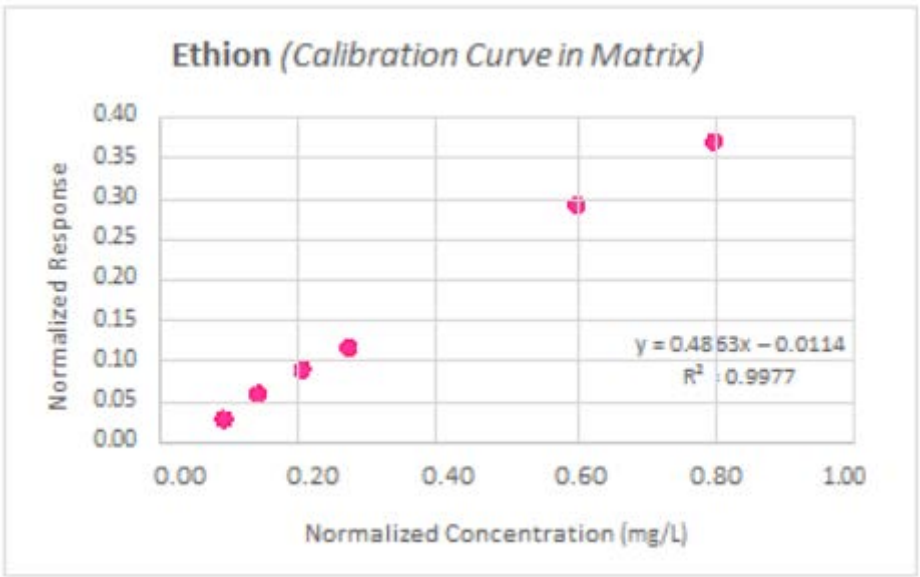
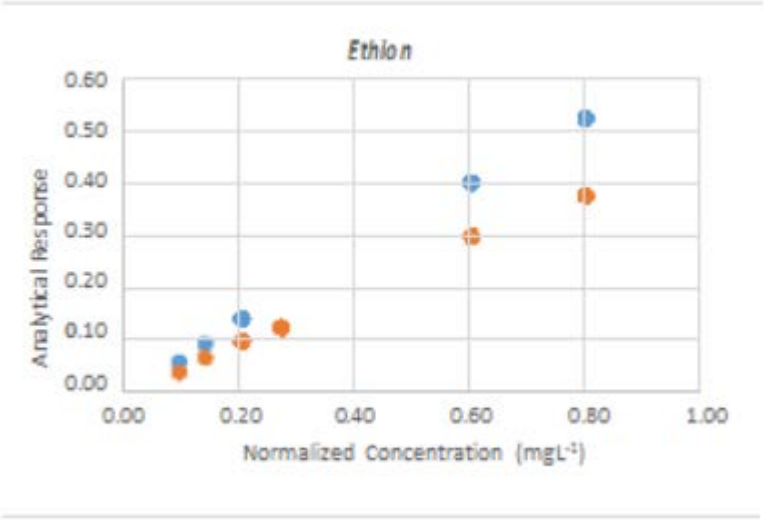


Figure 14. Calibration curves superimposed for visual evaluation (Gérez García *et.al*.,)



A matrix effect in fat of -26 % was obtained using the equation:

$$\text{Matrix effect } (\%) = \frac{\text{slope of the calibration curve in matrix}}{\text{slope of the calibration curve in solvent}} \times 100$$

Kidney

Accuracy

Table 12. Accuracy data for GC-ECD and GC-MS (Gérez García *et.al*.,)

Nominal spiked level (µg/kg)	Recovery (%)	RSD (%)
25	84	5
50	86	4
100	102	8

Table 13. Accuracy data for LC-MS/MS (Gérez García *et.al*.,)

Nominal spiked level (µg/kg)	Recovery (%)	RSD (%)
10	70	6
50	100	5
100	71	11

Table 14. Precision (GC-ECD) (Gérez García *et.al*.,)

Nominal spiked level (µg/kg)	Repeatability (%)	Reproducibility (%)
25	5	7
50	4	9
100	8	12

Table 15. Precision (LC-MS/MS) (Gérez García *et.al*.,)

Nominal spiked level (µg/kg)	Repeatability (%)	Reproducibility (%)
10	5	7
50	4	9
100	8	12

Figure 15. Method linearity (GC-ECD) (Gérez García *et.al*.,)

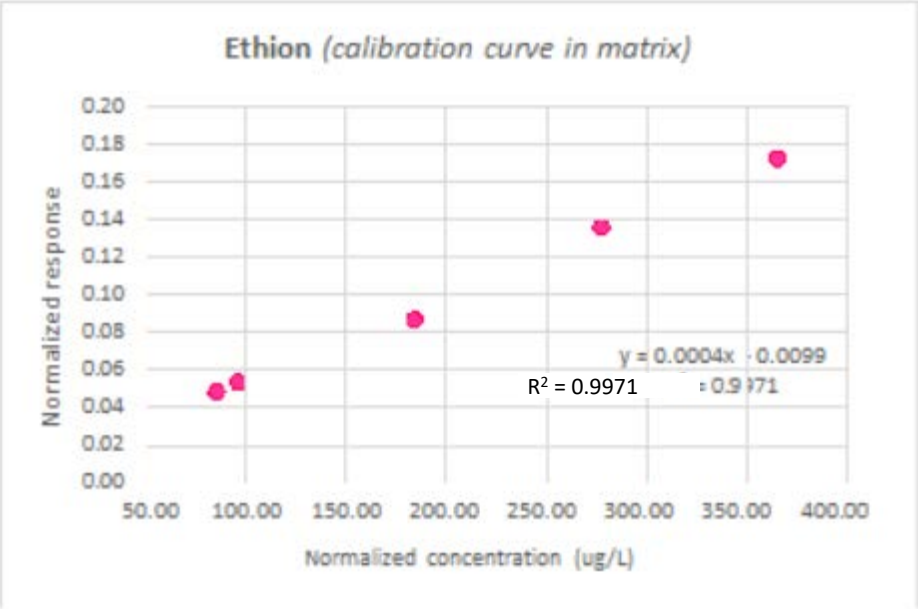
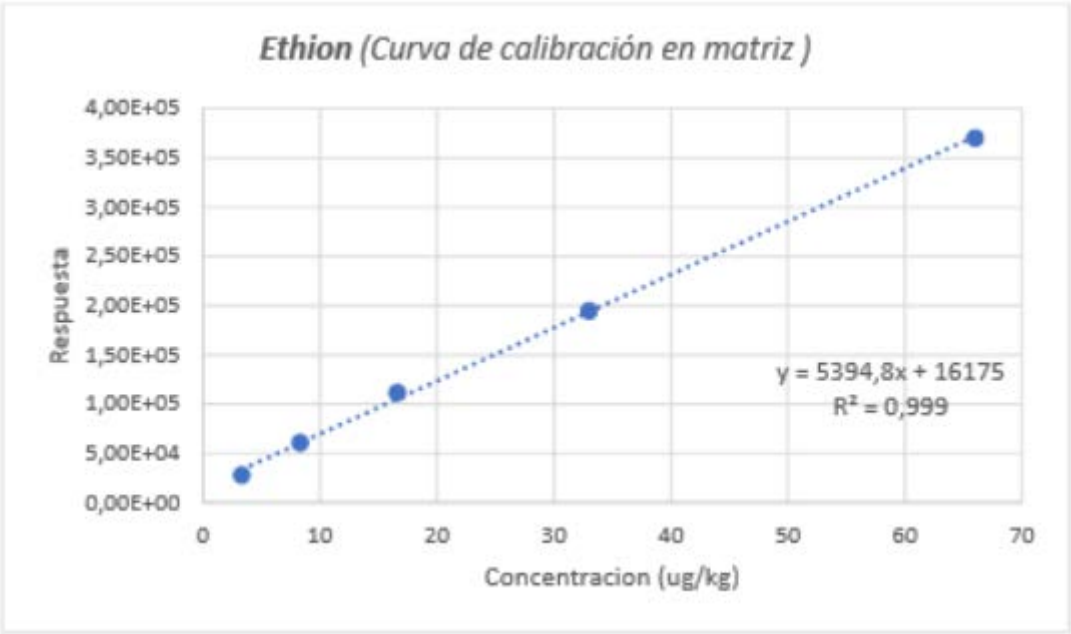


Figure 16. Method linearity (LC-MS/MS) (Gérez García *et.al*.,)



A matrix effect in kidney of -51 % was obtained using the equation:

$$\text{Matrix effect (\%)} = \frac{\text{slope of the calibration curve in matrix}}{\text{slope of the calibration curve in solvent}} \times 100$$

No further validation data were given in this report.

LC-MS/MS

The following data were provided in tables untranslated from the original Spanish. No validation report was provided. The LC-MS/MS method was used in the study reported by Bringas *et.al*., in cattle tissues.

Table 16. Summary of the validation data for the analytical method used in the study to determine residues of ethion in cattle tissues, as reported by Bringas *et.al*.,

Tissue	Muscle	Fat	Liver	Kidney
Intraday accuracy (% recovery)	5 µg/kg: 101 10 µg/kg: 98 100 µg/kg: 101	5 µg/kg: 104 10 µg/kg: 104 100 µg/kg: 99	5 µg/kg: 108 10 µg/kg: 106 100 µg/kg: 92	5 µg/kg: 111 10 µg/kg: 97 100 µg/kg: 95
Intraday precision (% CV)	5 µg/kg: 10 10 µg/kg: 8 100 µg/kg: 5	5 µg/kg: 4 10 µg/kg: 1 100 µg/kg: 9	5 µg/kg: 2 10 µg/kg: 2 100 µg/kg: 3	5 µg/kg: 3 10 µg/kg: 1 100 µg/kg: 4
Interday accuracy (% recovery)		5 µg/kg: 108.3 10 µg/kg: 103 100 µg/kg: 95.7	5 µg/kg: 108 10 µg/kg: 105.3 100 µg/kg: 94.3	
Interday precision (% CV)		5 µg/kg: 4.84 10 µg/kg: 1.09 100 µg/kg: 7.16	5 µg/kg: 1 10 µg/kg: 2.76 100 µg/kg: 2.69	
LOQ / LOD (µg/kg)	2 / 1	5 / 2	4 / 2	4 / 2
Analytical range	5 – 100 µg/kg	5 – 100 µg/kg	5 – 100 µg/kg	5 – 100 µg/kg
Linearity (r^2)	0.997	0.9974	0.9948	0.9958
Specificity	No interference	No interference	No interference	No interference
***Stability:** **a) Freeze-thaw**		a)	a)	
b) Room temperature (24 h)		b)	b)	
c) Storage (3 °C, 48h in liver and 72h in fat)		c) 10 µg/kg: -3.9 % 100 µg/kg: 6.5 %	c) 10 µg/kg: 2 % 100 µg/kg: 10 %	
d) Long term storage		d)	d)	

* It is stated that stability at room temperature for 24 h has been verified; however, no results have been provided. It has not been specified if the stability at room temperature was tested for the analyte in matrix samples or in the extract after the extraction procedure. In addition, neither freeze/thaw nor long term stability data have been provided.

Validation of the analytical methodology for the determination of Ethion residues in milk and bovine tissues by UPLC/MS/MS (Protocol number: RES-VAL-014/11). (Prazeres Gonçalves *et.al.*, 2012)

Description of the method

This analytical method is based on the coupling of ultra-high pressure liquid chromatography and tandem mass spectrometry (UPLC-MS/MS), and is prepared for the detection and quantification of ethion and fenthion (internal standard) in bovine tissue and milk samples.

Sample preparation:

After vortexing and ultrasound mixing, the samples are centrifuged at 8000 rpm and the phase containing the analyte is separated. This procedure is repeated twice and the extracts are pooled together. This extract is passed through a silica column and the filtrate is then dried under nitrogen atmosphere. The samples are then reconstituted with methanol, centrifuged at 15000 rpm and filtered on a 0.22 µm membrane just before injection into the UPLC system.

Validation of the method

Validation of the analytical methodology for the determination of Ethion residues in milk and bovine tissues by UPLC/MS/MS (Protocol number: RES-VAL-014/11).

Table 17. Summary of UPLC/MS/MS validation data (Prazeres Gonçalves *et.al*., 2012)

Tissue	Muscle	Fat	Liver	Kidney	Milk
Intraday accuracy (% bias)	5 µg/kg: 105 10 µg/kg: 95.88 70 µg/kg: 99.55 100 µg/kg: 100.30 250 µg/kg: 105.23 500 µg/kg: 97.03	5 µg/kg: 96.54 10 µg/kg: 107.41 70 µg/kg: 94 100 µg/kg: 103.7 250 µg/kg: 101.74 500 µg/kg: 96.61	5 µg/kg: 99.22 10 µg/kg: 100.21 70 µg/kg: 103.98 100 µg/kg: 111.01 250 µg/kg: 98 500 µg/kg: 87.58	5 µg/kg: 99.2 10 µg/kg: 100.33 70 µg/kg: 103.35 100 µg/kg: 112.29 250 µg/kg: 92.77 500 µg/kg: 92.06	5 µg/kg: 103.34 10 µg/kg: 93.17 70 µg/kg: 95.84 100 µg/kg: 107.65 250 µg/kg: 98.6 500 µg/kg: 101.39
Intraday precision (% CV)	10 µg/kg: ≤ 6.36 100 µg/kg: ≤ 2.35 250 µg/kg: ≤ 7.15	5 µg/kg: 5.43 10 µg/kg: 5.79 70 µg/kg: 6.91 100 µg/kg: 2.29 250 µg/kg: 1.97 500 µg/kg: 4.05	5 µg/kg: 6.45 10 µg/kg: 5.40 70 µg/kg: 2.77 100 µg/kg: 3.45 250 µg/kg: 3.39 500 µg/kg: 1.56	5 µg/kg: 4.72 10 µg/kg: 2.25 70 µg/kg: 4.96 100 µg/kg: 1.32 250 µg/kg: 1.26 500 µg/kg: 2.51	5 µg/kg: 1.47 10 µg/kg: 2.33 70 µg/kg: 2.02 100 µg/kg: 1.27 250 µg/kg: 3.13 500 µg/kg: 2.52
Interday precision	10 µg/kg: ≤ 3.88 100 µg/kg: ≤ 3.85 250 µg/kg: ≤ 1.86				
LOQ / LOD (µg/kg)	5 / 0.82	5 / 0.82	5 / 1.01	5 / 0.74	5 / 0.24 (µg/l)
Analytical range (µg/kg)	5 – 500	5 – 500	5 – 500	5 – 500	5 – 500 (µg/l)
Linearity (r^2)	0.9965	0.9936	0.9900	0.9914	0.9958
Specificity	< 1.5 %	No interference	No interference	< 0.52 %	< 0.25 %
Matrix effect	Significant	Significant	Significant	Significant	Significant
Ruggedness testing	3.66 %				
Stability (%):					
a) Freeze-thaw	a) 10 µg/kg: 4.19 250 µg/kg: -0.91	a) 10 µg/kg: -12.68 250 µg/kg: -12.59	a) 10 µg/kg: -0.51 250 µg/kg: -1.22	a) 10 µg/kg: 1.06 250 µg/kg: -4.22	a) 10 µg/kg: 4.89 250 µg/kg: 3.79
b) Room temperature	b) 10 µg/kg: -1.73 250 µg/kg: -0.91	b) 10 µg/kg: -14.26 250 µg/kg: -12.76	b) 10 µg/kg: 1.54 250 µg/kg: 4.61	b) 10 µg/kg: -10.11 250 µg/kg: -1.9	b) 10 µg/kg: 6.73 250 µg/kg: 1.43
c) Extract	c) 10 µg/kg: 0.68 250 µg/kg: -6.69	c) 10 µg/kg: -0.8 250 µg/kg: -1.15	c) 10 µg/kg: -2.17 250 µg/kg: 10.38	c) 10 µg/kg: -11.74 250 µg/kg: -6.86	c) 10 µg/kg: 0.87 250 µg/kg: -1.65
d) Long term storage	d) 10 µg/kg: -6.06 250 µg/kg: -4.91	d) 10 µg/kg: 4.75 250 µg/kg: -1.27	d) 10 µg/kg: -2.47 250 µg/kg: 0.47	d) 10 µg/kg: 5.52 250 µg/kg: -5.52	d) 10 µg/kg: 2.25 250 µg/kg: 1.38

The validation study was performed by LABTEC (Sao Paulo, Brazil). The final report is dated 13/09/2011. Declaration of conformity with GLP has been provided by the responsible person at the quality assurance team of LABTEC.

A detailed description has been given on how the mobile phase, extraction solution and stock solutions of ethion and fenthion are prepared for the validation study. Sample preparation is as described above.

Linearity, specificity/selectivity, matrix effect, limit of detection, limit of quantification, accuracy and stability were studied in order to validate the analytical method in bovine tissues (muscle, kidney, liver and fat) and milk. Precision and robustness of the method was also analysed in muscle tissues.

Linearity

Linearity of response was studied over the concentration range of 5, 10, 70, 100, 250 and 500 µg/kg (or µg/l for milk), with 7 sample replicates for each concentration. Linearity was confirmed for the method response in solvent, blank matrix and blank matrix extract, spiked with calibration standards. Correlation coefficient (r) was in all cases above 0.98 and residuals errors deviated less than 20 % from the nominal concentration.

Specificity

Specificity were confirmed by the absence of relevant chromatographic peaks in blank matrix samples, as compared to the peak areas of ethion and internal standard fenthion in spiked matrix samples for all the studied tissues.

Matrix effect

The possible interferences on the analysis caused by matrix components or sample extraction procedure were studied comparing the data obtained from the linearity test of calibration curves in blank spiked extracts and in standard solution. Statistical analysis (t-student) revealed significant differences in muscle, fat, kidney, liver and milk, meaning that these matrices have an effect on the precision of the method. Therefore, ethion residue concentrations must be quantified using the matrix calibration curve.

The Limit of Detection

The LOD was calculated by multiplying the standard deviation of the 7 replicates at the lowest concentration level (5 µg/kg) by the t-student t value with n = 7 and confidence level of 99 %. The calculated LODs for different tissues were:

- Muscle: 0.82 µg/kg
- Fat: 0.82 µg/kg
- Kidney: 0.74 µg/kg
- Liver: 1.01 µg/kg
- Milk: 0.24 µg/l

Limit of Quantification

The LOQ was set at 5 µg/kg for all tissues (5 µg/l for milk), corresponding to the lowest level at which accuracy and precision were demonstrated.

Precision (only for muscle tissue)

Repeatability and reproducibility were studied at three different concentrations (10, 100 and 250 µg/kg). For repeatability, six replicates of each concentration were analysed on three different days. Coefficient of variation (CV %) was lower than 2/3 of the theoretical value calculated with the Horwitz equation, except for 250 µg/kg samples of muscle on the second day that presented a slightly higher CV.

For reproducibility, the same number of samples were analysed by a different operator. Mean CV % did not exceed the limit value of 20 % for concentration levels between 10 and 100 µg/kg, or 15 % for concentration levels between 100 and 1 000 µg/kg.

Accuracy

Accuracy was studied by the means of recovery of 7 replicates of sampled fortified at 5, 10, 70, 100, 250 and 500 µg/kg (or µg/l for milk). All values reported and the mean values for each concentration level were within the accepted range for accuracy, irrespective of the type of matrix.

Robustness (only for muscle tissue)

Robustness of the analytical method was confirmed with the Youden test. Seven different parameters were slightly modified (methanol concentration, extraction volume, ultrasound time, vortex mixing time, temperature in the centrifuge, speed of the centrifuge and time in the centrifuge) over 8 analytical runs at 100 µg/kg with different combinations on each run. The mean concentration level obtained on the robustness analysis was similar to that obtained on repeatability studies, thus confirming that the method is robust.

Stability

Stability of the analyte was studied in spiked samples:

- after three consecutive freeze/thaw cycles
- after 24 h at -20 °C and a subsequent 4 h at room temperature
- after 25, 67,46, 115 and 63 h for muscle, fat, kidney, liver and milk, respectively, at 15 °C on the auto-injector
- after 218, 262, 273 and 241 days for muscle, fat, kidney and milk, respectively, stored at -20 °C

Variation was less than 15 % in all cases when compared to freshly extracted samples.

Comment:

The analytical method used for the quantification of ethion in bovine tissues and milk has been adequately validated in terms of linearity of the response, specificity, precision, accuracy, robustness and stability of the analyte (after freeze/thaw, after short and long term storage, and after extraction process on the auto-injector). LOD was set at 0.82, 0.82, 0.74, 1.01 and 0.24 µg/kg for muscle, fat, kidney, liver and milk, respectively. LOQ was set at 5 µg/kg for all matrices. A matrix effect was observed in all matrices; therefore, quantification of the analyte was done using the matrix calibration curves.

However, calculations for the stock solution preparation are not clear and there are some missing data from long term stability in liver.

Comprehensive Literature search

As part of the residue evaluation of ethion, the Committee performed a comprehensive literature search in April 2017 to identify any information relevant for its assessment. The following online databases were searched: Pubmed, B-ON, Springer Nature, Science Direct and Web of Science.

The following inclusion criteria were applied for determining study relevance:

- Any article regarding ethion concentrations in **plasma** of **cattle or other ruminants;**
- Any article regarding ethion concentrations in **edible tissues** of **cattle or other ruminants;**
- Any article regarding ethion residue determination **methods** for **cattle** plasma/tissue;
- Any article regarding ethion **metabolism / metabolites** in cattle
- Any article regarding **bioavailability** of ethion residues in animals
- Articles in all languages were included;

To determine the relevance of the published studies identified in the literature search, the following criteria were used when deciding to exclude studies from the assessment:

- Any article focusing on ethion **efficacy** against target parasites
- Any article focusing on **parasite resistance** to ethion
- Any article focusing on ethion use in food animal species **other than ruminants;**
- Any article focusing on kinetics/residues of **organophosphates other than ethion** (and not including ethion for comparison)
- Any article focusing on **pharmacokinetics or pharmacodynamics** of ethion in **parasite** species

Although no time limits were placed on the search results, studies published after 1994 were evaluated more thoroughly as these were not evaluated by the previous JMPR review of ethion.

The Committee noted that the majority of relevant papers for the ethion MRL evaluation in cattle concerned specific analytical methodologies, usually for use in national or regional surveillance of residues of pesticides in foods, and usually multi-residue methods. There was some potentially useful information on the stability of ethion in various matrices. There were no papers evaluating the pharmacokinetics or residues depletion of ethion in cattle.

A summary of the relevant recent publications follows:

Analytical methods (meat):

Determination of organophosphorus pesticides in bovine tissue by an on-line coupled matrix solid-phase dispersion–solid phase extraction–high performance liquid chromatography with diode array detection method. Tania M. Gutiérrez Valencia, Martha P. García de Llasera. Journal of Chromatography A. 2011. 1218:6869– 6877.

Summary: A miniaturised method based on matrix solid-phase dispersion coupled to solid phase extraction and high performance liquid chromatography with diode array detection (MSPD-SPE-HPLC/DAD) was developed for the trace simultaneous determination of organophosphorus pesticides (OPPs) in bovine tissue. The methodology was validated with liver samples. The LOD was 200 µg/kg, and the limit of quantification was 900 µg/kg.

Committee Comment: This is a well-validated multi-residue method for use in residues surveillance. However, the LOD and LOQ for ethion are not especially sensitive, and as such may not be able to detect or quantify ethion residues at concentrations suitable for MRL assessment. Additionally, it is likely that the target tissue for detection of ethion residues in cattle will be fat, and this method was only validated in liver, with confirmation of method ruggedness in muscle and lung tissues. Finally, the assay was designed for detection of only parent ethion (among other organophosphates), and not any ethion metabolites.

Analytical methods (milk):

1. Pesticide Residues in Bovine Milk in Punjab, India: Spatial Variation and Risk Assessment to Human Health. Bedi *et.al*., Archives of Environmental Contamination and Toxicology, 2015. 69:230-240.

Committee Comment: This paper describes a study of pesticide residues, one of which was ethion, in bovine milk in India (specifically the Punjab region), and their impact on human health through the consumption of milk. The study focused on the pesticide extraction and quantification in only one matrix (milk), and did not evaluate the presence of ethion (or its metabolites) in bovine tissues.

2. Development of a headspace solid-phase microextraction/gas chromatography–mass spectrometry method for determination of organophosphorus pesticide residues in cow milk. Rodrigues *et.al*., Microchemical Journal, 2011. 98:56-61.

Summary: A method based on solid-phase micro-extraction in mode headspace (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS) was developed and optimised through multivariate factorial design to determine residues of organophosphorus pesticides in cow's milk. The evaluated pesticides included ethion. To evaluate residues of these pesticides in milk, cows were exposed to the pesticides of interest and milk was collected after 24 h. The developed method was able to detect trace amounts of these pesticides in the collected milk samples. Ethion could be detected at concentrations below its LOQ (6.5 µg/L) up to 72 h after the spraying of cows.

Committee Comment: This paper demonstrates an analytical technique that could be used to determine ethion residues in milk. The LOD and LOQ (2.2 & 6.5 µg/L, respectively) would be sufficiently sensitive for regulatory purposes. However, it only evaluated organophosphate residues in milk (no bovine tissues), and was not designed for detection of ethion metabolites.

3. Combination of solid-phase extraction with dispersive liquid–liquid microextraction followed by GC–MS for determination of pesticide residues from water, milk, honey and fruit juice. Shamsipur *et.al*., Food Chemistry, 2016. 204:289-297.

Summary: A pre-concentration method for the extraction and determination of traces of multi-residue pesticides (including ethion) was developed using solid-phase extraction (SPE) coupled with dispersive liquid–liquid microextraction and GC–MS, resulting in a very sensitive method for a variety of liquid matrices.

Committee Comment: Another analytical methodology paper that that evaluated organophosphate residues in milk (and other liquids), but not bovine tissues.

4. Selective, solid-matrix dispersion extraction of organophosphate pesticide residues from milk. Di Muccio *et.al*., Journal of Chromatography A, 1996. 754:497-506.

Summary: Description of a single-step, selective extraction and clean-up of organophosphate (OP) pesticide residues from milk. Recovery experiments were carried out on homogenised commercial milk (3.6 % fat content), spiked with 24 OP pesticides, including ethion.

Committee Comment: Another analytical methodology paper that that evaluated organophosphate residues in spiked milk samples, but not bovine tissues.

Stability of ethion in various matrices:

Development and certification of reference materials for residues of organochlorine and organophosphorus pesticides in beef fat ACSL CRM 1 and 2. Armishaw *et.al*., Fresenius Journal of Analytical Chemistry. 1998. 360:630 - 639.

Summary: Beef fat samples were prepared and tested for three organophosphorus pesticides. Beef fat was spiked with pesticide solutions prepared from certified reference materials (e.g., ethion, 99 %). A sample was prepared containing close to 0.8 mg/kg of each of the organophosphorus pesticides diazinon, chlorpyrifos and ethion. No instability in any of these compounds was detected over a twelve month period.

Comment: This paper is relevant because it comments on the stability of ethion in beef fat; however, the beef fat used in the preparation of the candidate reference materials was first stabilised with 0.02 % w/w butylhydroxyanisol (BHA) to assist with long term storage. Furthermore, the stability results are from beef fat samples spiked with ethion, and not endogenous residues. No information regarding ethion residue depletion is present in the study. Ethion metabolites were not assessed.

The following published studies were identified in the literature search, but provided no information relevant to ethion residue assessment in bovine tissues:

Table 18. Unused papers from the literature search

Paper	Reason for exclusion
^{14}C-Ethion residues in soybean seeds: metabolic pathway, effect of processing, bioavailability, toxicity and protective action of artichoke leaf powder towards rats. Abdel-Gawad, H, *et.al*., 2013. Toxicological & Environmental Chemistry, 95:2:288-303.	Not relevant for the determination of residues in cattle matrices.
Residues of ^{14}C-Ethion Along the Extraction and Refining Process of Maize Oil, and the Bioavailability of Bound Residues in the Cake for Experimental Animals. H. Abdel-Gawad *et.al*., (2013). Environmental Contamination and Toxicology, 2013, 91:240–245.	Not relevant for the determination of residues in cattle matrices.
In vitro evaluation of acaricides efficiency to bovine's ticks of Rio Grande do Sul State, Brazil. Camillo *et.al*., Ciência Rural Santa Maria, 2009. 39:2:490-495	Assessment of ethion efficacy
Calculation of Pesticide Degradation in Decaying Cotton Gin Trash. Angus N. Crossan & Ivan R. Kennedy. The Bulletin of Environmental Contamination and Toxicology. 2008. 81:355–359.	Not relevant for the determination of residues in cattle matrices.

Effects of different spray formulations on the reproductive parameters of engorged Rhipicephalus (Boophilus) microplus females detached from experimentally infested cattle. Cruz *et.al*., Preventative Veterinary Medicine. 2015. 122:70-75.	Assessment of efficacy
LC-MS/MS Determination of Organophosphorus Pesticide Residues in Coconut Water. Deme *et.al*., Food Anal. Methods. 2013. 6:1162–1169.	Not relevant for the determination of residues in cattle matrices.
Distribution, fate and histopathological effects of ethion insecticide on selected organs of the crayfish, Procambarus clarkia. Desouky *et.al*., Food and Chemical Toxicology. 2013. 52:42-52.	Not relevant for the determination of residues in cattle matrices.
Extraction of organophosphorus pesticides by carbon-coated Fe_3O_4 nanoparticles through response surface experimental design. Maddah *et.al*., Journal of Separation Science. 2016. 39:256–263.	Analytical method development – too technical for this review.
Monitoring of organochlorine and organophosphorus pesticide residues in water during different seasons of Tighra reservoir Gwalior, Madhya Pradesh, India. Mamta *et.al*., Environmental Monitoring and Assessment. 2015. 187:684	Not relevant for the determination of residues in cattle matrices.
A single method for detecting 11 organophosphate pesticides in human plasma and breastmilk using GC-FPD. Naksen *et.al*., Journal of Chromatography B. 2016. 1025:92-104.	Not relevant for the determination of residues in cattle matrices.
Rapid determination of residues of pesticides in honey by µGC-ECD and GC-MS/MS: Method validation and estimation of measurement uncertainty according to document No. SANCO/12571/2013. Paoloni *et.al*., Journal of Environmental Science and Health, Part B. 2017. 51:3:133-142.	Not relevant for the determination of residues in cattle matrices.
Determination of coumaphos, chlorpyrifos and ethion residues in propolis tinctures by matrix solid-phase dispersion and gas chromatography coupled to flame photometric and mass spectrometric detection. Pérez-Parada *et.al*., Journal of Chromatography A. 2011. 1218:5852-5857.	Not relevant for the determination of residues in cattle matrices.
Validation of a matrix solid phase dispersion (MSPD) technique for determination of pesticides in lyophilized eggs of the chicken Gallus gallus domesticus. Reis Souza *et.al*., Microchemical Journal. 2013. 110:395-401.	Not relevant for the determination of residues in cattle matrices.
Determination of the Residue Levels of Some Commonly Used Organophosphorus Pesticides in Breast Milk. Şahin *et.al*., Ankara Medical Journal. 2017. 1:9-20.	Not relevant for the determination of residues in cattle matrices.
Determination of organophosphorus pesticide residues in vegetables using solid phase micro-extraction coupled with gas	Not relevant for the determination of residues in cattle matrices.

chromatography-flame photometric detector. Sapahin *et.al*., Arabian Journal of Chemistry. 2014. In press.	
Effect of acaricides on the activity of a Boophilus microplus glutathione S-transferase. Silva Vaz *et.al*., Veterinary Parasitology. 2004. 119:237–245.	Assessment of ethion efficacy
Rapid method for the determination of some organophosphorus insecticides in a small amount of serum in emergency and occupational toxicology cases. Singh & Dogra, Indian Journal of Occupational and Environmental Medicine. 2009. 13:2:84-87.	Not relevant for the determination of residues in cattle matrices.
Levels of select organophosphates in human colostrum and mature milk samples in rural region of Faizabad district, Uttar Pradesh, India. Srivastava *et.al*., Human and Experimental Toxicology. 2011. 30:10:1458–1463.	Not relevant for the determination of residues in cattle matrices.
Analysis of organophosphorus pesticides in whole blood by GC-MS-µECD with forensic purposes. Valente *et.al*., Journal of Forensic and Legal Medicine. 2015. 33:28-34.	Not relevant for the determination of residues in cattle matrices.
Effects of co-existed proteins on measurement of pesticide residues in blood by gas chromatography–mass spectrometry. Yue *et.al*., Journal of Chromatography B. 2010. 878:3089-3094.	Not relevant for the determination of residues in cattle matrices.
Short Communication: Evaluation of insecticide ear tags containing ethion for control of pyrethroid resistant Haematobia irritans (L.) on dairy cattle. Anziani *et.al*., Veterinary Parasitology. 2000. 91:147-151	Assessment of ethion efficacy
Silica nanoparticle based techniques for extraction, detection, and degradation of pesticides. Bapat *et.al*., Advances in Colloid and Interface Science. 2016. 237:1-14.	Analytical method development for pesiticide extraction from environmental samples.
Matrix solid phase dispersion (MSPD), Steven A. Barker The Journal of Biochemical and Biophysical Methods. 2007. 70:151–162.	Analytical method development.
Matrix solid-phase dispersion as a valuable tool for extracting contaminants from foodstuffs. Sara Bogialli & Antonio Di Corcia. The Journal of Biochemical and Biophysical Methods. 2007. 70:163–179.	Analytical method development.
Use of gas-liquid chromatography with electron-capture and thermionic-sensitive detection for the quantitation and identification of pesticide residues. Sicbaldi *et.al*., Journal of Chromatography A. 1997. 765:13-22.	Analytical method development.
Prioritization of pesticides based on daily dietary exposure potential as determined from the SHEDS model. Melnyk *et.al*., Food and Chemical Toxicology. 2016. 96:167-173.	Exposure assessment.

Long-term stability of pure standards and stock standard solutions for the determination of pesticide residues using gas chromatography, Avramides, EJ., Journal of Chromatography A. 2005. 1080:166 – 176.	Stability study for ethion stock solutions; analytical method was described but not validated.

Appraisal

Ethion is a small, lipid-soluble compound that can be absorbed by passive diffusion through the lungs, gastrointestinal tract, and skin. Absorption appears to be rapid by the oral route, but is slower via the dermal route due to its deposition in the epidermis and subcutaneous fat layer. Ethion is desulfurated by cytochrome P450 enzymes in the liver to its recognised active form, ethion monoxon, which causes acute toxicity due to its potent inhibition of neural acetylcholinesterase. Ethion and its monoxon metabolite are further metabolised by the action of esterases in the blood and liver, producing a variety of metabolites that have not been fully characterised. Elimination is mainly through excretion of water-soluble but uncharacterised metabolites in the urine.

No pharmacokinetic data were available for the target species, cattle.

Toxicokinetic parameters and cumulative excretion were studied in goats after intravenous, oral and dermal administration of unlabelled and ^{14}C-ethion (Mosha *et.al*., 1991).

- After IV injection of 2 mg/kg bw, the elimination half-life ($t_{1/2}$) was 2 h, total body clearance (Cl_t) was 3.2 L/kg/h and the volume of distribution (Vd_{ss}) was 9.4 L/kg. Plasma concentrations of ^{14}C-ethion (ethion + metabolites) were much higher and more persistent than those of parent ethion, likely indicating the presence of significant quantities of ethion metabolites in plasma. Cumulative excretion of ^{14}C-ethion was 78 % of the dose with 66 % in urine, 8 % in faeces and 4 % in milk over 14 days.
- Oral administration of 10 mg/kg bw resulted in low plasma concentrations of parent ethion and an oral bioavailability of less than 5 %. Cumulative excretion over 14 days was 80 % of the dose with 64 % in urine, 14 % in faeces and 1.7 % in milk.
- Dermal application of 100 mg/kg bw demonstrated a prolonged absorption half-life (85 h) and a bioavailability of 20 %. Only 0.05 % of the dose was excreted unchanged in milk.

It was concluded that (1) orally administered ethion is extensively metabolised in the gastrointestinal tract, (2) dermal application results in extended systemic absorption due to deposition in the dermal fat layer and (3) systemically absorbed parent ethion is rapidly metabolised. The metabolites were not identified in this study.

Residue data

No radiolabelled residue depletion studies in cattle, or any other species, were available for review.

There were no data available for pour-on or spray-on products.

There were four studies investigating the depletion of ethion residues in cattle tissues after dermal administration (via immersion bath or ear tag) provided by the requesting member states; these were reviewed by the Committee (see Table 7). None of these studies investigated the depletion of any of the metabolites and used parent ethion as the marker residue.

The Committee did not have access to the raw data for any of these studies, relying instead on either English translations of the study reports, which were originally in either Spanish or Portuguese, or on evaluation reports from the member states in which the products are authorised.

One study (Prazeres Gonçalves, 2012) was provided that investigated residues during and after treatment with an ear-tag product (40 g ethion per tag). Thirty cattle, divided into 6 groups of 5

animals each, were treated on day zero (D0), with one ear tag applied per animal and remaining in place for 120 days. The groups were slaughtered on days 7, 42, 65and 79 (whilst the tags were in place), and on days 120 and 142 (1 and 23 days, respectively, after removal of the ear tags on day 119). Samples of fat, muscle, kidney and liver were taken for analysis by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Residues were highest in fat samples, although it was not recorded from which anatomic location the fat samples had been taken (i.e., subcutaneous or perirenal). See Table 6 for complete results.

Three residue depletion studies that used immersion bath treatments were provided (Bringas *et.al*., (undated), Anon (2, undated), Gérez García *et.al*., 2017). These treatments consisted of almost total immersion of the cattle in the insecticide solution. All the products used were of the same quantitative composition with respect to the active substances (40 % ethion and 10 % cypermethrin concentrates, mixed in water to give a 400 ppm concentration of ethion at point of administration). Each of these studies used a different treatment protocol. In one study, the animals were treated once, in another study, they were treated twice, with a 9-day interval between treatments, and in the third study, the animals were treated three times, with 21-day intervals between treatments. In none of the studies was it possible to determine the exact dose received by each animal.

In the first study (Bringas *et.al*.,), the animals were treated once. Groups of four animals were slaughtered on days 15, 29, 43, 57, 69 and 92 days post treatment. There were limited useful data derived from this study, as detectable residues in fat were mostly outside the validated range of the LC-MS/MS analytical method used (5 - 100 µg/kg in fat), but it added to the evidence that residues were highest in fat samples, with very few detectable residues of ethion in any of the other edible tissues sampled (liver, kidney, muscle). Perirenal fat was sampled in this study.

In the second study (Anon 2), cattle were treated twice, 9 days apart. Groups of 4 animals (1 male and 3 female) were slaughtered on days 5, 10, 15 and 20 post treatment. Samples of muscle, kidney, liver, subcutaneous and perirenal fat were taken from each animal. Muscle samples were taken from the loin (both left and right); samples of subcutaneous fat were taken from along the back line. Residues were undetectable (LOD = 6.5 µg/kg) in all samples except one kidney sample at day 10 and all fat samples at all time points. The withdrawal periods used in the study were only up to 20 days, which is insufficient to cover the duration of approved withdrawal periods for immersion baths, but the depletion profile was comparable to the other data available.

In the third study (Gérez García *et.al*., 2017), cattle were treated three times, 21 days apart. Groups of 4 animals were slaughtered on days 13, 34, 70, 90, 105 and 117 post final treatment. Samples of muscle (two types; loin and thigh), fat (perirenal fat, plus subcutaneous fat from day 70 onwards), kidney and liver were analysed using GC-ECD, GC-MS and LC-MS/MS. The LOD was 1 µg/kg for all tissues and the LOQs were 5 µg/kg for fat (LC-MS/MS) and muscle, 10 µg/kg for fat (GC-MS), liver and kidney. Samples were analysed in triplicate. In addition, the samples of muscle and fat from slaughter day 34 onwards were re-analysed in a second laboratory. Samples were analysed by LC-MS/MS. The LOQ was 1 µg/kg and the LOD was 0.5 µg/kg for both tissues. No validation data were available for the analytical method used in the second laboratory. The results of analyses of the same samples between the two laboratories were inconsistent, bringing the reliability of these results into question.

It is clear that residues of parent ethion are detectable in fat at higher levels and for a much longer duration after treatment than any other edible tissue sampled. This is consistent with the half-life of the compound.

The Committee noted that the lack of qualitative or quantitative metabolite data is a major omission, and must be addressed before any MRLs can be determined for this substance. The toxicological assessment revealed that at least one metabolite (ethion monoxon) retains significant anticholinesterase activity, and therefore must be accounted for in the residue assessment. In addition, the available data did not identify all the metabolites of concern that lead to the identified reproductive toxicity. One option is to identify and quantify all active ethion metabolites in tissue residues, and include these metabolites along with parent ethion as the marker residue. Alternatively, a single substance can be selected as the marker residue. However, to estimate the toxicological activity of the total ethion residues (including metabolites), knowledge of the marker residue: total residue ratio over time will be required. As such data are not currently available, an accurate assessment of the total toxicological activity of ethion residues (and subsequent residue exposure assessment) cannot be performed.

Analytical methods

The Committee notes that there are analytical methods available for the determination of parent ethion in cattle tissues. However, as the marker residue has not yet been determined, the Committee cannot comment on the suitability of the analytical methods for use in residues control.

Dietary Exposure Assessment

Dietary exposure to ethion may occur through its use as a veterinary drug or its use as a pesticide. While ethion has been assessed by JMPR, no estimate of dietary exposure has been reported from its use as a pesticide.

No dietary exposure assessments were performed for ethion in cattle tissues due to the lack data regarding residue characterisation and total residue concentrations.

Maximum residue limits

In considering MRLs for ethion in cattle edible tissues, the Committee considered the following factors:

- An ADI of 0–0.002 mg/kg bw was established based on the NOAEL of 0.2 mg/kg bw per day in a developmental toxicity study in rats, and using a safety factor of 100.
- An acute reference dose (ARfD) of 0.02 mg/kg bw was established based on the NOAEL of 0.15 mg/kg bw for erythrocyte AChE inhibition in a repeated-dose study in male volunteers, and using a 10-fold intra-species safety factor.
- As the ADI was based on developmental effect and is appreciably lower than the ARfD, there is a potential concern for exposure of pregnant women. Therefore, exposure in high-percentile pregnant consumers or a suitable surrogate population should be addressed. This exposure scenario will also be protective of children given the nature of the end-point on which the ADI is based.
- The residues of concern include all residues derived from ethion, due to the lack of:
    - data relating the toxicological endpoint used to set the ADI to any specific residue or combination of residues, and
    - characterisation of metabolites, either in the laboratory species used in the toxicological studies, or in the edible tissues of the target species, cattle.
- No data regarding total ethion residues in cattle were provided.
- A suitable marker residue could not be determined.

The Committee was unable to recommend MRLs for ethion at this time.

Essential data needed to complete the assessment:

Pharmacokinetics and metabolism and residues depletion in cattle:

In order to enable a determination of a suitable marker residue(s), a metabolism study using radiolabelled ethion in cattle is required. The data should be sufficient to determine:

- The identity of the metabolites produced in cattle;
- The ratios of the parent compound and/or metabolites (i.e., potential marker residues) to the total residues over the residue depletion period in edible tissues (e.g., liver, kidney, muscle and fat).
- Such a study would also provide information on the relative concentration of the target compounds (i.e., marker residue; parent ethion and/or active metabolites) in the various edible tissues of cattle.

A comparison of cattle metabolites to metabolites seen in laboratory species should be conducted to ensure that all residues of toxicological concern produced in cattle have been covered by the available toxicology studies.

Analytical methods:

If it is shown that the marker residue(s) for ethion should be anything other than ethion itself (e.g., 'sum of ethion + ethion monoxon'), then analytical method(s) that can measure the marker residues in edible tissues (e.g., fat, kidney, liver, muscle) should be developed and validated in accordance with established guidance (CAC/GL71-2009). As there are numerous companies that market ethion formulations, and may perform future residue depletion studies, the use of a common (and suitable) analytical method will be desirable to facilitate comparisons between studies.

Marker residue depletion:

Once a suitable marker residue has been established for ethion, the MR: TRR ratios have been characterised in edible tissues, and a validated analytical method is available for quantifying the marker residue in edible tissues, a non-radiolabelled marker residue depletion study can be conducted. Interested member states should note that the label instructions and usage of ethion products varies significantly. Therefore a single ethion marker residue depletion study may not be suitable for establishing MRLs for all ethion products, due to significant differences in residue depletion and Good Veterinary Practices (label usage and withdrawal times) between formulations. For example, residue data generated from an ethion ear-tag study may not be suitable for establishing MRLs via immersion bath.

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