

Residues in animal commodities

The Meeting received a feeding study on lactating Holstein cows. The dosing regime involved a 2-day pre-conditioning phase, one week prior to the dosing period, at a dose level of 100 mg triazophos per cow. During the following period of 7 days, one cow was dosed with 50 mg triazophos (2.38 ppm in the feed) and the second cow with 100 mg (4.76 ppm in the feed), the third cow received untreated feed. Neither the pre-conditioning at 100 mg per cow and day nor the dosing of 50 and 100 mg per cow and day resulted in any residues above the LOQ of 0.05 mg/kg for milk and 0.01 mg/kg for muscle, fat, kidney and liver. The Meeting noted that because of the lack of an appropriate livestock metabolism study, a residue definition for animal products could not be determined and therefore the Meeting could not make use of the results of the feeding study.

The Meeting agreed to withdraw the previous recommendations for triazophos of 0.01 mg/kg in cattle meat and cattle milk.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of triazophos has resulted in recommendations for MRLs and STMR values for raw and processed commodities. Consumption data were available for 2 food commodities and were used in the dietary intake calculations. The results are shown in Annex 3.

The IEDIs for the 13 GEMS/Food regional diets, based on estimated STMRs were in the range 0–20% of the maximum ADI of 0.001 mg/kg bw (Annex 3). The Meeting concluded that the long-term intake of residues of triazophos from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The IESTI for triazophos was calculated for the food commodities for which maximum residue levels and HRs were estimated and for which consumption data were available. The results are shown in Annex 4.

The IESTI calculated for cotton seed oil for the general population and children were 2% and 5% of the ARfD (0.001 mg/kg bw), respectively. The IESTI calculated for soya bean (immature seeds with the pod) for the general population and children were 140% and 230% of the ARfD, respectively (Annex 4). The Meeting concluded that the short-term intake of residues of triazophos from the consumption of cotton seed oil is unlikely to present a public health concern. The information provided to the JMPR precludes an estimate that the dietary intake would be below the ARfD for consumption for soya bean (immature seeds with pod) by the general population and children.

The Meeting noted that the pod is not normally consumed and that no residue data relating to residues in the edible portion of soya bean pods or alternative GAP were submitted for soya bean (immature seeds with pod).

5.24 ZOXAMIDE (227)

TOXICOLOGY

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

Zoxamide has not been evaluated previously by the JMPR and was reviewed at the present Meeting at the request of the CCPR.

All the pivotal studies met the basic requirements of the relevant OECD or national test guideline and contained certificates of compliance with GLP.

Biochemical aspects

In rats given zoxamide, approximately 60% of a dose of 10 mg/kg bw was absorbed, with peak plasma concentrations of radioactivity occurring at 8 h after dosing. Zoxamide was extensively distributed among organs and tissues with highest concentrations reported in the liver. Excretion was primarily in the faeces, via the bile. The overall elimination half-life was 13–14 h. At 1000 mg/kg bw, there was some evidence of saturation of absorption, with C_{max} and AUC values being approximately 40–50 times those at 10 mg/kg bw, but with a similar elimination half-life. Females excreted approximately twice as much radiolabel in the urine as males. Very little radioactivity remained in tissues (< 0.2% of the administered dose) or carcass (< 2% of the administered dose) at 5 days after dosing. Pre-treatment of animals with diets containing zoxamide for 2 weeks or with five daily gavage doses of radiolabelled zoxamide did not significantly alter the absorption or distribution of radiolabel compared with that in untreated animals.

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

Toxicological data

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

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

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

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

study in females at 7500 ppm, equal to 255 mg/kg bw per day, and a deficit in body-weight gain (20%) was still present at the end of the study. Males receiving zoxamide at 7500 ppm also had reduced body-weight gain during the early stages of the study, but these animals had terminal body weights that were higher than those of the controls. Although food consumption was reduced transiently, there was no clear link between body weights of individual animals and food consumption. At the highest dose of 30 000 ppm, there were marked effects on body weight and food consumption, with females taking up to 7 weeks to regain their pre-test body weight. Reduced concentrations of serum albumin, and increases in liver and thyroid weights and serum alkaline phosphatase activities were also seen in both sexes at 30 000 ppm. The NOAEL in the 1-year study was 1500 ppm, equal to 48 mg/kg bw per day.

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

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

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

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

In long-term studies of toxicity in mice and rats, zoxamide exhibited no general toxicity and was not carcinogenic in either species. Increased liver weights (approximately 20%) in female rats killed after a 1-year exposure to zoxamide at a dietary concentration of ≥ 5000 ppm were not considered to be adverse as there were no associated histopathological or clinical chemistry findings at any time during the study. An apparent increase in thyroid C-cell lesions in male rats at the highest dose was not statistically significant, did not exhibit a dose-response relationship, was not reproduced in females and was within the range for historical controls. The NOAEL in mice was 7000 ppm, equal to 1021 mg/kg bw per day, and the NOAEL in rats was 20 000 ppm, equal to 1058 mg/kg bw per day, both values being based on the absence of treatment-related toxicity at the highest doses tested.

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

The reproductive toxicity of zoxamide has been investigated in a two-generation study in rats and studies of developmental toxicity in rats and rabbits. In the study of reproductive toxicity in rats, the NOAEL for effects on fertility, parental toxicity and pup development was 20 000 ppm, equal to 1474 mg/kg bw per day. Reductions in pup body-weight gain and spleen weights and reduced extramedullary haematopoiesis in the spleen were seen in F_{1a} , F_{1b} and F_{2a} offspring, but these effects appeared to be related to palatability as they were not evident in the F_{2b} generation, when pups and dams received equivalent exposures of zoxamide by gavage, rather than from the diet, from postnatal days 14 to 21. Increased relative liver weight was noted at doses of ≥ 5000 ppm in males and females, and in absolute liver weight only in males at 20 000 ppm. The changes in liver weight were not

associated with any histopathological or clinical chemistry change and were not considered to be adverse.

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

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

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

There are two reports of mild adverse effects following exposure to a diluted formulation containing zoxamide and mancozeb. In one case there was a report of skin irritation, in the other “flu-like” symptoms were reported. It is considered to be unlikely that these effects are related directly to exposure to zoxamide.

Toxicological evaluation

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

An ARfD was considered to be unnecessary for zoxamide as it is of low acute toxicity did not produce developmental effects and did not produce any other significant effects following acute exposures.

A toxicological monograph was produced.

Levels relevant to risk assessment

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

		Offspring toxicity	30 000 ppm, equal to 1474 mg/kg bw per day ^c	—
	Developmental toxicity ^b	Maternal toxicity	1000 mg/kg bw per day ^c	—
		Embryo/fetotoxicity	1000 mg/kg bw per day ^c	—
	Acute neurotoxicity ^b		2000 mg/kg bw per day ^c	—
Rabbit	Developmental toxicity ^a	Maternal toxicity	1000 mg/kg bw per day ^c	—
		Embryo/fetotoxicity	1000 mg/kg bw per day ^c	—
Dog	One-year study of toxicity ^a	Reduced body-weight gain	1500 ppm, equal to 48 mg/kg bw per day	7500 ppm, equal to 255 mg/kg bw per day

^a Dietary administration.

^c Highest dose tested.

^b Gavage administration.

Estimate of acceptable daily intake for humans

0–0.5 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Moderate (C_{max} , 8h); approximately 60% absorbed at 10 mg/kg bw
Dermal absorption	Approximately 1% from concentrate; 6% from dilution
Distribution	Extensive. Highest levels in liver.
Potential for accumulation	Low
Rate and extent of excretion	> 85% in 48 h. Urine (approximately 10–20%); bile (approximately 45%); faeces (approximately 50–80%).
Metabolism in animals	Extensive. Primarily via hydrolysis, dehalogenation, oxidation and conjugation.
Toxicologically significant compounds in animals, plants and the environment	Zoxamide.

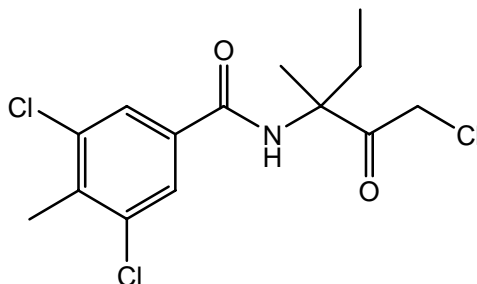
Acute toxicity

Rat, LD ₅₀ , oral	> 5000 mg/kg bw
Rat, LD ₅₀ , dermal	> 2000 mg/kg bw
Rat, LC ₅₀ , inhalation	> 5.3 mg/L
Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Slight transient irritant

Guinea-pig, skin sensitization (test method used)	A skin sensitizer (Buehler; Magnusson & Kligman)		
<i>Short-term studies of toxicity</i>			
Target/critical effect	Body-weight gain		
Lowest relevant oral NOAEL	1500 ppm (48 mg/kg bw per day) in a 1-year study in dogs		
Lowest relevant dermal NOAEL	< 107 mg/kg bw for local effects; 714 mg/kg bw per day for systemic effects.		
Lowest relevant inhalation NOAEC	No data (not required)		
<i>Genotoxicity</i>			
	Not genotoxic in vivo		
<i>Long-term studies of toxicity and carcinogenicity</i>			
Target/critical effect	None.		
Lowest relevant NOAEL	7000 ppm (1021 mg/kg bw per day) in mice (highest dose tested)		
Carcinogenicity	Not carcinogenic		
<i>Reproductive toxicity</i>			
Reproduction target/critical effect	None		
Lowest relevant reproductive NOAEL	20 000 ppm (1047 mg/kg bw per day) in rats (highest dose tested)		
Developmental target/critical effect	None		
Lowest relevant developmental NOAEL	1000 mg/kg bw per day in rats and rabbits (highest dose tested)		
<i>Neurotoxicity/delayed neurotoxicity</i>			
	No indications of neurotoxicity in studies of acute toxicity or repeat-doses		
Acute neurotoxicity	NOAEL was 2000 mg/kg bw in rats (highest dose tested)		
<i>Other toxicological studies</i>			
	RH-141,452 Rapid excretion essentially unmetabolized. Oral LD ₅₀ in mice > 5000 mg/kg bw Negative in an Ames test.		
	RH-141,455 Rapid excretion essentially unmetabolized. Oral LD ₅₀ in mice > 5000 mg/kg bw Negative in an Ames test		
<i>Medical data</i>			
	Two reports (one case of irritation & one of flu-like symptoms) following exposure to a diluted formulation of mancozeb/zoxamide. Unlikely to be directly related to zoxamide.		
Summary			
	Value	Study	Safety factor
ADI	0–0.5 mg/kg bw	Dog, 1-year study	100
ARfD	Unnecessary	—	—

RESIDUE AND ANALYTICAL ASPECTS

Zoxamide, a benzamide fungicide, was identified as a priority new compound at the 38th Session of the CCPR (ALINORM 06/29/24) for evaluation by the 2007 JMPR. The Meeting received information on physical and chemical properties, animal and plant metabolism, environmental fate, analytical methods, storage stability, use patterns, supervised trials and processing.



(*RS*)-3,5-dichloro-*N*-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide

In this appraisal, the following abbreviated names were used for metabolites.

RH-127450	3,5-dichloro- <i>N</i> -(1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide
RH-129151	2-(3,5-dichloro-4-methylphenyl)-4-ethyl-4-methyl-4H-1,3-oxazin-5(6H)-one
RH-139432	3,5-dichloro-4-methylbenzamide
RH-141288	3,5-dichloro- <i>N</i> -(3-hydroxy-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide
RH-1452	3,5-dichloro-4-hydroxymethylbenzoic acid
RH-1455	3,5-dichloro-1,4-benzene-dicarboxylic acid
RH-149736	3,5-dichloro-4-hydroxymethylbenzamide
RH-149737	4-carboxy-3,5-dichlorobenzamide
RH-150721	(3-amino-3-methyl-2-oxo)pentyl-(3,5-dichloro-4-methyl)benzoate
RH-163353	3,5-dichloro- <i>N</i> -(2-carboxy-1-ethyl-1-methyl-2-oxoethyl)-4-methylbenzamide
RH-24549	3,5-dichloro-4-methylbenzoic acid

Animal metabolism

The Meeting received information on the fate of orally-dosed zoxamide in a lactating goat.

When [¹⁴C-phenyl]zoxamide was administered orally at a dose equivalent to a dietary concentration of 60.7 ppm to a lactating goat once a day for 7 consecutive days, 95% of the recovered radioactivity (77.5% of the administered dose) was found in urine (37.1%) and faeces (36.1%). None of individual tissues or cumulative milk sample on day 7 contained more than 3% of the administered dose. On day 4 the radioactive residues in milk was the highest at 0.24 mg/kg in parent equivalents.

Unextracted radioactivity was less than 10% of total radioactive residues (TRR) (< 0.05 mg/kg) in all samples except liver (12%).

No parent compound was found in any of tissues or milk sample. A number of metabolites were detected in milk and tissues. In fat, RH-127450 was found at 0.13 mg/kg in parent equivalent. However, as the dose administered in the study was about 14 times the highest concentration found in

any commodity after treatment of the respective crop in accordance with GAP, significant residue concentrations are unlikely to occur in milk or any tissues in practice.

Zoxamide was extensively metabolized and readily eliminated following oral administration to a lactating goat. Once administered orally, zoxamide underwent dechlorination, then oxidation of either position 4 of the benzene ring or the end of the side-chain and further hydrolysis.

The metabolism of zoxamide in the lactating goat was qualitatively similar to that described in the toxicology section (see page 282).

Plant metabolism

The Meeting received information on the fate of zoxamide after foliar application of [U-¹⁴C-phenyl]zoxamide to grapes, cucumber, tomato and potato.

When grape vines were sprayed at a rate of 1.9 kg ai/ha three times at 30 day intervals, grapes harvested 1 day after the last application contained 0.74 mg/kg of radioactive residues. The parent compound was the major residue at 0.43 mg/kg (58% of TRR). RH-129151, RH-139432, RH-141288, RH-149736, RH-149737 and RH-150721 were identified but all were less than 0.021 mg/kg in parent equivalents ($\leq 2.8\%$ of TRR).

Cucumber plants were sprayed three times at the rate of 1.3 kg ai/ha at a 7 day intervals and foliage and fruit samples were harvested 1 day after the last application. While an average radioactive residue in foliage was 108 mg/kg in parent equivalent, that in fruits was 1.5 mg/kg, which indicates that translocation of zoxamide, was not significant one day after the final application. Extraction of foliage and fruit samples with acetonitrile-water mixture solubilised 100% of the total radioactivity and there were no volatile or unextracted residues. Zoxamide accounted for 87% of TRR in fruits and 89% in foliage indicating that the parent is predominant. Minor metabolites were identified in fruits and foliage. Among them, RH-150721 and RH-157450 were present at the highest concentrations but still less than 0.1 mg/kg ($< 5\%$ of TRR).

Tomato plants received three foliar applications at 0.86 mg/kg with 18 day intervals and tomato fruits were collected 1 day after the last application. The TRR was 0.29 mg/kg in green tomato and 0.50 mg/kg in red tomato. The parent was the major component of residues amounting to 0.14 mg/kg (48% of TRR) in green tomato and 0.22 mg/kg (44% of TRR) in red tomato. Minor amounts of metabolites were identified but none exceeded 3% of TRR. RH-1452 and RH-141288 were identified in two different fractions but their actual concentrations were not determined.

Three foliar applications were made at the rate of 0.9 kg ai/ha on potato plants with the first application at 39 days after planting, and the second and third made at intervals of 21 and 17 days respectively. Mature potato tubers were harvested 14 days after the last application. The TRR was 0.18 mg/kg parent equivalents. Unlike other plants tested, the parent compound was not found in the harvested commodity, i.e., the potato tuber. The metabolites RH-1455 and RH-1452 were found at 0.069 and 0.037 mg/kg accounting for 39% and 21% of the TRR, respectively.

The nature of minor metabolites suggests that zoxamide, when applied to plants, underwent dechlorination and hydrolysis or oxidation. Zoxamide was the major residue in grape, cucumber and tomato when harvested one day after the last application. However, the parent compound was not found in potato sampled 14 days after the last application.

Environmental fate in soil

The Meeting reviewed information on aerobic soil metabolism and rotational crop study as zoxamide was intended for protection of potatoes.

Aerobic soil metabolism studies were conducted using [U-¹⁴C-phenyl]zoxamide applied to various soils which were then incubated under aerobic conditions at 20 or 25 °C. Under aerobic conditions, zoxamide applied to soil was rapidly degraded. After 120–122 days, only small amounts (0.6–10%) of applied zoxamide remained as the parent. Carbon dioxide was steadily evolved from all soils and accounted for 34–58% of the dose applied after 120–122 days. RH-127450, RH-129151,

RH-24549, RH-139432 and RH-163353 were formed and then degraded during the study periods. Unextracted radioactivity, 0.4-3.3% of the applied dose (3.3% in silt loam dosed at 1.5 mg/kg; for other soils tested 0.4-0.8%) on day 0, increased steadily to reach 24-38% of the applied dose on day 120-122. Several other degradates were observed at very low concentrations. These results indicate that none of zoxamide or its identified metabolites are persistent in soil.

Residues in succeeding crops

In an outdoor confined rotation study, mustard, radish, turnip, sorghum and soya bean were planted at 30, 137, 210, 365 days following the last of four applications of [¹⁴C-phenyl]zoxamide. Zoxamide was applied to bare soil between mid April and early June (18 day intervals) at a rate of 0.5 kg ai/ha. Crops were harvested at an intermediate stage and when mature.

TRRs were very low for all samples at all plant back intervals. In general, the amount of extractable residues was low in all the crop samples. Between 7% and 40% of the TRR was recovered in the polar MeOH/H₂O fractions for all the crops grown on treated soil. About 2 to 36% of the TRR was found in the organic extracts (CHCl₃, CH₃CN and hexane) of all the crops. The concentrations in these samples did not exceed 0.023 mg/kg. The values of extracts for all the crop samples showed a significant fraction of unextracted residues: generally 49% or greater.

Concentrations of RH-1452 and one other metabolite were generally below 0.01 mg/kg. The second metabolite was not fully identified. Other metabolites were detected at lower concentrations in some crops.

Zoxamide residues are not expected to occur in succeeding crops.

Methods of analysis

Analytical methods for determination of residues of zoxamide were developed for a wide range of matrices including cucurbits, grapes, tomato, potato and their processed commodities and byproducts.

In most of the methods for determination of zoxamide only, zoxamide was extracted with organic solvent or a mixture of organic solvent and aqueous solution specific to the matrix; cleaned up with liquid-liquid partition followed by solid phase extraction using carbon, alumina, Florisil and silica singly or in combination; and analysed by gas chromatography using electron capture detection (GC/ECD) for quantitation and mass selective detection (GC/MSD) for confirmation. For detection, ELCD or NPD may also be used. These methods were validated in independent laboratories. Most of the methods were suitable as enforcement methods with the limit of quantification at 0.01 mg/kg. One method for potato and its products has an LOQ of 0.02 mg/kg.

The existing multi-residue enforcement methods, one of FDA screen methods and DFG S19 were also tested to be suitable for analysing zoxamide.

The methods for potato and its processed commodities determine zoxamide and two metabolites, RH-1452 and RH-1455. While zoxamide is extracted in the organic phase in liquid-liquid partition, these metabolites were extracted in the aqueous phase. After methylation of these metabolites using diazomethane, and further clean-up, they were analysed using GC/ECD or GC/MSD. The LOQ was 0.02 mg/kg.

Stability of residues in stored analytical samples

Stability of zoxamide (0.1-2 mg/kg) in homogenized samples of grapes (433 days), cucumbers (868 days), tomatoes (810 days), and potatoes (708 days); grape juice (858 days); dried grapes (789 days); wine (8 months); tomato juice (832 days); tomato paste (237 days); and tomato puree (228 days) stored in deep freezer at a temperature below -10 °C was investigated.

No decrease of zoxamide was observed in all samples of cucumbers, tomatoes and its processed products and potatoes during the test periods.

In the case of grapes and its products, in particular grape juice, relatively large fluctuations were observed in the percentage of remaining zoxamide during the test period. However, the Meeting

concluded that zoxamide was sufficiently stable for 14 months in grapes, 28 months in grape juice, 26 months in dried grapes and 8 months in wine.

RH-1452 and RH-1455 were shown to be stable for 29 months of storage while frozen.

Definition of the residue

In grapes, zoxamide represented 58% of the TRR with no metabolite exceeding 5% of the TRR. Also in cucumber and tomato, zoxamide is the major residue component: 87% of TRR in cucumber, 48% of TRR in green tomatoes and 44% of TRR in red tomatoes. No metabolite was found to be more than 10% of the TRR in all cucumber and tomato samples. Most metabolite residues were present at less than 5% of the total residues. These indicate that the residue of concern in grapes, cucurbit and tomato be defined as parent although samples analysed were taken only one day after the last application.

In potato, however, no parent zoxamide was detected. RH-1452 and RH-1455, comprising 21% and 39% of the total residue, respectively, were the major components of the residue. Another 16% of the residue was identified as glucose and/or other sugars. No other metabolites were present at or higher than 10% of TRR. In supervised field trials in Northern and Southern Europe, the United States, Canada and Mexico, samples were analysed for zoxamide, RH-1452 and RH-1455. In all trials, residues of parent were below the LOQ and concentrations of the metabolites were also below the LOQ in all but two trials where zoxamide was found at 0.02 mg/kg.

Methods of analysis are available for determination of zoxamide in grapes, cucurbits, tomatoes and potatoes and their processed products. A method is available also for determination of RH-1452 and RH-1455 in potatoes.

The current Meeting concluded that only zoxamide is toxicologically significant.

In the lactating goat study, the main components of residues were RH-127450 in milk and fat, glucuronic acid conjugates of 4-hydroxymethyl-RH-141288 in liver, with the highest concentration of 0.13 mg/kg parent equivalents of RH-12740 in liver. However, as the administered dose was 14 times higher than the highest residue concentration found in the reported trials, no residue was expected to be found in animals given feed with incurred residues of zoxamide. No method of analysis is currently available for these metabolites. For these reasons, the Meeting concluded that it was not in a position to recommend a residue definition for animal commodities.

In the lactating goat study, the concentration of radioactive residues expressed in parent equivalent in fat was about 4 times that in muscle but about one half of that in kidney or liver. Therefore, the Meeting considered residues not fat-soluble.

In countries where there are MRLs for zoxamide, the residue definition was mostly “zoxamide” except in the USA where it is zoxamide including its metabolites RH-1452 and RH-1455 for potato and its products.

The Meeting recommended the following residue definition for zoxamide in plant commodities.

For plants: Definition of the residue (for compliance with the MRL and for estimation of dietary intake): zoxamide

Results of supervised residue trials on crops

The Meeting received supervised trial data for zoxamide uses on grapes, cucurbits, tomato and potato.

Grapes

Numerous residue trials were conducted on grapes in Brazil, Canada, Germany, France, Greece, Italy, Republic of Korea, Spain and the USA.

The trials conducted in Germany used six applications rather than four as on the label. The Meeting decided to use the results of these trials for MRL estimation as the last applications contribute most to the residue concentration at harvest. In 12 German trials in accordance with German GAP

(maximum rate of 0.24 kg ai/ha in 800-1600 L/ha, 4 applications, with a PHI of 56 days) (except application number), zoxamide residues in rank order were: 0.34, 0.38, 0.39, 0.41, 0.41, 0.45, 0.49, 0.55, 0.59, 0.60, 0.66 and 0.72 mg/kg.

The trials conducted in France used ten applications rather than three on the label. The Meeting decided to use the results of these trials for MRL estimation as it is the last applications that contribute the most to the residue concentration at the harvest. In 21 Northern French trials in accordance with French GAP (0.12 kg ai/ha, 3 applications, PHI 28 days)(except application number), zoxamide residues in rank order were: 0.09, 0.17, 0.19, 0.19, 0.33, 0.35, 0.45, 0.47, 0.48, 0.50, 0.50, 0.51, 0.55, 0.56, 0.67, 0.77, 0.77, 0.81, 0.88, 1.31, 1.55 mg/kg. In 15 Southern French trials conducted in accordance with French GAP, zoxamide residues in rank order were: 0.21, 0.21, 0.33, 0.42, 0.42, 0.46, 0.49, 0.54, 0.58, 0.61, 0.63, 1.07, 1.11, 1.53 and 2.84 mg/kg. Since the residue populations in the Northern and Southern France are similar and there is a uniform GAP for the whole of France, the Meeting considered it appropriate to combine the results from 36 trials in France: 0.09, 0.17, 0.19, 0.19, 0.21, 0.21, 0.33, 0.33, 0.35, 0.42, 0.42, 0.45, 0.46, 0.47, 0.48, 0.49, 0.50, 0.50, 0.51, 0.54, 0.55, 0.56, 0.58, 0.61, 0.63, 0.67, 0.77, 0.77, 0.81, 0.88, 1.07, 1.11, 1.31, 1.53, 1.55 and 2.84 mg/kg.

In 15 Italian trials conducted in accordance with Italian GAP (maximum rate of 0.17 kg ai/ha, 0.017 kg ai/hL, 5 applications, PHI 28 days), zoxamide residues in rank order were: 0.24, 0.28, 0.29, 0.30, 0.33, 0.48, 0.48, 0.54, 0.59, 0.65, 0.66, 0.81, 0.82, 1.37 and 1.56 mg/kg.

In six Spanish trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.36, 0.53, 1.17, 1.21, 1.42 and 1.92 mg/kg.

In four Greek trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.27, 0.32, 0.34 and 0.64 mg/kg.

Combined residues from Italian, Spanish and Greek trials in accordance with Italian GAP in rank order were: 0.24, 0.27, 0.28, 0.29, 0.30, 0.32, 0.33, 0.34, 0.36, 0.48, 0.48, 0.53, 0.54, 0.59, 0.64, 0.65, 0.66, 0.81, 0.82, 1.17, 1.21, 1.37, 1.42, 1.56 and 1.92 mg/kg.

Six trials were conducted in Canada but none was in accordance with Canadian GAP (0.19 kg ai/ha, 6 applications, PHI 66 days). However, four trials were in accordance with US GAP (maximum rate of 0.22 kg ai/ha, 8 applications, PHI 14 days). Residues in rank order were: 1.12, 1.46, 1.52 and 1.69 mg/kg.

Among numerous US trials, 17 trials were conducted in accordance with US GAP. Zoxamide residues in rank order were: 0.22, 0.31, 0.34, 0.34, 0.42, 0.46, 0.49, 0.52, 0.61, 0.66, 0.83, 0.91, 1.08, 1.18, 1.61, 1.65 and 4.34 mg/kg.

Combined residues from the US and Canadian trials conducted in accordance with US GAP (ranked order, median underlined) were: 0.22, 0.31, 0.34, 0.34, 0.42, 0.46, 0.49, 0.52, 0.61, 0.66, 0.83, 0.91, 1.08, 1.12, 1.18, 1.46, 1.52, 1.61, 1.65, 1.69 and 4.34 mg/kg.

In seven Brazilian trials conducted in accordance with Brazilian GAP (maximum rate of 0.13 kg ai/ha, 600–1000 L/ha, PHI 7 days), zoxamide residues in rank order were: 0.07, 0.08, 0.14, 0.14, 0.15, 0.16 and 0.36 mg/kg.

Three trials conducted in the Republic of Korea seemed to be in accordance with Korean GAP (0.01 kg ai/ha, three applications, PHI 7 days). The residues were: 0.05, 0.06 and 0.08 mg/kg.

Among results of these trials, residues from US trials would lead to the highest maximum residue level. Based on the results from US and Canadian trials, the Meeting estimated a maximum residue level and an STMR for zoxamide in grapes of 5 and 0.83 mg/kg respectively.

Fruiting Vegetables, cucurbits

Protected supervised trials were conducted on cucumber in France and Spain and field trials in the Republic of Korea and the USA. Supervised trials were also conducted in the USA for cantaloupe and squash.

Six supervised indoor trials on cucumber in France were in accordance with Polish GAP (maximum rate of 0.15 kg ai/ha, 700–800 L/ha, 3 applications, PHI 4 days) although five applications were made. Residues from these trials in rank order were: 0.01, 0.03, 0.04, 0.06, 0.06 and 0.48 mg/kg. In three Spanish trials conducted in accordance with Polish GAP, zoxamide residues in rank order were: 0.25, 0.44 and 0.45 mg/kg. Combined residues in rank order (median underlined) were: 0.01, 0.03, 0.04, 0.06, 0.06, 0.25, 0.44, 0.45 and 0.48 mg/kg.

Seven outdoor trials were conducted in the USA but only one trial was in accordance with the current US GAP for cucurbits (maximum rate of 0.19 kg ai/ha, 8 applications, PHI 5 days). The residues were: 0.04 mg/kg.

Four trials were conducted in the Republic of Korea on cucumber but none were in accordance with Korean GAP.

Seven trials on cantaloupe and six on summer squash were conducted in the USA but only one each was in accordance with the current US GAP. The residue level in one cantaloupe trial was 0.04 mg/kg and that in one squash trial was 0.09 mg/kg.

On the basis of indoor trials in Europe, the Meeting estimated a maximum residue level and an STMR for zoxamide in cucumber at 1 and 0.06 mg/kg respectively.

Tomato

Protected supervised trials were conducted on tomato in Greece and Spain; and field trials in Brazil, Italy, Spain and the USA.

In 10 Spanish indoor trials conducted in accordance with Italian GAP (maximum rate of 0.17 kg ai/ha, 0.017 kg ai/hL, 5 applications, PHI 3 days), zoxamide residues in rank order were: 0.07, 0.08, 0.09, 0.09, 0.10, 0.12, 0.12, 0.15, 0.24 and 0.29 mg/kg.

In two French indoor trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.28 and 0.31 mg/kg.

In three Greek indoor trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.15, 0.30 and 0.30 mg/kg.

Combined residues from the indoor trials in Spain, France and Greece in accordance with Italian GAP were: 0.07, 0.08, 0.09, 0.09, 0.10, 0.12, 0.12, 0.15, 0.15, 0.24, 0.28, 0.29, 0.30, 0.30 and 0.31 mg/kg.

In 12 Italian outdoor trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.12, 0.13, 0.14, 0.15, 0.16, 0.18, 0.18, 0.20, 0.22, 0.24, 0.24 and 0.30 mg/kg. In five Spanish outdoor trials conducted in accordance with Italian GAP, zoxamide residues in rank order were: 0.03, 0.04, 0.04, 0.05 and 0.05 mg/kg. Combined residues were: 0.03, 0.04, 0.04, 0.05, 0.05, 0.12, 0.13, 0.14, 0.15, 0.16, 0.18, 0.18, 0.20, 0.22, 0.24, 0.24 and 0.30 mg/kg.

Eighteen US outdoor trials were considered to have been conducted in accordance with US GAP (maximum rate of 0.19 kg ai/ha, 8 applications, PHI 5 days) although application number was mostly 10 up to 13 despite the label specification of 8 applications; however the Meeting concluded that the last applications contribute the most to residue concentrations at harvest. Zoxamide residues in ranked order (median underlined) were: 0.07, 0.10, 0.11, 0.12, 0.13, 0.16, 0.18, 0.18, 0.19, 0.20, 0.21, 0.21, 0.22, 0.23, 0.32, 0.38, 0.40 and 1.0 mg/kg.

In five Brazilian outdoor trials conducted in accordance with Brazilian GAP (maximum rate of 0.13 kg ai/ha, applied in 800 L/ha, with a PHI of 7 days), zoxamide residues in rank order were: 0.01, 0.02, 0.02, 0.03 and 0.14 mg/kg.

Among results from the above trials, those from US trials would lead to the highest maximum residue level. Based on the US data, the Meeting estimated a maximum residue level and an STMR for zoxamide in tomato of 2 and 0.195 mg/kg respectively.

Potato

Supervised trials were conducted on potato in Argentina, Brazil, Canada, France, Germany, Greece, Italy, Republic of Korea, Mexico, the Netherlands, Spain, the UK and the USA.

In six trials in Northern France, seven in Germany, one in the Netherlands and 11 from the UK conducted in accordance with GAP in Ireland, the Netherlands and the UK (maximum rate of 0.15 kg ai/ha, 200–600 L/ha, 10 applications, PHI 7 days), zoxamide residues were all < 0.02 mg/kg (25).

In six trials in Southern France, four in Greece, seven in Italy and six in Spain conducted in accordance with Italian GAP (maximum rate of 0.17 kg ai/ha (0.017 kg ai/hL), 5 applications, PHI 7 days), zoxamide residues were all < 0.02 mg/kg (23).

Twelve Canadian trials were considered to have been conducted in accordance with Canadian GAP (0.19 kg ai/ha, 6 applications, PHI 3 days) although 10 applications were made; however the Meeting concluded that the later applications contribute the most to residue concentrations at harvest. The residues were all < 0.02 mg/kg (12). A total of 27 USA trials were considered to be in compliance with US GAP (0.19 kg ai/ha, 6 applications, PHI 3 days) although 10 applications were made. The residues were all below the LOQ (0.02 mg/kg) (4) or LOD (0.006 mg/kg) (22) and hence < 0.02 mg/kg (27). Even with double rate applications, residues were below the LOQ.

Eight trials were conducted in Mexico but samples were taken 13 or 14 days after the last application instead of the PHI of 7 days as specified on the label.

In two Argentine trials, conducted in accordance with Argentine GAP (0.15 kg ai/ha, 400–1000 L/ha, PHI 7 days), zoxamide residues were < 0.05 mg/kg (2).

In six Brazilian trials, conducted in accordance with Brazilian GAP (maximum rate of 0.13 kg ai/ha, 650 L/ha, PHI 7 days), zoxamide residues (ranked order, median underlined) were: < 0.01 (4) (two were below the LOD) and 0.02 mg/kg (2).

Six trials were conducted in the Republic of Korea but none were in compliance with Korean GAP.

On the basis of the Brazilian trials and the fact that, other than two Brazilian trials, residues from trials done in accordance with respective GAP were all below the LOQ, the Meeting estimated a maximum residue level and an STMR at 0.02 and 0.02 mg/kg.

Fate of residues during processing

The Meeting received information on processing of grapes to dried grapes, juice, wine and pomace, tomatoes to puree and paste, and potatoes into flakes, chips and peel.

Processing factors were calculated for grapes (dried grapes, juice, wine and pomace), tomato (puree and paste) and potato (peel) and are shown in the Table below. Processing factors could not be calculated for potato flakes or chips because the residue concentrations were below the LOQ in both potatoes and processed products.

Mean processing factors and STMR-P for food and feed.

Commodity	Processing factor	Median or best estimate	STMR-P mg/kg
Grapes			0.83
Unclarified juice	0.10, 0.16	0.13	0.11
Dried grapes	2.2, 3.5	2.9	2.4
Wine	< 0.01, < 0.01, < 0.01, < 0.01, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.03, < 0.03, < 0.04	< 0.02	0.02
Pomace, wet	0.01, 0.02, 0.05, 0.05, 0.13, 0.79, 1.1, 1.5, 3.1	1.3 ¹	1.1
Tomato			0.195
Puree	0.43	0.43	0.08
Paste	0.97	0.97	0.19
Potato			0.02
Peel	> 3.0	3.0	0.06

¹ As the spread of processing factors of wet pomace calculated from trial results is very large, the Meeting decided to take a conservative approach and use four values at the higher end to provide the best estimate of the processing factor.

Dried grapes are expected to contain higher residues than grapes. Multiplying the highest residue concentration found in the supervised trials (4.34 mg/kg) by the processing factor of 2.9, resulted in an estimate of 12.6 mg/kg, the Meeting estimated a maximum residue level at 15 mg/kg.

Residues in animal commodities

Potato wet peel and wet grape pomace may be fed to dairy cattle and beef cattle but not as major feed ingredients. The calculated maximum and mean livestock animal burden was 0.03–1.50 ppm.

In the metabolism study, in which zoxamide equivalent to 60.7 ppm in the diet was orally administered to a lactating goat for 7 consecutive days, no parent compound was found in any tissue or milk. A number of metabolites were present in tissues and milk but mostly below 0.1 mg/kg parent equivalents. Given the low estimated animal burden, about one fortieth of the administered level, no zoxamide or its metabolite is expected to be present at detectable levels in tissues or milk.

The Meeting agreed that no maximum residue level was necessary for commodities derived from mammals.

The livestock dietary burden was also calculated for layers and broilers with potato wet peel and wet grape pomace and were 0–0.73 ppm. No information was available on the fate of zoxamide in poultry. In addition, no method of analysis was submitted for zoxamide or metabolites in commodities derived from poultry.

The Meeting agreed that no maximum residue level could be estimated for commodities of poultry origin.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of zoxamide were calculated for the 13 GEMS/Food cluster diets using STMRS/STMR-Ps estimated by the current Meeting (Annex 3). The ADI is 0–0.5 mg/kg bw and the calculated IEDIs were all 0% of the maximum ADI. The Meeting concluded that the long-term intakes of residues of zoxamide, resulting from the uses considered by the current JMPR, are unlikely to present a public health concern.

Short-term intake

The 2007 JMPR decided that an ARfD is unnecessary. The Meeting therefore concluded that the short-term intake of residues of zoxamide is unlikely to present a public health concern.