MESOTRIONE (addendum)

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Explanation

Mesotrione (2-(4-mesyl-2-nitrobenzoyl)cyclohexane-1,3-dione) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2014, when an acceptable daily intake (ADI) of 0–0.5 mg/kg body weight (bw) was established. The Meeting concluded that it was not necessary to establish an acute reference dose (ARfD) for mesotrione (Annex 1, reference *133*).

The 2014 Meeting also assessed data on two metabolites of mesotrione: AMBA (2-amino-4-methylsulfonylbenzoic acid) and MNBA (2-nitro-4-methylsulfonylbenzoic acid). On the basis of the "Plant and animal metabolite assessment scheme" of JMPR (WHO, 2015), the 2014 Meeting concluded that these two metabolites were unlikely to be a safety concern (Annex 1, reference *133*).

Following a request for additional maximum residue levels by the Codex Committee on Pesticide Residues, mesotrione was placed on the agenda of the present Meeting, which assessed additional toxicological information available since the last review.

Several toxicological studies on the two metabolites of mesotrione, AMBA and MNBA, were submitted to the present Meeting, including a study on systemic exposure to AMBA from MNBA, two-generation reproductive toxicity and developmental toxicity studies with MNBA, and an in vivo micronucleus assay with AMBA. No new information on mesotrione, the parent compound, was submitted.

All critical studies contained statements of compliance with good laboratory practice and were conducted in accordance with relevant national or international test guidelines, unless otherwise specified. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects of metabolites

1.1 Systemic exposure to AMBA and MNBA

AMBA is a metabolite of MNBA that is postulated to be predominantly formed in the gut from unabsorbed MNBA by intestinal microflora and is then absorbed into the systemic circulation. To investigate and quantify the systemic exposure to AMBA in blood and plasma following exposure to MNBA, female Han Wistar rats were administered a single oral gavage dose of MNBA (purity 99.8%; batch no. 694472) in 1% weight per volume (w/v) aqueous carboxymethylcellulose at 75 mg/kg bw. A blood sampling schedule (0.25–72 hours), capable of fully characterizing the systemic exposure to both MNBA and AMBA in the rat, was defined by a preliminary pharmacokinetics study. A temporary tail vein cannula and use of volumetric absorption microsampling allowed an appropriate number of 10 µL serial blood samples to be taken from individual animals to characterize the concentration–time profiles of both MNBA and AMBA. Larger volumes (approximately 100 µL) of blood were removed at 0.5, 10, 24 and 48 hours to allow for plasma analysis. Concentrations of MNBA and AMBA in blood and plasma were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS), using validated bioanalytical methods. To obtain more consistent data and simplify their interpretation, the systemic exposure (area under the concentration–time curve [AUC]) to each analyte in rat blood was derived from MNBA and AMBA concentration data up to 24 hours post-dosing.

The AUCs of MNBA and AMBA and the ratios of their concentrations in blood and plasma are summarized in Table 1.

Table 1. Systemic exposure to MNBA and AMBA in rats treated orally with MNBA at a dose of 75 mg/kg bw

Parameter		MNBA		AMBA	
$AUC_{0–24\ h}$ (ng·h/mL)					
		Replicate A[a]	Replicate B[b]	Replicate A[a]	Replicate B[b]
	No. of rats	*5*	*5*	*5*	*5*
	Mean ± SD	3 492 ± 902	4 050 ± 343	9 378 ± 6 089	6 784 ± 1 382
Plasma concentration (ng/mL)					
Nominal time after dosing (hours)	0.5	2 355 ± 860		12.2 ± 3.54	
	10	241 ± 223		1 244 ± 1 043	
	24	NC		139 ± 201	
	48	NC		NC	
Blood:plasma concentration ratio					
Nominal time after dosing (hours)	0.5	0.71 ± 0.33		1.14 ± 0.72	
	10	0.90 ± 0.82		0.88 ± 0.20	
	24	NC		0.80 ± 0.13	
	48	NC		NC	

AMBA: 2-amino-4-methylsulfonylbenzoic acid; $AUC_{0–24\ h}$: area under the concentration–time curve from 0 to 24 hours; bw: body weight; MNBA: 2-nitro-4-methylsulfonylbenzoic acid; NC: not calculable, as no plasma data were available for these time points

[a] Results generated from data within the recognized stability period.

[b] Results generated from data 16 days beyond the recognized stability period. However, as the concentrations of MNBA in rat blood, determined by analysis of replicates A and B, were generally similar, the calculation of the $AUC_{0–24\ h}$ from the replicate B samples was justified and suggests that MNBA is stable in rat blood for up to 24 days.

Source: Punler (2017)

Following single oral gavage administration of MNBA to female Han Wistar rats at a dose of 75 mg/kg bw, the concentration–time profiles of both MNBA and AMBA, although variable, were sufficiently well characterized to confirm the systemic exposure to AMBA, with systemic concentrations of AMBA being reported from 0.25 to 72 hours post-dosing. Comparison of the systemic exposure quantified in terms of blood $AUC_{0–24\ h}$ shows a 2- to 3-fold higher exposure to AMBA compared with MNBA over the 24-hour period following dosing with MNBA. Concentrations of MNBA and AMBA in rat plasma were similar to those observed in blood, indicating that both compounds distribute into red blood cells, but are also still freely available in plasma (Punler, 2017).

2. Toxicological studies on metabolites

2.1 AMBA

(a) In vivo genotoxicity

AMBA was assessed for its potential to cause damage to chromosomes or cell division apparatus or to cause cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of young adult rats. In all phases, the dosing of the vehicle and test item was by oral (gavage in 1% aqueous carboxymethylcellulose) administration twice, approximately 24 hours apart.

In the range-finding study, three male and three female Crl:WI(Han) rats were given a single dose of AMBA (purity 98.6%; lot/batch no. 924777) at 2000 mg/kg bw, the limit dose, by gavage. The maximum tolerated dose was confirmed to be greater than the limit dose of 2000 mg/kg bw in both sexes, and, as there was no difference in toxicity between the sexes, the main study was conducted in males only. Proof of bone marrow exposure to AMBA was confirmed as part of the range-finding study via LC-MS/MS analysis using the whole blood of animals given AMBA.

For the main study, six male Crl:WI(Han) rats were dosed with AMBA at 0, 500, 1000 or 2000 mg/kg bw by gavage. In a positive control group, six male rats were given a single 15 mg/kg bw dose of cyclophosphamide monohydrate by gavage. Bone marrow was harvested from all range-finding and main study animals approximately 24 hours after the final dose administration, and smears were prepared. The stained slides prepared for the main study were coded, 6000 polychromatic erythrocytes per animal were scored for the presence of micronuclei, and the group frequencies were statistically analysed.

There were no statistically significant increases in micronucleus frequency in male rats administered AMBA at any dose.

AMBA gave negative results in the rat bone marrow micronucleus assay in vivo (Dunton, 2016).

2.2 MNBA

(a) Reproductive toxicity

In a two-generation reproductive toxicity study, Crl:WI(Han) rats (30 of each sex per group) were given MNBA (purity 99.8%; lot/batch no. 694472) in 1% (w/v) aqueous carboxymethylcellulose vehicle at a dose of 0, 100, 300 or 1000 mg/kg bw per day via oral gavage. Evaluations of the male and female rat reproductive systems included gonadal function, estrous cycle, mating behaviour, conception, gestation, parturition, lactation and weaning, and the growth and development of the offspring over two successive generations.

There were no treatment-related changes in clinical signs, body weight or feed consumption in either generation. Kidney weights (absolute and relative to body weight) were increased at 1000 mg/kg bw per day in male rats in the parental generation; however, the increase was not statistically significant. At 1000 mg/kg bw per day, organ weights of bilateral kidneys were statistically significantly increased in males and females of the first filial generation. However, their magnitudes were slight (males: 9%

and 8% for left and right absolute kidney weights; 8% and 6% for left and right relative kidney weights; females: 6% and 5% for left and right absolute kidney weights). No corresponding histopathological changes were observed in the kidney in either generation. Therefore, the slight increases in kidney weights were not considered to be toxicologically relevant. There were no effects on reproductive function or performance, mating behaviour, conception or pup development at any dose. In addition, there were no macroscopic findings or histopathological changes related to the test substance at any dose, as evaluated in either adults or offspring.

The no-observed-adverse-effect level (NOAEL) for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested (Gilmore, 2016).

(b) Developmental toxicity

In a developmental toxicity study, female Crl:WI(Han) rats (22 per group) were given MNBA (purity 99.8%; lot/batch no. SMO3C0689) suspended in 1% (w/v) aqueous carboxymethylcellulose vehicle at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage once daily from day 6 to day 19 of gestation. During the study, clinical observations were recorded, and body weights and feed intake were measured. On day 20 of gestation, the dams were killed, and the live fetuses were removed from the uterus and weighed. The sex of the fetuses was determined, and the fetuses were examined for external, visceral, skeletal and cartilaginous abnormalities. Placental and gravid uterine weights were also recorded.

There were no deaths during the study, and no clinical observations considered to be related to MNBA treatment were noted. Overall mean body weight gain was slightly lower (within 10%) at 1000 mg/kg bw per day, but there was no statistical significance or dose–response relationship. Feed intake at 1000 mg/kg bw per day was statistically significantly decreased only from gestation day 15 to gestation day 18, but the decrease was very slight in magnitude (25.1, 23.5, 22.7 and 23.2 g at 0, 100, 300 and 1000 mg/kg bw per day, respectively). As this slight decrease in feed intake had minimal effects on body weight, it was not considered to be adverse. There were six non-pregnant females, two in each of the groups given 100, 300 or 1000 mg/kg bw per day. The uterine and fetal data were unaffected by the treatment. There was no adverse effect of MNBA on the incidence of major or minor external, visceral, skeletal or cartilaginous fetal abnormalities or variations.

The NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Pottle, 2016).

2.3 Comparison of toxicity of metabolites with toxicity of parent compound

The toxicity profiles for the metabolites AMBA and MNBA and mesotrione are compared in Table 2.

Table 2. Comparison of toxicity profiles for mesotrione and its metabolites AMBA and MNBA

	LOAEL/NOAEL		
Study end-point	**Mesotrione**	**AMBA**	**MNBA**
Acute toxicity (rats)	LD_{50} >5 000 mg/kg bw	LD_{50} >5 000 mg/kg bw	LD_{50} >5 000 mg/kg bw
Primary toxicity profiles	Effects on eye, kidney, liver and thyroid due to inhibition of 4-HPPD	Covered by in situ production in MNBA studies	Minor clinical chemistry changes
Ninety-day toxicity (rat)	0.63/0.41 mg/kg bw per day	ND	263.7/50.6 mg/kg bw per day

Study end-point	LOAEL/NOAEL		
	Mesotrione	**AMBA**	**MNBA**
Genotoxicity	Negative in in vivo micronucleus assay	Negative in in vivo micronucleus assay	Negative in in vitro and in vivo assays, including micronucleus assay
Reproductive toxicity (rat)			
Parental toxicity	1.1/0.3 mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Reproductive toxicity	297/11.7 mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Offspring toxicity	0.3/– mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Developmental toxicity (rat)			
Maternal toxicity	100/– mg/kg bw per day	ND	–/1 000 mg/kg bw per day
Embryo/fetal toxicity	100/– mg/kg bw per day	ND	–1 000 mg/kg bw per day

/–: no NOAEL (i.e. effects observed at all doses); –/: no LOAEL (i.e. NOAEL is the highest dose tested); 4-HPPD: 4-hydroxyphenylpyruvate dioxygenase; AMBA: 2-amino-4-methylsulfonylbenzoic acid; bw: body weight; LD_{50}: median lethal dose; LOAEL: lowest-observed-adverse-effect level; MNBA: 2-nitro-4-methylsulfonylbenzoic acid; ND: no data; NOAEL: no-observed-adverse-effect level

Source: Annex 1, reference *133*, and current monograph

Based on these toxicity profiles, it is apparent that these two metabolites are of much lower toxicity than the parent compound, consistent with their very weak 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibiting activities. The Meeting noted that although there were no repeated-dose studies on AMBA, its toxicity was considered to be addressed by studies with MNBA, owing to the high levels of AMBA detected following dosing with MNBA.

Comments

Biochemical aspects of metabolites

Systemic exposure to AMBA was confirmed following a single oral gavage administration of MNBA to rats at 75 mg/kg bw. Exposure to AMBA was 2- to 3-fold higher than exposure to MNBA based on blood $AUC_{0–24\,h}$. Both metabolites were detected in red blood cells and plasma (Punler, 2017).

Toxicological data on metabolites

AMBA (plant, livestock and rat metabolite)

AMBA was negative in an in vivo micronucleus assay in rats. Taken together with the results of the in vitro genotoxicity tests evaluated by the 2014 Meeting (Annex 1, reference *133*), the current Meeting concluded that AMBA is unlikely to be genotoxic in vivo.

MNBA (plant, livestock and rat metabolite)

In a two-generation reproductive toxicity study in rats treated with MNBA at 0, 100, 300 or 1000 mg/kg bw per day via oral gavage, the NOAEL for parental, offspring and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested (Gilmore, 2016).

In a developmental toxicity study in rats treated with MNBA at 0, 100, 300 or 1000 mg/kg bw per day by gavage from day 6 to day 19 of gestation, the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Pottle, 2016).

Toxicological evaluation

The Meeting concluded that it was not necessary to revise the ADI or establish an ARfD for mesotrione. In addition, the Meeting confirmed the previous conclusion by the 2014 JMPR that MNBA and AMBA are unlikely to be a safety concern.

Critical end-points for setting guidance values for exposure to mesotrione metabolites

Studies on metabolites	
MNBA	
Systemic exposure to MNBA and AMBA in the rat following oral exposure to MNBA	Administration of MNBA leads to systemic exposure to both MNBA and AMBA
Two-generation reproductive toxicity study	
Parental toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Offspring toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Reproductive toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity study	
Maternal toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Embryo/fetal toxicity NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
AMBA	
In vivo rat micronucleus assay	No evidence of genotoxicity

References

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