5. Evaluation of data for acceptable daily intake and acute reference dose for humans, maximum residue levels and other values

5.1 ACETOCHLOR (280)

TOXICOLOGY

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide) was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2015, when an acceptable daily intake (ADI) of 0–0.01 mg/kg body weight (bw) and an acute reference dose (ARfD) of 1 mg/kg bw were established (Annex 2, reference *136*).

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

In order to predict the genotoxicity of metabolite A (acetochlor *tert*-sulfinyllactic acid) and metabolite B (acetochlor 1-hydroxyethyl *sec*-oxanilic acid), which are soybean metabolites, the sponsor provided in silico data on general toxicity and genotoxicity for acetochlor, metabolites A and B and related metabolites considered by the 2015 Meeting. The present Meeting applied this information to the "Plant and animal metabolite assessment scheme" of JMPR for metabolites A and B.

Toxicological data on metabolites

In silico predictions of the general toxicity and genotoxicity of metabolites A and B were submitted to the present Meeting. The predictions were prepared using Derek Nexus, Leadscope and the Organisation for Economic Co-operation and Development (OECD) Quantitative Structure–Activity Relationship (QSAR) Toolbox.

Based on their structural similarity to the metabolites of acetochlor evaluated by the 2015 JMPR, metabolites A and B were predicted to be less toxic than acetochlor on the basis of likely lower systemic absorption following oral exposure, rapid excretion, minimal metabolism and lack of tissue distribution or localization.

Metabolite A was predicted to be non-genotoxic on the basis of modelling using both Derek Nexus and Leadscope, a lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox and a literature search of substances containing the hydroxycarboxylic acid functional group, which found only non-genotoxic substances.

Metabolite B was predicted to be non-genotoxic on the basis of modelling using both Derek Nexus and Leadscope and the lack of genotoxicity alerts of potential concern identified by the OECD QSAR Toolbox.

Toxicological evaluation

On the basis of in silico data, the Meeting concluded that metabolites A (acetochlor *tert*-sulfinyllactic acid) and B (acetochlor 1-hydroxyethyl *sec*-oxanilic acid) are unlikely to be genotoxic. Following the "Plant and animal metabolite assessment scheme" of JMPR, the Meeting concluded that, for chronic toxicity, these two metabolites could be assessed using the threshold of toxicological concern (TTC) approach. Both metabolites are categorized in Cramer class III, and therefore a TTC of 1.5 µg/kg bw per day applies.

The Meeting concluded that the information provided was insufficient to conclude definitively on the general toxicity of metabolites A and B relative to that of acetochlor.

An addendum to the toxicological monograph was prepared.

RESIDUE AND ANALYTICAL ASPECTS

Acetochlor is a selective herbicide belonging to the chloroacetanilide class that was first and last evaluated for residues and toxicological aspects by the 2015 JMPR, when an ADI of 0–0.01 mg/kg bw and an ARfD of 1 mg/kg bw were established. The residue definition for compliance with the MRL and for dietary risk assessment (for animal and plant commodities) is the sum of compounds hydrolysable with base to 2-ethyl-6-methylaniline (EMA) and 2-(1-hydroxyethyl)-6-methylaniline (HEMA), expressed in terms of acetochlor. The residue is not fat soluble.

Acetochlor was scheduled at the Fiftieth Session of the CCPR for the evaluation of additional uses by the 2019 Extra JMPR. The Meeting received new information on metabolism in soya bean, analytical method data, and residue trials on soya bean and alfalfa (forage and hay).

Metabolism in plants

The present Meeting received information on the identification of metabolites in soya bean seed extracts from a metabolism study on soya beans after pre-plant or post-emergence applications that had been previously evaluated by the Meeting. The identified acetochlor metabolites were its *tert*-sulfinylacetic acid, *tert*-sulfinyllactic acid and 1-hydroxyethyl *sec*-oxanilic acid, which were also previously identified in soya bean feed commodities. These metabolites are covered by the current definition of the residue based on the common moieties EMA and HEMA.

Methods of analysis

The methods developed to quantify residues of acetochlor in plant and animal matrices involve hydrolytic conversion of metabolites to the EMA or HEMA chemophores, which are quantified and expressed as total acetochlor residues. They involve extraction with methanol/water mixture, followed by hydrolysis of residues with aqueous hydroxide solution. The main differences between the previous and the new methods are the clean-up conditions, sample sizes and instrumentation for quantification (LC-MS/MS in more recent versions). LOQs are typically 0.025 mg/kg each for EMA and HEMA. Representative compounds that generate EMA (*tert*-sulfonic acid) and HEMA (1-hydroxyethyl-tert-oxanilic acid) on base hydrolysis are used as reference materials for fortification and method validation. The methods are suitable for analysis of acetochlor and related metabolites in plant and animal matrices.

Stability of residues in stored analytical samples

The stability of incurred residues analysed as EMA and HEMA in the soya bean samples after more than eight years of frozen storage was estimated based on the analysis conducted when the study was performed (2007/8) and when the samples were again analysed in 2016. The results were submitted to the present Meeting. On average (n=8), the percent remaining was 122% for EMA and 149% for HEMA, probably due to modifications in the LC-MS/MS analytical method used in the original study. The Meeting concluded that acetochlor residues in soya bean seeds are stable for at least 8 years.

In 2015, JMPR concluded that acetochlor residues were also stable in several plant matrices including alfalfa forage and clover hay for at least 330 days under freezer storage conditions (-20 ºC).

Results of supervised residue trials on crops

Soya bean, dry

The critical GAP for acetochlor on soya bean in the USA is pre-plant/pre-emergence, and post-emergence (before the R2 growth stage, full flowering) at up to 1.7 kg ai/ha and not exceeding a maximum rate per year of 3.4 kg ai/ha. Supervised trials were conducted in the USA in 2007. In 13 independent trials conducted according to GAP, total residues in soya bean seeds were < 0.05, 0.05, 0.10, 0.11, 0.12, 0.14, 0.15, 0.19, 0.20, 0.22, 0.23, 0.25 and 0.91 mg/kg.

The Meeting estimated a maximum residue level of 1.5 mg/kg and a STMR of 0.15 mg/kg for soya bean, dry.

Alfalfa

The critical GAP for acetochlor in alfalfa in the USA is pre-plant/at-planting/pre-emergence and post-emergence (up to or at the 4th-trifoliate stage - new stands - or following spring green-up - fall-planted or established stands - or between cuttings), with a maximum rate of 3.4 kg ai/ha per year and a PHI of at least 20 days. Supervised trials were conducted in the USA in 2013 and 2014. In eight trials conducted according to GAP, total residues in alfalfa forage were 0.82, 0.92, 1.1, 1.9, 2.5, 2.9, 4.0 and 5.8 mg/kg, and in alfalfa hay were 2.0, 2.8 (2), 4.4, 4.7, 5.0, 6.9 and 13.0 mg/kg (fresh weight basis).

The Meeting estimated a maximum residue level of 30 mg/kg (dry basis) for alfalfa hay.

The Meeting withdrew the previous recommendation for legume animal feed of 3 mg/kg and recommended a maximum residue level of 3 mg/kg for legume animal feed, except alfalfa hay.

The Meeting also estimated a median residue of 4.55 mg/kg and a highest residue of 13 mg/kg for alfalfa hay (fresh weight basis), a median residue of 2.2 mg/kg and a highest residue of 5.8 mg/kg for alfalfa forage.

Fate of residues during processing

The processing factors for soya bean oil, meal and hulls estimated by the 2015 JMPR are 0.11, 1.2 and 0.72, respectively. Therefore, considering a STMR of 0.15 mg/kg for soya bean seeds, the Meeting estimated a STMR-P of 0.016 mg//kg for soya bean oil, a median residue of 0.18 mg/kg for soya bean meal and of 0.108 mg/kg for soya bean hulls.

Animal feedstuffs

Estimation of livestock dietary burdens

Dietary burden calculations for beef cattle, dairy cattle and poultry are provided below. The dietary burdens were estimated using the OECD diets listed in Appendix IX of the 3rd edition (2016) of the FAO Manual. Considering the items estimated by the 2015 and present JMPR, livestock dietary burdens were estimated for cattle and poultry.

Summary of livestock dietary burden (ppm acetochlor equivalents of dry matter diet)

	US-Canada		EU		Australia		Japan	
Commodity	**Max**	Mean	**Max**	Mean	**Max**	Mean	**Max**	Mean
Beef cattle	2.33	0.87	12.27	4.47	16.57[a]	6.29[c]	1.63	0.68
Dairy cattle	4.28	1.43	7.77	2.65	10.75[b]	3.87[d]	3.82	1.44
Poultry - broiler	0.11	0.11	0.16	0.16	0.10	0.10	0.08	0.08
Poultry - layer	0.11	0.11	0.61[e]	0.18[f]	0.10	0.10	0.07	0.07

[a] Highest maximum beef or dairy cattle dietary burden suitable for maximum residue level estimated for mammalian tissues

[b] Highest maximum dairy cattle dietary burden suitable for maximum residue level estimated for mammalian milk

[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimated for mammalian tissues.

[d] Highest mean dairy cattle dietary burden suitable for STMR estimated for milk.

[e] Highest maximum poultry dietary burden suitable for maximum residue level estimated for poultry tissues and eggs.

[f] Highest mean poultry dietary burden suitable for STMR estimated for poultry tissues and eggs.

Animal commodity maximum residue levels

Based on the estimated dietary burden and the results of farm animal feeding studies evaluated by the 2015 JMPR, the calculations used to estimate highest total residues for use in estimating maximum residue levels, STMR and HR values are shown below.

	Feed level	Residues	Feed level	Residues (mg/kg) in			
	(ppm) for milk residues	(mg/kg) in milk	(ppm) for tissue residues	Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study [a]	-		15	-	< 0.02	0.04	-
	50	< 0.02	50	< 0.02	0.02	0.09	<0.02
Dietary burden and high residue	10.75	< 0.0043	16.57	<0.0007	0.02	0.0418	<0.02
STMR beef or dairy cattle							
Feeding study[b]			5	-	-	<0.02	-
			15	-	<0.02	0.03	-
	50	< 0.02	50	<0.02	0.02	0.07	<0.02
Dietary burden and median residue estimate	3.87	< 0.0015	6.29	<0.0025	0.02	0.0213	<0.0025

[a] Highest residues for tissues and mean residues for milk

[b] Mean residues for tissues and mean residues for milk

The Meeting estimated a maximum residue level of 0.05 mg/kg, a STMR of 0.0213 mg/kg and a HR of 0.0418 mg/kg for edible offal (mammalian) to replace the previous recommendation.

The Meeting confirmed its previous recommendations for meat (mammalian except marine mammals), mammalian fat (except milk fat) and milks.

No residues were observed in eggs and poultry tissues on dosing laying hens at up to 50 ppm in the diet for 28 days. Considering the poultry dietary burden of 0.61 ppm (highest maximum) and 0.18 ppm (highest mean), the Meeting confirmed its previous recommendation for poultry commodities.

RECOMMENDATIONS

On the basis of the data obtained from supervised trials the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Residue definition for compliance with the MRL and for dietary risk assessment for plant and animal commodities: *Sum of compounds hydrolysable with base to 2-ethyl-6-methylaniline (EMA) and 2-(1-hydroxyethyl)-6-methylaniline (HEMA), expressed in terms of acetochlor*.

The residue is not fat soluble

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for acetochlor is 0–0.01 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for acetochlor were estimated for the 17 GEMS/Food Consumption Cluster diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2019 Extra JMPR Report.

The IEDIs accounted for 0 to 4% of the maximum ADI. The Meeting concluded that the long-term dietary exposure to residues of acetochlor from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The ARfD for acetochlor is 1 mg/kg bw. The International Estimate of Short Term Intakes (IESTIs) for acetochlor were calculated for the food commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting. The results are shown in Annex 4 of the 2019 Extra JMPR Report.

The IESTIs were 0% of the ARfD for the general population and for children. The Meeting concluded that the acute dietary exposure to residues of acetochlor from uses considered by the present Meeting is unlikely to present a public health concern.

Threshold of toxicological concern (TTC) approach for metabolites

Acetochlor *tert*-sulfinyllactic acid and acetochlor 1-hydroxyethyl *sec*-oxanilic acid are unlikely to be genotoxic, and could be assessed using the TTC Cramer Class III of 1.5 µg/kg bw per day.

The metabolites acetochlor tert-sulfinyllactic acid and acetochlor 1-hydroxyethyl sec-oxanilic acid were identified in metabolism studies, found in maize grain, soya bean seed and poultry commodities (<10% TRR). They belong to the group of metabolites that are hydrolysed in the analytical methods for plant and animal commodities to form EMA and HEMA.

The maximum IEDI calculated for acetochlor (based on total EMA and HEMA) from commodities considered by the JMPR (Annex 3) was 0.385 µg/kg bw. The Meeting concluded that dietary exposure to residues of acetochlor tert-sulfinyllactic acid and acetochlor 1-hydroxyethyl sec-oxanilic acid from the uses considered by the JMPR is unlikely to present a public health concern.