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Pesticide residues in food 2007

Joint FAO/WHO Meeting on Pesticide Residues

REPORT 2007

World Health Organization

Food and Agriculture Organization of the United Nations

Pesticide residues in food 2007

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Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues
Geneva, Switzerland, 18–27 September 2007

WORLD HEALTH ORGANIZATION
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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CONTENTS

T, toxicological evaluation; R, residue and analytical aspects; D, dietary risk assessment

* New compound

** Evaluated within the Periodic Re-evaluation Programme of the Code Committee on Pesticide Residues

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2007 Joint FAO/WHO Meeting on Pesticide Residues

GENEVA, 18–27 SEPTEMBER 2007

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ABBREVIATIONS

ADI	acceptable daily intake
ai	active ingredient
ARfD	acute reference dose
AST	aspartate aminotransferase
AUC	area under the curve for concentration–time
$BMDL_{10}$	benchmark-dose lower 95% confidence level
bw	body weight
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCN	Codex classification number (for compounds or commodities)
CCPR	Codex Committee on Pesticide Residues
CSAF	chemical-specific assessment factor
C_{max}	maximum concentration
EC_{50}	the concentration of agonist that elicits a response that is 50% of the possible maximum
F_1	first filial generation
F_2	second filial generation
FAO	Food and Agricultural Organization of the United Nations
GAP	good agricultural practice
GC	gas chromatography
GGT	gamma-glutamyltransferase
GEMS/Food	Global Environment Monitoring System–Food Contamination Monitoring and Assessment Programme
GnRH	gonadotropin-releasing hormone
hCG	human chorionic gonadotrophin hormone
HPLC	High Performance Liquid Chromatography
HR	highest residue in the edible portion of a commodity found in trials used to estimate a maximum residue level in the commodity
HR-P	highest residue in a processed commodity calculated by multiplying the HR of the raw commodity by the corresponding processing factor
IC_{50}	concentration required to inhibit activity by 50%
IEDI	international estimated daily intake
IESTI	international estimate of short-term dietary intake
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry

JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint Meeting on Pesticide Residues
JMPS	Joint FAO/WHO Meeting on Pesticide Specifications
LC	liquid chromatography
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LH	luteinising hormone
LOAEC	lowest-observed-adverse-effect concentration
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
MTD	maximum tolerated dose
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MEQ	methylethoxyquin
MIC	minimum inhibitory concentration
MRL	maximum residue limit
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
OECD	Organization for Economic Co-operation and Development
PHI	pre-harvest interval
PPARα	peroxisome proliferator-induced receptor alpha
ppm	parts per million
STMR	supervised trials median residue
STMR-P	supervised trials median residue in a processed commodity calculated by multiplying the STMR of the raw commodity by the corresponding processing factor
TIPA	triisopropylammonium
TLC	thin-layer chromatography
THPI	1,2,3,6-tetrahydrophthalimide
TRR	total radiolabelled residue
TSH	thyroid stimulating hormone
TMDI	theoretical maximum daily intake
WHO	World Health Organization

USE OF JMPR REPORTS AND EVALUATIONS BY REGISTRATION AUTHORITIES

Most of the summaries and evaluations contained in this report are based on unpublished proprietary data submitted for use by JMPR in making its assessments. A registration authority should not grant a registration on the basis of an evaluation unless it has first received authorization for such use from the owner of the data submitted for the JMPR review or has received the data on which the summaries are based, either from the owner of the data or from a second party that has obtained permission from the owner of the data for this purpose.

PESTICIDE RESIDUES IN FOOD

REPORT OF THE 2007 JOINT FAO/WHO MEETING OF EXPERTS

1. INTRODUCTION

A Joint FAO/WHO Meeting on Pesticide Residues (JMPR) was held at the headquarters of the World Health Organization (WHO), Geneva, Switzerland, from 18 to 27 September 2007. The Meeting brought together the Food and Agriculture Organization of the United Nations (FAO) Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group.

The Meeting was opened by Dr Maria Neira, Director, WHO, on behalf of the Director-General of WHO and the Director-General of FAO. Dr Neira acknowledged the important role played by the work of the Meeting in the establishment of international food safety standards and its contribution to sustainable development. She informed the Meeting of the six-point agenda (promoting development, fostering health security, strengthening health systems, harnessing research, information and evidence, enhancing partnerships, and improving performance) that the new Director-General of WHO, Dr Margaret Chan, had proposed for the organization. That agenda also referred to using evidence to define strategies and measure results, and in that context the work of the Meeting was an important contribution to the goals of WHO. Dr Neira informed the Committee that the 50th World Health Assembly had approved an increased budget for food safety and nutrition and for public health and the environment, which illustrated the importance given by the Member States to these areas of work. She emphasized that FAO and WHO considered the provision of scientific advice in food safety an important and core activity.

The Meeting was held in pursuance of recommendations made by previous Meetings and accepted by the governing bodies of FAO and WHO that studies should be undertaken jointly by experts to evaluate possible hazards to humans arising from the occurrence of residues of pesticides in foods. The reports of previous Meetings (see Annex 5) contain information on acceptable daily intakes (ADIs), acute reference doses (ARfDs), maximum residue limits (MRLs), and the general principles that have been used for evaluating pesticides. The supporting documents (residue and toxicological evaluations) contain detailed monographs on these pesticides and include evaluations of analytical methods.

During the Meeting, the FAO Panel of Experts was responsible for reviewing residue and analytical aspects of the pesticides under consideration, including data on their metabolism, fate in the environment, and use patterns, and for estimating the maximum levels of residues that might occur as a result of use of the pesticides according to good agricultural practice. The estimation of MRLs and supervised trials median residue (STMR) values for commodities of animal origin was elaborated. The WHO Core Assessment Group was responsible for reviewing toxicological and related data in order to establish ADIs, and ARfDs, where necessary and possible.

The Meeting evaluated 31 pesticides, including 6 new compounds and 10 compounds that were reviewed within the Periodic Re-evaluation Programme of the Codex Committee on Pesticide Residues (CCPR) for toxicity or residues, or both. Toxicological evaluation and development of MRLs was also performed for 12 additional pesticides

The Meeting established ADIs and ARfDs, estimated MRLs and recommended them for use by the CCPR, and estimated STMR and highest residue (HR) levels as a basis for estimating dietary intakes.

The Meeting also estimated the dietary intakes (both short-term and long-term) of the pesticides reviewed and, on this basis, performed a dietary risk assessment in relation to their ADIs or ARfDs. Cases in which ADIs or ARfDs may be exceeded were clearly indicated in order to facilitate the decision-making process by the CCPR. The rationale for methodologies for long-term and short-term dietary risk assessment are described in detail in the reports of the 1997 JMPR (Annex 5,

reference *80*, section 2.3) and 1999 JMPR (Annex 5, reference *86*, section 2.2). Additional considerations are described in the report of the 2000 JMPR (Annex 5, reference *89*, sections 2.1–2.3).

The Meeting also considered a number of general issues addressing current issues related to the risk assessment of chemicals, the evaluation of pesticide residues and the procedures used to recommend maximum residue levels.

The Meeting responded to a number of specific concerns raised by CCPR.

The tentative agenda on the list of compounds for evaluation by the 2007 JMPR was amended as followings:

- Due to the lack of data in support of the residue evaluation of permethrin and the toxicological evaluation of vinclozolin, these compounds were removed from the agenda;
- In response to the 39th CCPR, residue evaluations of profenofos and dimethoate were rescheduled to the 2008 JMPR;
- The residue evaluation of tebuconazole was postponed to 2008 due to unforeseen circumstances.

1.1 DECLARATION OF INTEREST

The Secretariat informed the Committee that all experts participating in the 2007 JMPR had completed declaration-of-interest forms, and that no conflicts had been identified. The Meeting was informed of the following potentially relevant interests: Professor Boobis and Dr McGregor were or had been consulting on compounds not on the agenda of this meeting, but for companies that had submitted data for other compounds evaluated by this meeting; Professor Ray's research group had received funding from the pesticide industry for mechanistic studies on the toxicity of certain pyrethroids, none of which were on the agenda of the present Meeting.

2. GENERAL CONSIDERATIONS

2.1 SHORT-TERM DIETARY INTAKE ASSESSMENT: FURTHER CONSIDERATIONS

The 2006 Meeting had discussed the uncertainties in the calculation and interpretation of international estimated short-term intake (IESTI) (Annex 5, reference *107*, general item 2.4). In this context, the present Meeting welcomed the publication of an Opinion by the European Food Safety Authority (EFSA) on 'Acute dietary intake assessment of pesticide residues in fruit and vegetables'.[1]

The Meeting acknowledged the usefulness of the detailed analysis performed by EFSA, which was based on supervised field-trial data provided to the European Union, available national monitoring data and food-consumption databases from nine European countries covering the Global Environment Monitoring System—Food Contamination Monitoring and Assessment Programme (GEMS/Food) cluster diets F (Scandinavian), E (central European) and B (Mediterranean). Inter alia, the EFSA Opinion addressed the effect of choosing the default value of 3 for the variability factor and also the effect of replacing the highest residue (HR) in the IESTI equation by the maximum residue limit (MRL).

The MRL is the maximum concentration of a pesticide residue that the Codex Alimentarius Commission recommends should be legally permitted in a food.[2] MRLs are based on data on good agricultural practice (GAP) and foods derived from commodities that comply with the respective MRLs are intended to be safe for human consumption.[2] Whether a particular food commodity is safe for human consumption is assessed by comparing estimated extreme (high-end) intakes using the IESTI equation and comparing the estimated intake with the ARfD.

MRLs are based on maximum residue levels (a residue definition for enforcement purposes) estimated by JMPR as the maximum concentration of residue expected in food commodities when GAP is used. The HR is the highest residue value (a residue definition for risk-assessment purposes) in the edible portion of a food commodity and is estimated as the highest concentration of residue occurring in samples from valid supervised trials at maximum GAP. Maximum residue levels and HRs are derived from the same set of supervised field trials, in which residues have ideally been measured both in the whole commodity and in the edible portion. When no information is available on the residue in the edible portion, the residue in the whole commodity can be used as a worst-case estimate, provided that the residue definitions for enforcement and risk assessment are the same. When the residue definitions are the same and the whole food commodity is the edible portion, the maximum residue level is typically higher than the HR.

The Meeting noted that in the EFSA analysis, replacing the HR with the MRL in the IESTI equation had a slightly greater effect on the overall distribution of the percentage of person-days with intakes at or below the ARfD than did changing the variability factor from 7 or 5 to 3, the value used by JMPR since 2003. However, the Meeting noted that the intake on the vast majority of person-days would be less than the ARfD.

There is a public perception that small differences in estimated intake are real differences in terms of food safety (e.g., 120% ARfD is unacceptable, 80% ARfD is acceptable). Such differences could potentially arise from use of the HR versus the MRL in the IESTI equation. However, there is conservatism in the derivation of the ARfD and in the estimation of intake. For example, a safety

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[1] European Food Safety Authority (2007) *Opinion of the Scientific Committee on plant protection products and their residues on acute dietary intake of pesticide residues in fruit and vegetables*. Adopted on 19 April 2007 (http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178629328713.htm).

[2] Joint FAO/WHO Codex Alimentarius Commission (2006) *Codex Alimentarius Commission Procedural Manual*, 16th edition. FAO, Rome, (http://www.codexalimentarius.net/web/procedural_manual.jsp).

factor for inter-individual variation is included when the ARfD is established, and as such the ARfD is designed to protect those individuals at the upper-end of human susceptibility. The Meeting further noted that there is likely to be very limited overlap between the population with the greatest sensitivity to a particular pesticide and the population with estimated intake of residues greater than the ARfD. Therefore in cases where the ARfD is exceeded, additional considerations should be taken into account, e.g., the amount by which the ARfD is exceeded, the basis on which the ARfD has been established likely conservatism and possible consequences, and the uncertainties in the estimate of intake.

The Meeting concluded that, overall, IESTI using the HR as an input is a satisfactory indicator for assessing the acceptability of MRLs for the assessment of short-term dietary intake. However, from the perspective of public perception there may be benefits in estimating the IESTI from the MRL.

If using the MRL in the IESTI equation, adjustments and alternatives would be needed in situations where the edible portion is different from the commodity to which the MRL applies, where the risk-assessment definition is different from the enforcement definition and in situations where there are no detectable residues in the edible portions.

The Meeting reiterated its recommendation from 2006 that FAO and WHO should host a consultation to address the issues identified in the reports of the present Meeting and of the Meeting of the previous year, with the participation of relevant stakeholders. The main objectives would be the continued refinement of the estimation of the short-term dietary intake of pesticides and of the interpretation of the outcome of short-term dietary risk assessment conducted by JMPR. The Meeting recommended investigation of the practicalities of using the MRL in IESTI calculations, because allowance would be needed for residues in edible portions, for the risk-assessment residue definition and in situations where no residues are detectable in the edible portion. Furthermore, the issue of whether it is appropriate to use the IESTI equations for evaluating the safety of individual consignments should be further investigated. The discussion should include how to improve communication between JMPR and risk managers and the public on the output of the risk assessment conducted by the Meeting.

2.2 CODEX MAXIMUM RESIDUE LIMITS FOR COMPOUNDS NO LONGER SUPPORTED BY COMPANIES/SPONSORS

When a pesticide is scheduled under the Periodic Re-evaluation programme for review, the entire toxicology and residue chemistry data bases must be supplied to the JMPR by the sponsors, usually the manufacturer(s). Recently two scheduled periodic re-evaluations could not be conducted because companies declined to support the review or to supply the necessary studies to FAO and WHO. Vinclozolin and permethrin had to be removed from the JMPR schedules because toxicology or residue studies, respectively, were not provided. In other instances, only partial data packages were submitted, for example, support of only one isomeric mixture of a pesticide which is marketed as two or more different isomeric mixtures.

The JMPR recommendations are based only on the results of the scientific assessment of the data supplied. In the absence of sufficient toxicological and residue data the Meeting cannot make recommendations for maximum residue levels. The importance of complete data submissions was addressed by the 2006 JMPR (General Consideration 2.1, JMPR Report 2006). It is the prerogative of the CCPR to accept or reject those recommendations, including recommendations to withdraw previous maximum residue levels suitable for use as MRLs. The CCPR has the option to consider other factors that it deems appropriate in retaining MRLs.

2.3 TOXICOLOGICAL RELEVANCE OF TRIAZOLE FUNGICIDES AND THEIR COMMON METABOLITES

The following triazole fungicides have been evaluated by the Meeting:

- Bitertanol
- Difenoconazole
- Fenbuconazole
- Flusilazole
- Hexaconazole
- Myclobutanil
- Penconazole
- Propiconazole
- Tebuconazole
- Triadimefon + Triadimenol

Variable amounts of the common metabolites, 1,2,4-triazolyl acetic acid and 1,2,4-triazolyl alanine are formed in plants, and of 1,2,4-triazole in plants and animals. The amount of 1,2,4-triazole found in rat urine varies from approximately 1% to 65% of the dose administered, depending on the parent compound.

1,2,4-Triazolyl alanine was evaluated toxicologically by the 1989 JMPR. The Meeting at that time concluded from the available data that residues of triazole alanine arising from the use of triazole fungicides do not present a toxicological hazard.

There was less information available on 1,2,4-triazolyl acetic acid, but it is likely to have low toxicity similar to 1,2,4-triazolyl alanine.

1,2,4-Triazole exhibits a number of toxicological effects, but the no-observed-adverse-effect levels (NOAELs) for relevant end-points were higher than those for the respective parent compounds.

In 2004, the Meeting evaluated triadimenol and triadimefon. In this context the plant metabolites 1,2,4-triazole, 1,2,4-triazolyl acetic acid and 1,2,4-triazolyl alanine were also evaluated. The following conclusion was reached:

> Since 1,2,4-triazolyl alanine and 1,2,4-triazolyl acetic acid were of low systemic toxicity and developmental effects with 1,2,4-triazole occur at doses of ≥ 100 mg/kg bw per day, these metabolites were judged not to pose an additional risk to humans.

In a situation in which the metabolites arise from multiple triazole fungicides, they cannot be included in the residue definition. Since the metabolites cannot be linked to a specific triazole fungicide, they would have to be evaluated on their own. The Meeting did not have sufficient information to judge levels that would be without potential effect in consumers. However, the Meeting is aware that a number of studies of toxicity are available on the three common triazole metabolites. Therefore the Meeting recommended that a full evaluation of these metabolites should be performed.

In addition to the possibility of combined exposure to common triazole metabolites, the Meeting wished to draw attention to the possibility of combined exposures to more than one parent triazole. Some, but not all, of the triazole fungicides cause certain toxicological effects (e.g., developmental) that may share a common mode of action. Hence, this should ideally be taken into account when performing a refined intake assessment. The Meeting was aware of ongoing national and regional work on common mechanism groups for triazole fungicides and would welcome regular updates on this. The Meeting therefore recommended that work be undertaken to identify which triazole fungicides should be considered together in a cumulative risk assessment. This would most likely be undertaken at national or regional level, where adequate intake data would be available.

2.4 SETTING OF REFERENCE VALUES FOR ORGANOPHOSPHORUS PESTICIDES: RELEVANCE OF THE BIOCHEMICAL CHARACTERISTICS OF THE INDIVIDUAL COMPOUNDS

The present Meeting established ADIs and ARfDs for the organophosphorus insecticides azinphos-methyl (a di-methyl organophosphate) and profenofos (an ethyl-isopropyl organophosphate). The ADI was 0–0.03 mg/kg bw for both compounds, while the ARfDs were 0.1 and 1 mg/kg bw for azinphos-methyl and profenofos, respectively. The Meeting noted that the ARfD : ADI ratio is about 3.3 for azinphos-methyl and about 33 for profenofos. The Meeting also noted that the median (of results from available studies) oral median lethal doses (LD_{50}s) for azinphos-methyl and profenofos in rats were 13 mg/kg bw and 620 mg/kg bw, respectively, and hence the LD_{50} : ARfD ratio was about 130 for azinphos-methyl and about 620 for profenofos.

The likely explanation for these unexpectedly large differences between these compounds showing the same mode of action is given below.

General characteristics of inhibition of acetylcholinesterase activity by organophosphorus compounds

The molecular target of organophosphorus insecticides is acetylcholinesterase present in the nervous system. Typical clinical signs appear when acetylcholinesterase activity in the nervous system is inhibited by more than about 50% and, in the absence of treatment with antidote, death occurs when acetylcholinesterase activity is inhibited by more than 90%. Inhibition of brain acetylcholinesterase activity (or of its surrogate, erythrocyte acetylcholinesterase activity) by 20% or more is the relevant end-point with which to identify the NOAEL to be used for establishing the ADI or the ARfD.

The inhibited (organophosphorylated) acetylcholinesterase enzyme may either age (i.e., lose a side-chain of the phosphoryl residue), becoming resistant to spontaneous and pharmacologically-mediated reactivation, or may spontaneously reactivate.

Dimethyl-phosphorylated acetylcholinesterase reactivates with a half-life of about 1 h and ages relatively slowly (with a half-life of about 4 h). Hence most of the inhibited acetylcholinesterase will reactivate within a few hours after peak effect and only a relatively small fraction will age (i.e., will be irreversibly inhibited).

Reactivation of di-ethyl phosphorylated acetylcholinesterase is very slow (with a half-life of about 2 days) and there is no measurable reactivation of di-isopropyl phosphorylated acetylcholinesterase. Hence, essentially all acetylcholinesterase inhibited in this way will age.

Given these differences, it is expected that to reach the same level of effect via accumulation of inhibited acetylcholinesterase will require higher and possibly more doses of a di-methyl organophosphate than of a di-ethyl or di-isopropyl organophosphate.

Azinphos-methyl and profenofos

Table 1 shows the oral LD_{50}s in rats and NOAELs in animals given a single dose or repeated doses of azinphos-methyl or profenofos.

From the table it can be seen that, as expected, the dose of azinphos-methyl that was without significant effect on acetylcholinesterase activity after repeated daily doses was only about three times lower (2 versus 0.7) than the single dose that was without significant effect. On the other hand, the daily non-effective dose of profenofos was about 30 times (100 versus 3) lower than the single non-effective dose. This difference is consistent with the biochemical characteristics of inhibited

acetylcholinesterase as described above. However, differences in toxicokinetic parameters, not discussed here, might also play a role in this difference.

The present Meeting used a safety factor of 10 to set the ADI and the ARfD for azinphos-methyl in the light of the availability of reliable data from humans. Based on these data, the Meeting established an ARfD of 0.1 mg/kg bw and an ADI of 0–0.03 mg/kg bw. No relevant data from humans were available for profenofos and the standard safety factor of 100 was therefore applied to the data from animals.

However, while there is an approximately 130-fold difference between the ARfD and the oral LD_{50} in rats for azinphos-methyl and about a 620-fold difference for profenofos, it should be noted that the ARfD for azinphos-methyl is less uncertain since it is based on data from humans.

The Meeting concluded that due attention should be given to the structure of the organophosphorus compound under evaluation in order to properly understand the results of studies with single or repeated doses. Such understanding, together with the availability of adequate toxicokinetics data, might help in defining the chemical-specific assessment factor and in judging the adequacy of the available toxicological data.

Table 1. Oral LD_{50}s in rats and NOAELs for azinphos-methyl and profenofos

Substance	Median oral LD_{50} in rats[a] (mg/kg bw)	NOAEL in animals		Ratio of NOAEL for single dose versus NOAEL for repeated doses
		Single dose (mg/kg bw)	Repeated doses (mg/kg bw per day)	
Azinphos-methyl	13	2	0.7	2.9
Profenofos	620	100	3	33
Ratio for profenofos : azinphos-methyl	48	50	4.3	—

[a] Median of results for available studies.

2.5 CONSIDERATION OF SELECTION OF RESIDUE DATA FROM SUPERVISED TRIALS

The objective in evaluating residue trial data is to select residue values representing the GAP so as to estimate the maximum, median and high residues occurring in commodities treated according to the maximum GAP.

The estimation of STMR and HR values relies on the selection of residue data from trials within GAP. No more than one data point for each value is selected from each trial. A sufficient number of trials approximating GAP are needed to represent field and cultural practice variability (*FAO Manual* 2nd ed. 71).

When considering residue data from trials any one of the following may apply when several residue values are described as “replicates”:

1. replicate laboratory samples taken from a field sample
2. replicate field samples (each sample is taken randomly through a whole sprayed plot)
3. samples from replicate plots or sub or split-plots (the whole trial is subject to the same spraying operation, but it is divided into 2 or more areas that are sampled separately)
4. samples from replicate trials (trials from the same site that are not independent may be considered as replicate trials)

By definition, the Codex MRLs refer to the average residue in the bulk sample taken according to the Codex sampling procedure (Recommended method of sampling for the determination

of pesticide residues for compliance with MRLs
(www.codexalimentarius.net/download/standards/379/cxs_229.pdf).

Consequently, those results which best represent the average residue in independent trials single samples should be selected.

It can be assumed that the normally large samples taken in supervised trials correspond to the size of bulk samples specified by the Codex procedure. Accordingly the maximum residue level for plant commodities can be estimated from the residues measured in composite samples, having a standard deviation of s_i.

Where the average residue measured in replicate random samples taken from one field would be used as a single residue value the true distribution of the residues would be apparently reduced proportional to the square root of the number of replicate field samples.

According to the sampling theory the standard deviation (also called 'standard error') of mean residue in n samples taken from the populations of "i" samples is:

$$S_{\bar{n}} = \frac{S_i}{\sqrt{n}}$$

Consequently if we use the average of two randomly selected replicate field samples taken from a plot, we reduce the standard deviation of the residues by 1.41.

Based on the above considerations, the Meeting decided to use the highest residues measured in replicate field samples taken from one experimental plot in future evaluations.

The Meeting will continue to apply the procedures described in the FAO Manual for the other situations. The Meeting will calculate the average of the analysis results of replicate test portions (replicate laboratory sample), and will select the highest residue value from the various definitions of replicates as the single value for purpose of identifying the STMR or HR value or recommending the maximum residue level.

2.6 RECONSIDERATION OF ALTERNATIVE GAPS

At the 37th Session of the CCPR in 2005 it was proposed that when an estimated exposure for a particular GAP and pesticide/commodity combination exceeds the ARfD, the JMPR should consider alternative GAPs with adequate supporting field trials (ALINORM 05/28/24; para. 67, 68 and 81). The procedures for examining alternative GAPs are identified by the JMPR as either a retrospective or a prospective approach. These were discussed in 2005 and 2006 by the JMPR and CCPR at its 38th Session (ALINORM 06/29/24; para 29).

At the 39th Session of CCPR in 2007 the delegation of the USA presented a paper which summarizes the suggestions made by the CCPR and JMPR from 2005 to 2006 and proposes an explicit process to be used in future (ALINORM 07/30/24; para.21, 22, 41, 42). The Committee noted that the proposed procedure included a number of activities involving JMPR, and agreed that the document would be forwarded to the 2007 JMPR for consideration and advice. The Committee would also consider the alternative GAP procedure at its next session in the light of the advice received from JMPR (ALINORM 07/30/24, para. 43).

The 2007 JMPR welcomed the document which summarizes the lessons learned from the past and gives guidance on the way to proceed. Since the Meeting noted that it had already used a similar procedure for the retrospective and prospective approach in its residue evaluations in 2006 and 2007, the Meeting agreed with the proposals in general.

However, the Meeting was cautious about the proposal to derive an "acceptable highest residue" for the situation, where an alternative GAP is not available. Prospective approach, #2: "*The JMPR should also indicate an approximate acceptable Highest Residue (HR) as one of the*

conclusions of their analysis, i.e., a value that would yield an acceptable IESTI calculation. This information should be in the JMPR Report. This would provide a benchmark for interested parties and would help to alleviate the submission of non-relevant data to JMPR".

The JMPR's concern is that an "acceptable highest residue" would only consider the ARfD and consumption data. Such a value is not based on an existing GAP and on appropriate supervised residue trials. Residues arising from the use of pesticides in agriculture should generally not be present in food unless there is a GAP and then the estimation of maximum residue levels should be based on supervised residue trials data that support the GAP. Therefore an evaluation of pesticide residue data for exposure estimation and trade purposes has to be made on a scientific basis. A theoretical calculated value cannot be used to estimate a maximum residue level. There is an ethical obligation for data submitters to provide a full data set including all the supervised residue trials data related to the relevant GAPs (General Item 2.1, JMPR Report 2006).

The Meeting emphasized that the work of the JMPR is based on the best available scientific information. JMPR considers for its residue evaluations all aspects of the use and the fate of the pesticide and its residues, which implies that all studies that provide such information are necessary.

2.7 MRLS FOR PROCESSED FOODS (ESTABLISHMENT OF MRLS AND/OR PROCESSING FACTORS FOR PROCESSED AND READY-TO-EAT FOODS)

The 39th CCPR (2007) agreed to refer agenda paper CX/PR 07/39/8 (and other relevant documents such as CRD 22) to the 2007 JMPR on the understanding that the JMPR comments would be considered at the 2008 session of CCPR where it would be decided whether to develop guidelines on the application of processing factors (ALINORM 07/30/24 - Rev. 1).

The current policy of the JMPR is that MRLs for raw agricultural commodities should also apply to all processed foods and feeds derived from them (without adjustment), and that separate MRLs are not recommended for processed commodities unless residues are shown to concentrate during processing.

The Meeting reiterated its support for the existing policy and confirmed that it is impractical to establish maximum residue limits for all processed commodities. However, in light of the discussions of the CCPR it is clear that guidance is required to clarify when processing studies may be required, when maximum residue levels should be recommended for processed commodities and the appropriate use of default processing factors.

The Meeting considered there are two main uses of processing studies:

- determining whether or not residues concentrate during processing to the extent that residues legitimately present in the processed commodity occur at levels that may disrupt trade if a maximum residue limit is not established. In this situation processing studies are used in the estimation of a maximum residue level.
- refining dietary intake estimates used by the Meeting in assessing safety to consumers.

It was recalled by some members that the protocol for the superseded Theoretical Maximum Daily Intake (TMDI) calculations required the use of MRLs as estimates of residues. To facilitate refinement of TMDI calculations, maximum residue levels were recommended for a variety of processed commodities with lower residues than the raw agricultural commodity (RAC). International recognition of more realistic long-term exposure estimates such as the International Estimated Dietary Intake (IEDI) which uses high residues (HRs and HR-Ps) and Supervised Trial Median Residues (STMRs and STMR-Ps), negates the need for recommendations where concentration does not occur.

Internationally, refinement of dietary intake estimates is often constrained by a lack of information on consumption of processed commodities. The ability of JMPR to utilise processing factors to refine dietary intake calculations is hampered by an understandable lack of precise

consumption figures. This has been compounded by the recent changes from using 5 regional diets to the use of the 13 cluster diets developed by GEMS/Food. The food balance and other information available in developing 13 cluster diets has led to a smaller number of processed commodities for which consumption figures are available.

Despite the current constraints on the effective use of processing studies by the Meeting when considering dietary intake, it was felt that processing studies should continue to be documented.

Identification of metabolites/degradation products that might form upon processing is an important aspect of an evaluation. Unless processing involves fermentation where the residue may be exposed to metabolism by organisms different from that observed in crop, animal and soil metabolism studies, studies on the pH and temperature dependent hydrolysis and photolysis of the residues should be sufficient to indicate if different degradation products need to be considered in processed commodities.

Specific comments on each of the four recommendations of CCPR agenda paper CX/PR 07/39/8 are provided below:

(1) Processing studies should be mandatory for a relatively short list of commodities (e.g., the 16 commodities proposed by the US). Draft CXLs for the RACs do not advance to Step 8 without the submission to and acceptance by JMPR of the requisite processing studies.

The potential to disrupt trade is likely to be the major driver for processing studies, e.g., concentration of residues in wheat bran. The use of processing studies in dietary risk assessment is typically to enable refinement of the intake estimate (reduction) to obtain more realistic values. While highly desirable, the provision of processing studies is generally not essential for performing risk assessment at the international level. In some cases processing studies allow the refinement of intake estimates to acceptable levels.

The Meeting reviewed processing factors reported in a German database (http://www.bfr.bund.de/cd/579 [3]) obtained from JMPR reports 1995–2006, selected EU-Draft Assessment Reports 2003–2005, BVL: Food monitoring data 2002 and ATLANTA: Citrus fruit data 2007. The review, supported by the experience of panel members, indicates that few processes result in concentration of residues in the processed commodity. These generally involve the separation of the RAC into different components such as bran, hulls and husks from grains, skins and pulp from flesh after juicing, extraction of the oil component from oilseeds and olives and removal of water by dehydration.

Additional factors such as significance in trade and the diet, when combined with likelihood of concentration in the processed commodity, are suitable criteria for establishing a list of commodities requiring processing studies to enable CCPR as risk managers to make appropriate decisions on maximum residue recommendations. The JMPR currently relies on the Codex Alimentarius Classification as its guide to identify major internationally traded processed commodities.

The Meeting observed that many regulators currently require processing studies for commercial processing of RAC that are known to give rise to concentration of residues for some pesticides. JMPR will evaluate the data provided.

If residue levels are low or not detected in the RAC there would be no value in requiring a processing study (see JMPR 1999, page 13).

To assist the CCPR in their discussions the Meeting developed a list of commodities for which processing studies should routinely be submitted as experience suggests residues may concentrate in processed commodities that are in trade (Table 2). The Meeting recognized that it is a CCPR decision whether to advance maximum residue limits for commodities listed in Table 2

[3] At present, the BfR processing factor database contains 1042 processing factors for foods for 116 pesticides and 433 processing factors for feedstuffs for 91 pesticides.

depending on the availability of suitable processing studies. As there are a potentially large number of commodities for which processing studies might be required, the Meeting explored the potential for extrapolation of processing of commodities within a commodity group.

The number of processing studies conducted with a single pesticide on different members of a commodity group is generally small and inadequate to provide guidance on extrapolation of processing studies. However, for a limited number of commodities, the Meeting considered that the available data together with the nature of the related commodities and the similarity of the process would allow pragmatic decisions on extrapolation to be made. These proposals are also indicated in Table 2.

(2) CXLs or processing factors should be established or recommended for those processed commodities where a significant increase (more than 1.3 times) of residue of concern occurs from RAC to processed commodity. It should be decided in advance for which commodities CXLs and for which processing factors will be established.

The Meeting considered that processing studies should only routinely be provided for commodities listed in Table 2. Table 2 also indicates the Meetings suggestions on commodities for which maximum residue levels should be recommended if concentration occurs upon processing.

The JMPR's experience suggests that the accuracy and precision of processing studies is such that a PF of 1.3 cannot be adequately distinguished from a value of 0.7. Concentration of 1.3× is considered not to represent "significant increase" in residues. Where application of the PF to residues from trials leads to estimates in the processed commodity that are less than the proposed maximum residue level for the unprocessed commodity, the JMPR would not normally recommend a separate maximum residue level for the processed commodity.

(3) CXLs or processing factors should be established or recommended for those processed commodities where a significant decrease in residue occurs from RAC to processed commodity, and this should be decided in advance. The processing factor must be considered in order to achieve a satisfactory dietary exposure assessment.

The Meeting was of the opinion that maximum residue levels are not required for processed commodities where residues do not concentrate. In these cases it should be sufficient to document the processing factors used in decision making and in dietary intake (e.g., estimation of STMR-P and HR-P values) in line with current JMPR practice. Table 3 lists types of processing procedures that the Meeting considered would be useful for refining dietary intake calculations but not essential for estimating maximum residue levels. Suggested extrapolations for the use of processing data in dietary intake calculations are also listed.

An exception to not recommending a maximum residue level would be if the acute intake estimate for consumption of the processed commodity was close to the acute reference dose in which case a maximum residue level would be recommended to CCPR. This is considered to be an unlikely event.

(4) A limited number of default (generic) processing factors should be established or recommended for some predefined common processes, starting with dehydration (e.g., dried vegetables, spices, fruits herbal infusions, milk powder). These can be used nationally and internationally for risk assessment purposes.

The Meeting agreed that default dehydration factors are of use in dietary intake assessment (where processing study results are not available) and is aware of a range of default factors employed by the US EPA that would be suitable. Table 4 details a range of default factors that are considered reliable and could be used by the JMPR. Use of default factors would be restricted to situations where there was no other form of processing involved and would not apply to situations such as whole milk → skim milk powder (change in fat content prior to dehydration) or sugar beet → beet pulp, dry (juice extracted prior to dehydration).

Default values assume that no loss of pesticide occurs during drying. For some pesticides, losses by volatilization and decomposition may occur. Defaults would always be superseded by factors based on data. Defaults are not applicable where the process generates a relevant compound e.g., dithiocarbamates → ETU.

Table 2. Industrial processes involving well-defined procedures typically practised on a large scale for major commodities. Most regulatory authorities consider studies for these processes essential for estimation of maximum residue levels.

Raw Agricultural Commodity	Processing	Processed commodity	Required	Extrapolations	Purpose		
					MRL	Diet	Animal feed
Cereal grain – oats, rye, triticale, wheat	Milling	Bran	Y	Wheat → small grains (oats, rye, triticale) except rice	✓	✓	✓
		Flour	Y			✓	
		Germ	Y			✓	
		Wholemeal	O			✓	✓
		Bread	O			✓	
Cereal grain - Rice	Milling	Husked rice	Y	None	✓		✓
		Bran	Y		✓		✓
		Hulls	Y				✓
		Polished rice	O			✓	
Cereal grain – maize	Milling wet/dry	Oil	Y	Maize (dry milling only) → grain sorghum	✓	✓	
		Flour	Y			✓	
		Meal	Y			✓	✓
Citrus fruit[1]	Juicing	Juice	Y	Orange → all citrus		✓	
		Pulp	Y				✓
		Peel	O			✓	
		Molasses	O		✓		
Pome fruit	Juicing	Juice	Y	Apple → pome fruit		✓	
		Pomace, wet or dry	Y		✓		✓
		Sauce	O			✓	
Grapes	Juicing/ Dehydration	Juice	Y	None		✓	
		Pomace, wet or dry	Y		✓		✓
		Raisins	Y		✓	✓	
		Wine (fermentation)	Y			✓	
Plums	Dehydration	Prunes	Y	None	✓	✓	
Tomato	Juicing	Juice	Y	None		✓	
		Paste	O			✓	
		Purée	O			✓	
Sweet corn		Kernels	Y	None		✓	
		Cannery waste	Y				✓
Oilseeds	Solvent extraction/ crushing	Oil refined	Y	Soy bean ↔ rape (canola) ↔ cottonseed ↔ sunflower ↔ sesame ↔ linseed (flax) ↔ peanut ↔	✓	✓	

Raw Agricultural Commodity	Processing	Processed commodity	Required	Extrapolations	Purpose		
					MRL	Diet	Animal feed
				safflower			
		Hulls	Y				✓
		Meal	Y		✓		✓
	Cold press	Oil	Y	Soy bean ↔ rape (canola) ↔ cottonseed ↔ sunflower ↔ sesame ↔ linseed (flax) ↔ peanut ↔ safflower	✓	✓	
		Hulls	Y				✓
		Meal	Y		✓		✓
Olives	Pressing/ extraction	Oil	Y	None	✓	✓	
Potato		Peel /processing waste	Y	None			✓
		Granules	O			✓	
		Chips	O			✓	
		Crisps	O			✓	
Sugar beet, Sugarcane	Press	Sugar	Y	Sugar beet ↔ sugarcane		✓	
		Molasses	Y		✓		✓
		Beet pulp, dry	Y		✓		✓
		Cane bagasse	O				✓

Y=yes; O=optional

[1] The Meeting noted that processing of citrus fruit to produce citrus oil often results in concentration of residues, however citrus oil was not included in the table as there is no Codex Commodity Classification. Citrus oil is used as flavouring and is an extremely minor component in the diet.

Table 3. Additional household preparation, processing procedures and possible extrapolations for refinement of dietary intake (all studies optional, i.e., not essential for estimation of maximum residue levels)

Processing procedure	Explanations	Examples of major crop[1]	Extrapolations[6]
Distribution in the edible / non edible portion	Normally covered by the residue trials	Citrus Tropical fruits (with inedible peel) e.g. Banana Winter squash, Melon	Orange ↔ grapefruit ↔ lime ↔ lemon ↔ tangerine (clementine, mandarin) Banana ↔ plantains Melons → all inedible peel cucurbits
Preparation of vegetable juice other than tomato		Carrot	
Infusions and extractions	Infusions, including green and black tea. Roasting and extraction (including instant coffee)	Tea Cacao Coffee	
Preparation of canned and frozen fruit		*Canned:* Apple/Pear Peach Pineapple *Frozen:* Strawberry Apple Peach Blueberry	Any one canned fruit ↔ all canned fruits Any one frozen fruit ↔all frozen fruits
Preparation of other fruit	Includes production of	Pome fruit	Any one fruit ↔ other major fruits

Processing procedure	Explanations	Examples of major crop[1]	Extrapolations[6]
products (primary processes only)	marmalade, jam, jelly, sauce/puree	Stone fruit Grape Citrus (orange)	
Preparation of alcoholic beverages	Fermentation Brewing/distillation	Rice Barley Other Cereals (wheat, maize, rye) Sugar [for grapes see table 2]	Grapes[4] → all wine-producing RACs except rice Rice (beer, wine) → None Barley ↔ all beer-producing RACs (except rice, including hops) Barley ↔ all whiskey-type producing RACs
Cooking vegetables, pulses and cereals in water		Carrots Beans Peas (succulent and dry) Potatoes Spinach	
Preparation of canned and/or frozen vegetables	Both commercial-type canning and freezing procedures should be demonstrated.	Common (green or snap) bean Corn (sweet) Pea (garden, succulent) Potato Spinach Beet (garden, table) Broccoli (frozen) Tomato	Common bean, corn, pea, or spinach → all vegetables Potato → sweet potato
Miscellaneous preparations of other vegetable products	Frying Microwaving	Potatoes	Potatoes ↔ all vegetables
Processing of products of animal origin including preparation of meat and fish[2]	Boiling Pasteurisation Baking Smoking Frying Fermentation Poaching	Milk, eggs Milk Eggs Meat, fish Meat Milk Eggs, fish	
Dehydration[3]	Removal of water	Fruits (other than grapes, plums) Vegetables Grasses	None
Fermentation of soya beans, rice and others (except alcoholic beverages)	Fermentation	Cabbage Soya (soy bean) Rice	
Pickling	Brining or corning, the process of preserving food by anaerobic fermentation in salt solution	Cucumber	

[1] The crops mentioned are only examples giving some important crops for this kind of study. The selection of crop depends on the use pattern of the pesticide.

[2] Conducted only if a veterinary use is requested

[3] Default processing factors may be used in lieu of processing studies for some dehydration processes (see Table 2). No extrapolations are possible as each commodity contains a different percentage of water.

[4] Processing studies are necessary for both red and white wine grapes.

[5] Processing need to be similar e.g., Canning of peeled fruit

[6] Wider extrapolation may be possible depending on the pesticide, e.g., thermal or hydrolytic degradation of the pesticide to give no residues

Table 4. Products with known dehydration factors.

Raw agricultural commodity	Processed product	Dry matter content in RAC	Dry matter in dried product	Theoretical processing factor
FT 0297 Figs	Fruit, dried	22%	74%	3.4
FB 0269 Grapes	Fruit, dried	18%	85%	4.7
Grass	Hay	20%	86%	4.3
FS 0014 Plums	Prunes	20%	70%	3.5
FP 226 Apple	Fruit, dried	17%	68%	4.0
FS 0240 Apricot	Fruit, dried	14%	69%	4.9
FP 0230 Pear	Fruit, dried	16%	73%	4.6
VO 0448 Tomato	Tomato, sun dried	6.1%	85%	14
VO 0445 Peppers Sweet	Sweet pepper, dry	9%	92.9%	10
VO 0444 Peppers chilli	Chilli pepper, dry	13%	92.9%	7

2.8 CROP GROUPS AND COMMODITY GROUP MRLS

The FAO Manual explains that the establishment of commodity group MRLs as opposed to MRLs for individual commodities has long been considered an acceptable procedure at both the national and international levels.

The Meeting was aware of the progress being made by the CCPR on the Revision of the Codex Classification of Foods and Animal Feeds.[4]

In November 2005, an FAO/WHO consultation[5] was held in The Netherlands to update the principles and methods of risk assessment relating to the establishment of MRLs for pesticides and veterinary drugs. The Consultation encouraged the wider use of group MRLs.

The 2006 JMPR report [6] explained the uses of MRLs and suggested a more liberal extrapolation to group MRLs.

JMPR recommended to CCPR:

After dietary intake assessment, commodity group MRLs may be proposed on the following minimum conditions:

(1) The pesticide is registered or authorized for use on the crop group; and

(2) Relevant and adequate residue data are available for at least one major commodity of the group. (However, all relevant data for the commodities of the group should be taken into account.)

At its 39th Session, CCPR agreed to the revised procedure[7].

Commodity group MRLs are the most practical way of introducing MRLs for commodities of minor crops. For full implementation, the directions for use on pesticide product labels will need close attention and changes to the Codex Commodity Classification will be needed.

We should note the distinction between the crop group and the commodity group. The distinction is not always clear because we use the same words to describe the crop and the

[4] CCPR. 2007. *Report of the 39th Session of the Codex Committee on Pesticide Residues, Beijing, China, 7 - 12 May 2007.* ALINORM 07/30/24 - Rev. 1. Para 151-154.

[5] FAO/WHO. 2006. Updating the Principles and Methods of Risk Assessment: MRLs for Pesticides and Veterinary Drugs. http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/bilthoven_2005.pdf

[6] Annex 5, reference 107.

[7] CCPR. 2007. *Report of the 39th Session of the Codex Committee on Pesticide Residues, Beijing, China, 7 - 12 May 2007.* ALINORM 07/30/24 - Rev. 1. Paragraph 34.

commodity, e.g., in one context, "pineapples" can mean the crop in the field and in another context "pineapples" can mean the fruit itself.

This ambiguity sometimes suggests that “crop” and “commodity” are the same, e.g., a sentence in a recent circular letter[8] states: “By adding minor commodities in the crop groups, minor crops could be more easily added on the plant protection products labels”. The intention is probably to add commodities (produced by minor crops) to commodity groups, so that the minor crops could be added to suitable corresponding crop groups.

For field uses, pesticides are applied to the crop, so it is the crop or crop group that should appear on pesticide product labels.

MRLs and residues are expressed on commodities, so commodities and commodity groups appear in MRL tables.

To be useful on pesticide labels, crops within a crop group should have similar growth pattern and production characteristics, similar cultural practices and similar pests that require the same pesticide treatment. Such a crop group then needs a corresponding commodity group so that a group MRL can be established.

Examples of crop groups on labels and corresponding commodity groups suitable for group MRLs.

Crop group	Commodity group
Cereals	GC 0080 Cereal grains
Citrus	FC 0001 Citrus fruits
Grain legumes	VD 0070 Pulses
Pome fruit	FP 0009 Pome fruits
Vegetables - leafy	VL 0053 Leafy vegetables

Some crop groups on labels do not readily translate to corresponding commodity groups, e.g.:

Field crops	Very broad – no corresponding commodity group.
Crucifers	Does not match a Codex commodity group.
Legumes	Commodity groups could be pulses, legume vegetables or legume animal feeds
Nuts	Commodities could be peanuts and various tree nuts.
Asian fruiting vegetables	No clear commodity group.

Some of the current Codex Commodity Groups do not relate to practical crop groups for label instructions. For example, the crops relating to "Assorted tropical and sub-tropical fruits inedible peel" have diverse characteristics and possibly different pests; similarly for "Fruiting vegetables other than cucurbits".

“Tree nuts” is a somewhat artificial crop group, because the various crops within the group do not have similar characteristics and are not expected to be susceptible to the same pests and diseases requiring the same pesticide treatment.

In practice, residues in tree nuts (expressed on edible portion) from field use are often low or not detectable irrespective of the pesticide use, so a tree nut group MRL can be achieved.

The situation is more straightforward for post-harvest uses, where the product label directions refer to commodities. For example, “tree nuts” and “cereal grains” are suitable commodity groups for post-harvest uses and related group MRLs.

[8] CAC. 2007. Request for comments on the proposals for ‘Bulb vegetables’ and ‘fruiting vegetables’. Circular letter CL 2007/36 – PR. Paragraph 3.

Examples of commodity group MRLs from the 2006 JMPR

Recommendations from the 2006 JMPR were examined for MRL grouping and MRL mutual support without grouping.

When the data from a number of commodities are put together for mutual support, it suggests that the use pattern on the crops was similar and the residues on the commodities were similar, but extrapolation to the wider commodity group could not be justified.

'Commodity group except xxx' MRLs suggest that, at least in the specific case, there was some problem that prevented the inclusion of those excepted commodities.

Examination of 'mutual support' decisions may provide starting points for practical smaller groups or sub-groups of crops and corresponding commodities that would be useful on product labels and in MRL tables respectively.

The following commodity groups readily lend themselves to group MRLs:

- citrus fruits;
- pome fruits;
- stone fruits;
- cucurbit fruiting vegetables; and
- Brassica vegetables.

The following are notable difficulties, mostly because the use patterns are not the same on the crops producing the commodities:

- Grapes do not group well with berries and other small fruits.
- Rice does not group well with other cereal grains.
- Sweet corn and mushrooms do not group well with fruiting vegetables other than cucurbits.
- 'Tropical-fruits-inedible-peel' is too broad – smaller sub-groups are needed.

Commodity group and mutual support groupings from 2006 JMPR

Compound	Commodities with data supporting MRL	Group or commodities with MRL recommendation	Code
Pirimicarb	mandarin, orange	citrus fruits	FC
Thiabendazole	mandarin, orange	citrus fruits	FC
Bifenazate	apple, pear	pome fruits	FP
Fludioxonil	apple, pear	pome fruits	FP
Pirimicarb	apple	pome fruits	FP
Thiacloprid	apple, pear	pome fruits	FP
Bifenazate	apricot, cherry, peach	stone fruits	FS
Pirimicarb	cherry, nectarine, peach, plum	stone fruits	FS
Pyraclostrobin	cherry, peach, plum	stone fruits	FS
Thiacloprid	peach, sweet cherry	stone fruits	FS
Pirimicarb	currant, gooseberry, raspberry	berries and other small fruits (except grapes and strawberries)	FB
Thiacloprid	currant, raspberry, strawberry	berries and other small fruits (except grapes)	FB
Endosulfan	avocado, custard apple, mango, papaya	mutual support: avocado, custard apple, mango, papaya	FI
Endosulfan	litchi, persimmon	mutual support: litchi, persimmon	FI
Pirimicarb	broccoli, Brussels sprouts, cauliflower, cabbage	Brassica vegetables	VB
Bifenazate	cantaloupe, cucumber, summer squash	cucurbit fruiting vegetables	VC
Propamocarb	cucumber, melon, summer squash	cucurbit fruiting vegetables	VC
Pirimicarb	cucumber, summer squash	cucurbit fruiting vegetables (except melons and watermelons	VC

Compound	Commodities with data supporting MRL	Group or commodities with MRL recommendation	Code
Thiacloprid	melon, watermelon	mutual support: melon, watermelon	VC
Pirimicarb	sweet peppers, tomato	fruiting vegetables other than cucurbits (except mushrooms, fungi, sweet corn)	VO
Pirimicarb	beans, peas	legume vegetables (except soya beans)	VP
Propargite	dry beans, dry broad-bean, dry chick-pea, dry lupin	mutual support: dry beans, dry broad-bean, dry chick-pea, dry lupin	VD
Pirimicarb	dry beans, dry peas	pulses (except soya beans)	VD
Endosulfan	potato, sweet potato	mutual support: potato, sweet potato	VR
Pirimicarb	carrot, potato, sugar beet	root and tuber vegetables	VR
Endosulfan	hazel nuts, Macadamia nuts	mutual support: hazel nuts, Macadamia nuts	TN
Bifenazate	almond, pecan	tree nuts	TN
Thiacloprid	almond, pecan, walnut	tree nuts	TN
Aminopyralid	barley, oats, wheat	barley, oats, wheat, triticale	GC
Pirimicarb	barley, maize, wheat	cereal grains (except rice)	GC
Pirimicarb	barley straw, maize fodder, wheat straw	straw and fodder of cereal grains except rice	AS
Aminopyralid	barley straw, oats straw, wheat straw	straw of barley, oats, wheat, triticale	AS

Recommendations

CCPR should note the different purposes for crop groups (directions for use on pesticide product labels) and commodity groups (MRLs) and should aim for an integrated system that will, in practice, produce more crop group registrations and corresponding commodity group MRLs.

Ideally, there should be crop groups

1. that have similar pesticide product label directions within each crop group,

AND

2. that produces commodity groups with similar residue characteristics within each commodity group.

Registration authorities, industry and researchers should give careful consideration to using crop groups and commodity groups that meet the above criteria.

2.9 STATISTICAL METHODS FOR THE ESTIMATION OF MRL

The JMPR estimates maximum residue levels that it recommends for use as Codex MRLs. The recommendation is accompanied by an evaluation of the pesticide's toxicity and a determination of the human dietary exposure. The recommendations may become Codex MRLs only where the exposure does not exceed established ADIs or ARfDs. The maximum residue levels are derived from the a set of values composed of the highest result, usually in mg/kg, for residues found in a series of supervised field trials conducted under maximum GAP conditions. The set will contain a variable number of data points (field trials), typically from 3 to 50 or more. Additionally, sets of data from trials conducted in different countries or regions may be combined where the GAPs are similar and the sets are deemed not to be from different populations. This is ascertained by use of the Mann-Whitney U Test, which compares the medians. Sets are combined to provide more data points upon which to estimate the maximum residue level or to allow estimation of a maximum residue level for a commodity group, e.g., combining pear and apple data to estimate pome fruit.

The maximum residue level must be estimated "somewhat" above the highest residue value (HR). The issue is defining "somewhat." Until recently, the Meeting relied upon professional judgment only. The maximum residue level tended to be estimated near the high residue where there

were many data points (≥ 12) and more distant from the HR where there were few data point (n=3 to 8) and/or those points were clustered near the HR. The maximum residue level needs to be set such that it represents the residue level not likely to be exceeded when the pesticide is applied according to the relevant GAP. Setting it too low risks disrupting trade where the pesticide has been used according to GAPs reviewed by the JMPR; setting it too high may encourage misuse of the pesticide.

Some national regulatory authorities utilize statistical methods to assist in the determination of the appropriate maximum residue level. The JMPR has been investigating this approach and most recently considered the NAFTA statistical calculation (General Item 2.5, JMPR Report 2005; General Item 2.10, JMPR Report 2006) and the binomial calculation (General Item 2.10, JMPR Report 2006). Following these considerations the JMPR adopted the NAFTA method as a tool, to be used in determining MRL estimates. The Meeting previously agreed that where this statistical tool is used, class rounding (scaling) is not appropriate. Rather the result should be rounded up to one significant figure.

The Meeting welcomed the availability of the NAFTA paper *Statistical Basis of the NAFTA Method for Calculating Pesticide maximum Residue Limits from Field Trial Data*[9]. The document details the development and testing of the NAFTA Method and also gives some explanation of and comparison to other available methods for calculating MRL estimates from a residue data set.

The 2006 JMPR also considered the binominal procedure for MRL calculation and found that it generally gave results comparable to those from the NAFTA method. However, the NAFTA paper details both theoretical and practical problems with the binomial procedure. Because of these concerns, simulations with normal and log normal data sets were conducted. It was found that the binominal method does not give unbiased estimates of the 95th percentile.

The 2007 Meeting performed a simple analysis of the maximum residue level estimations obtained from the NAFTA spreadsheet, using the default value provided by the logic tree of the spreadsheet (rounded up to one significant figure), and the estimate made independently by the entire FAO panel expert group.

There were a total of 94 plant commodity data sets considered. Excluded were data sets for livestock commodities (meat, milk, poultry, eggs), as these are not generally amenable to statistical calculation. Also excluded were plant commodity data sets with more than 60% < LOQ values (censored data, n=36). Of those considered, 44% resulted in the same estimate by the NAFTA spreadsheet and the Panel judgment. An additional 16% differed by 20% or less (difference/Panel judgment × 100). The Panel judgment was less than and more than the spreadsheet rounded estimate in about equal amounts of 28%.

There were no clear trends with which to correlate the differences in spreadsheet and professional judgment. One disagreement was a spreadsheet estimate below the HR. The Meeting previously agreed that a maximum residue estimate would not be made below the HR where the HR is judged to be scientifically valid. For difenoconazole on celery there were nine trial points with a high residue of 2 mg/kg. The spreadsheet suggested 1.18 mg/kg (log normal), but the Meeting considered a value somewhat above the high residue appropriate, or 3 mg/kg. This situation was repeated more dramatically for cyromazine on cabbage, where there were six trial points with a high residue of 6.1 mg/kg. The NAFTA spreadsheet recommended 3 mg/kg (log normal), i.e., considerably below the HR. The Meeting considered that given only 6 data points, the estimate should be substantially above the high residue and estimated 10 mg/kg.

There were other cases where the spreadsheet seemed to overestimate the maximum residue level. For example, for dimethomorph on corn salad (lambs lettuce) there were 6 residue values with

[9] Statistical Basis of the NAFTA Method for Calculating Pesticide maximum Residue Limits from Field Trial Data 1 (US EPA and Canada PMRA, May, 2007: http://www.pmra- arla.gc.ca/english/pdf/nafta/docs/nafta_mrls-e.pdf)

an HR of 7.1 mg/kg. The NAFTA spreadsheet recommended (log normal) 24 mg/kg. The Meeting recommended 10 mg/kg.

A particular continuing concern is the estimation of MRLs for very small data sets. The general JMPR policy is to require at least six independent trials (n=6), but often recommendations are made on a case-by-case basis for as few as three trials (n=3). The latter typically occurs for minor commodities which show a small spread (low RSD) in residue values. It may also occur for commodities where all results are at or near the LOQ and which have other supporting information, such as the results of metabolism studies and residue levels from exaggerated rate treatments. The NAFTA Paper states for apparent log normal data sets, the minimum acceptable value for n is 6. Below 6 values the estimate of the 95th percentile is extremely unreliable. An analysis paper on the NAFTA model from Australia [10] suggests an underestimation of the 95th percentile happens somewhere between 20 and 10 samples. Clearly extra caution must be taken in interpreting statistically generated MRL estimates with small data sets (n=3 to 15).

Another special case is the situation where many of the data points in the supervised field trial data set are < LOQ, or censored data. The NAFTA Paper recommends assigning values to the < LOQs by using a maximum likelihood estimate (MLE) procedure, but only up to about 60% censored data. The MLE approach assumes a log normal distribution. The Meeting questions if there are other procedures for handling the censored data that do not require assumptions on the distribution.

The Meeting also noted that currently available statistical spreadsheets/methods for the estimation of MRLs do not address the issue of application of such tools to data sets that are the composite of two or more subsets. The NAFTA model is exactly suited to NAFTA field trial data, where the data are from a *single population* and *proportional to production as distributed among zones and represent one GAP*. Such is often not the case with the data voluntarily submitted to JMPR. Data from various countries or regions may be *combined* for a given crop where the GAPs are comparable and apparently not from different populations.

The Meeting considered that expert judgment must be the primary factor in estimating maximum residue levels. No statistical approach can accommodate the scientific and technical aspects of residue data sets that go beyond mathematical distributions. Consider the hypothetical data set (n=12): 1, 1.1, 1.3, 3.1, 3.9, 5.0, 5.6, 5.8, 7.6, 8.2, 8.5, 9.6 mg/kg. The NAFTA spreadsheet estimates 30 mg/kg for the maximum residue level based on the 99th percentile log normal. Additional information available indicates that the three highest values were from sampling 1 day less than the PHI of 4 days, but within the variability in GAP allowed by JMPR. Thus, the residues might be somewhat overstated in the three samples with highest residues. Also, several trials conducted at exaggerated application rates (1.6 ×) and not included in the set gave residues of 8–11 mg/kg. These additional facts lead the evaluators to consider 30 mg/kg excessive and to estimate the maximum residue level at 20 mg/kg. This illustrates the importance of professional judgment.

The Meeting affirmed that reviewers will continue the practice of preparing summary calculation sheets for each compound and its commodities under review. This will include the various estimations from the NAFTA spreadsheet (EU normal, EU nonparametric, NAFTA log normal (3 types), mean plus 3 standard deviations). The Meeting will routinely consider these values as a part of its deliberations in making maximum residue level estimates.

The Meeting concluded that:

I. Any statistical method of maximum residue level (MRL) estimation from a data set is a tool and not the controlling factor in making the estimate. Professional judgment of the JMPR FAO Panel and transparent evaluation of the data set must be performed.

II. The binominal procedure should not be used at this time, pending resolution of apparent deficiencies.

[10] Stern, S. (2007) Comparing Two Methodologies for Setting MRLs for Field Trial Data, DSI Consulting, Australia.

III. Regulatory authorities and other interested parties are encouraged to give additional consideration to:

a. Appropriate techniques for handling data sets with substantial censored data (< LOQ values).

b. Combination of multiple data sets into one set for MRL estimation and the appropriateness of statistical MRL estimation in such situations.

2.10 OECD LIVESTOCK FEED TABLES - JMPR CALCULATION OF LIVESTOCK DIETARY BURDEN

In 2006, JMPR was advised of the development of OECD livestock feed tables intended as guidelines for manufacturers to determine livestock dietary burdens during the planning of livestock feeding or transfer studies[11]. The tables of OECD feedstuffs were included as Annex 6 to the JMPR Report. Subsequently, the full OECD report[12] was issued.

The OECD tables include data for beef cattle, dairy cattle, sheep, lambs, swine, broilers, layers and turkeys. Data are available from three different geographic regions: US-Canada, EU and Australia. Feedstuff categories in the OECD tables were chosen to ensure that the highest residue levels are estimated and a realistic although not nutritionally optimal livestock diet is composed. The primary purpose of the tables was to estimate a highest livestock dietary burden from the geographic regions which would then be used to set appropriate dosing for a livestock feeding study. JMPR is using the livestock diets in the tables to estimate livestock dietary burdens from available residue data.

Information is included on typical dry matter content for each commodity, allowing calculation of residues on a dry weight basis when measured dry matter or moisture content is not available. The tables also suggest, for each feed commodity, whether to use the highest residue or STMR for calculation of the maximum dietary burden.

The Meeting agreed to use the OECD tables as a replacement for those previously used – originating from the USA and published in the *FAO Manual*[13].

Feeding studies are normally available for lactating dairy cattle and laying hens. For this situation, livestock dietary burdens will be calculated for beef and dairy cattle, broiler and layer hens.

Essentially the same process is followed as described in the *FAO Manual*.

The aim is to calculate the dietary burden from the livestock feed tables and available residue data in such a way as to estimate the highest dietary burden. Starting with the feed item with the largest residue concentration on a dry weight basis, the percentage of livestock diet for each feed is allocated using the OECD Table. One feed commodity from each Codex Commodity Group is used or, if more than one, only up to the highest percentage % feed allocation for a commodity of that Group. Feeds are allocated a percentage of the livestock diet for each animal until no more than 100% of the diet is used. The residue contribution of each feed (mg/kg) is then calculated using the residue on a dry weight basis and the corresponding percentage of the diet. All residue contributions for each animal are then summed to determine the total dietary burden (see worked example).

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[11] Annex 5, reference 107.

[12] OECD. 10-Oct-2006. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Series on Testing and Assessment. Number 64. Series on Pesticides. Number 32. Guidance Document on Overview of Residue Chemistry Studies. Document ENV/JM/MONO(2006)32.

[13] FAO. 2002. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. Appendix IX. Maximum proportion of agricultural commodities in animal feed. *FAO Plant Production and Protection Paper*, 170.

Highest residue, STMR and STMR-P values are chosen as indicated in the OECD table for the maximum burden calculation. STMR and STMR-P values are chosen for the mean dietary burden.[14] [15] In the maximum burden calculation for some raw agricultural commodities (RACs), e.g., cereal grains, STMR values are used for residues from pre-harvest pesticide uses because of bulking and blending of the RAC, but highest residue values are used for post-harvest uses (could occur after bulking and blending).

Calculations are made for the maximum and mean dietary burdens for the 3 geographic regions for beef and dairy cattle, layers and broiler chickens, i.e., 24 calculated values. Highest values among the regions are chosen from the mean and maximum calculations to represent the dietary burdens. One proviso is that the dietary burdens related to residues in milk and eggs must come from dairy cattle (not beef cattle) and laying hens (not broilers) respectively.

The Meeting noted differences in consumption of specific feed items between the previously used tables and the new OECD tables. For example, the new tables assign 5% of the diet of beef cattle in US-Canada to cotton gin by-products, whereas the previous value was 20% of the diet of beef and dairy cattle assigned to cotton gin by-products.

The meeting also noted some new commodities in the tables that were not previously designated as feed items for the purpose of calculating livestock dietary burdens: e.g., cabbage, kale, bean seed, grape pomace and sugarcane bagasse.

JMPR 2006 agreed to use the new data tables for livestock dietary burden estimation beginning in 2007. Estimations from previous years would not be revised unless a new evaluation was required for some reason such as a new data submission.

Livestock dietary burden calculations for 2007 will be included as an annex to the JMPR Report.

Worked example (difenoconazole)

First, dietary burdens are calculated from commodity percent of diet and residues expressed on dry weight.

Estimated maximum dietary burden of livestock

BEEF CATTLE MAX

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue dw mg/kg	*Diet content (%)*			Residue contribution (ppm)		
						US-CAN	*EU*	*AU*	US-CAN	EU	AU
Sugar beet leaves or tops	AM AV	0.95	highest residue	23	4.130		*20*			0.83	
Apple pomace, dry	AB	1.65	STMR-P	100	1.650	*20*	*20*	*20*	0.33	0.33	0.33
Wheat straw and fodder	AS	1.2	highest residue	88	1.364	*10*	*20*	*80*	0.14	0.27	1.09
Cabbage heads, leaves	VC	0.19	HR	15	1.267		*20*			0.25	
Carrot culls	VR	0.13	HR	12	1.083	*10*	*15*		0.11	0.16	
Oilseed rape fodder	AM AV	0.14	highest residue	100	0.140	*20*			0.03		
Potato culls	VR	0.01	HR	20	0.050	*20*	*5*		0.01	0.00	
Rape seed (for meal)	SO	0.02	STMR	88	0.023	*15*			0.00		
Soya bean seed	VD	0.02	STMR	89	0.022	*5*			0.00		
Total						*100*	*100*	*100*	0.62	1.85	1.42

[14] Annex 5, reference 92.

[15] Annex 5, reference 101.

DAIRY CATTLE MAX

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue dw mg/kg	*Diet content (%)* *US-CAN*	*EU*	*AU*	Residue contribution (ppm) US-CAN	EU	AU
Sugar beet leaves or tops	AM AV	0.95	highest residue	23	4.130		*30*			1.24	
Apple pomace, dry	AB	1.65	STMR-P	100	1.650	*10*	*10*	*10*	0.17	0.17	0.17
Wheat straw and fodder	AS	1.2	highest residue	88	1.364	*10*	*20*	*20*	0.14	0.27	0.27
Cabbage heads, leaves	VC	0.19	HR	15	1.267		*20*			0.25	
Carrot culls	VR	0.13	HR	12	1.083	*10*	*15*	*5*	0.11	0.16	0.05
Grape pomace, dry	AB	0.36	STMR-P	100	0.360			*10*			0.04
Oilseed rape fodder	AM AV	0.14	highest residue	100	0.140	*20*		*40*	0.03		0.06
Potato culls	VR	0.01	HR	20	0.050		*5*	*5*		0.00	0.00
Rape seed (for meal)	SO	0.02	STMR	88	0.023	*15*		*10*	0.00		0.00
Soya bean seed	VD	0.02	STMR	89	0.022	*15*			0.00		
Total						*80 (note)*	*100*	*100*	0.44	2.10	0.59

Note: Insufficient feed items with residues from the use of the pesticide are available to reach 100% of this diet.

Estimated mean dietary burden of livestock

BEEF CATTLE MEAN

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue dw mg/kg	*Diet content (%)* *US-CAN*	*EU*	*AU*	Residue contribution (ppm) US-CAN	EU	AU
Apple pomace, dry	AB	1.65	STMR-P	100	1.650	*20*	*20*	*20*	0.33	0.33	0.33
Sugar beet leaves or tops	AM AV	0.25	STMR	23	1.087		*20*			0.22	
Wheat straw and fodder	AS	0.685	STMR	88	0.778	*10*	*20*	*80*	0.08	0.16	0.62
Carrot culls	VR	0.05	STMR	12	0.417	*10*	*15*		0.04	0.06	
Grape pomace	AB	0.36	STMR-P	100	0.360						
Cabbage heads, leaves	VC	0.035	STMR	15	0.233		*20*			0.05	
Oilseed rape fodder	AM AV	0.06	STMR	100	0.060	*20*			0.01		
Potato culls	VR	0.01	STMR	20	0.050	*20*	*5*		0.01	0.00	
Rape seed (for meal)	SO	0.02	STMR	88	0.023	*15*			0.00		
Soya bean seed	VD	0.02	STMR	89	0.022	*5*			0.00		
Total						*100*	*100*	*100*	0.48	0.81	0.95

DAIRY CATTLE MEAN

Commodity	Commod group	Residue mg/kg	Basis	% Dry matter	Residue dw mg/kg	*Diet content (%)* *US-CAN*	*EU*	*AU*	Residue contribution (ppm) US-CAN	EU	AU
Apple pomace, dry	AB	1.65	STMR-P	100	1.650	*10*	*10*	*10*	0.17	0.17	0.17
Sugar beet leaves or tops	AM AV	0.25	STMR	23	1.087		*30*			0.33	
Wheat straw and fodder	AS	0.685	STMR	88	0.778	*10*	*20*	*20*	0.08	0.16	0.16
Carrot culls	VR	0.05	STMR	12	0.417	*10*	*15*	*5*	0.04	0.06	0.02
Grape pomace	AB	0.36	STMR-P	100	0.360			*20*			0.07
Cabbage heads, leaves	VC	0.035	STMR	15	0.233		*20*			0.05	
Oilseed rape fodder	AM AV	0.06	STMR	100	0.060	*20*		*40*	0.01		0.02
Potato culls	VR	0.01	STMR	20	0.050		*5*	*5*		0.00	0.00
Rape seed (for meal)	SO	0.02	STMR	88	0.023	*15*			0.00		
Soya bean seed	VD	0.02	STMR	89	0.022	*15*			0.00		
Total						*80*	*100*	*100*	0.30	0.76	0.44

The calculations for poultry follow the same approach, but are not shown here.

The calculations are then summarized and the highest dietary burdens (underlined) are selected for MRL and STMR estimates on animal commodities.

	Livestock dietary burden, difenoconazole, ppm of dry matter diet					
	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.62	0.48	1.85	0.81	1.42	<u>0.95</u>
Dairy cattle	0.44	0.30	<u>2.10</u>	<u>0.76</u>	0.59	0.44
Poultry - broiler	0.01	0.01	0.12	0.05	0.01	0.01
Poultry - layer	0.01	0.01	<u>0.54</u>	<u>0.20</u>	0.01	0.01

Select the highest maximum and highest mean dietary burdens for estimating animal commodity MRLs and STMRs respectively.

	Dietary burden, ppm or mg/kg dry matter	
	for estimating MRLs	for estimating STMRs
Mammalian meat	2.10	0.95
Milk	2.10	0.76
Poultry meat	0.54	0.20
Eggs	0.54	0.20

These values are then used in combination with data from livestock feeding studies to estimate the MRLs and STMRs for meat, milk and eggs. The procedure is described on pages 80–81 of the *FAO Manual*.

2.11 STATUS REPORT FROM THE OECD EXPERT GROUP ON RESIDUE CHEMISTRY GUIDELINES

The purpose of the OECD Expert Writing Group (EG) and its implications for JMPR were explained in the 2006 JMPR Report (General Consideration 2.11). The Meeting was presented with an update of the EG efforts in 2007.

Guidelines on (1) the nature of the residue in processed commodities and (2) storage stability and a guidance document on analytical methods have been completed and approved. Additionally, an advanced draft of the magnitude of the residue in processing guideline, provided to the Meeting, is under EG comment/revision and will most likely be submitted for OECD approval before the end of 2007.

The magnitude of the residue in field trials guideline drafting group is now starting work. The initial (2007–2008) activities will centre on details of conducting a residue trial. A critical aspect will be a delineation of the variables that enter into a specific GAP and the degree of variation in these variables that will be allowed in designating different GAPs among OECD countries as equivalent. The group will also address methods for deriving an MRL estimate from a data set and procedures for combining equivalent data sets. This will include consideration of the possible applicability of the proportionality concept, that is, that the residue concentration on the crop is proportional to the rate of application. Upon completion of the core document, attention will be given (2008) to zoning and its implications on the geographic location of field trials. New work by the US EPA and reanalysis of the FAO/OECD zoning project results will be considered.

The Meeting was presented with draft versions of templates for crop field trials, nature of the residue in processing, magnitude of the residue in processing, analytical methods, and storage stability. These are designed to be used by sponsors/manufacturers in summarizing studies for submission.

The JMPR reiterated that the OECD documents will be utilized in the preparation of future versions of the *FAO Manual*. Such utilization will promote maximum harmonization and will facilitate work share. It was noted that the OECD livestock feeding tables were adopted in 2006 and utilized by the 2007 Meeting.

2.12 RESIDUES IN DRIED CHILLI PEPPERS

The 2004 JMPR, taking into account the water content of fresh peppers and the estimated water content of dried chilli peppers, as well as the decision of CCPR, applied a generic factor of 10 for conversion of residues in fresh peppers to dried chilli peppers. For the estimation of maximum residue

levels, in or on dried chilli peppers, the Codex MRLs established primarily for fresh sweet peppers were used.

The delegation of the Republic of Korea opposed the proposed Codex MRLs (Step 8) for dried chilli peppers at the 38th Session of the CCPR and offered to submit compound- specific processing factors which were much lower than the default factor (10) used by the JMPR.

The effects of the drying of chilli peppers on the residues of azinphos-methyl, chlorfenapyr, clothianidin, diazinon, diethofencarb, EPN, folpet, imidacloprid, indoxacarb, metalaxyl, methomyl, methoxyfenozide, tetraconazole, and vinclozolin were reported by the Republic of Korea. The results were evaluated by the present Meeting.

Methods of residue analysis

The pesticide residues were extracted with acetone, partitioned with dichloromethane, except diethofencarb for which hexane was used. Following the liquid-liquid partitioning, the concentrated extracts were cleaned up on Florisil columns with various elution mixtures.

Methomyl was determined with a different procedure based on extraction with acetonitrile and cleanup on solid phase microextraction cartridges. The cleaned extracts were analysed with GC-ECD or HPLC DAD or FLD.

Recovery tests were performed at 0.2 and 1 mg/kg levels in three replicates. The LOQs claimed were 0.02 mg/kg for all pesticides in fresh red peppers and 0.04 mg/kg in dried red peppers. The recovery tests were performed at 10 and 50 times higher concentrations than the corresponding LODs. On the other hand, the spike levels were much lower than the actual residues measured in fresh and dried peppers, which were between 1–6.5 mg/kg and 1.7–20.6 mg/kg, respectively. The report on the validation of the method was not submitted.

The processing of the samples started between 9 and 30 days after the sampling. Test portions of fresh and dried chilli peppers were fortified at 0.5 mg/kg residue levels at the time of receiving the samples, and the residues were determined, together with the analysis of the samples. No analytical recovery tests were performed at the time of analysis of samples. The results available indicate that the residue levels did not change during the storage of the samples.

The fresh red chilli peppers were processed as per the conventional method used in Korea, which is also specified by the Korean Food Code. A 4 kg portion of a sample was dried for 35 h at 60 °C. The seeds were removed and the dried flesh was ground to powder. The water content of powdered red chilli peppers was determined by drying 5 g of material at 105 °C for 5 h. The water content of fresh and dried peppers was determined in triplicate.

The average water contents of fresh red chilli peppers and powdered red chilli peppers were 84.04 ± 0.55 and 31.25 ± 0.47%, respectively.

Residues in fresh and dried chilli peppers

Field trials were performed at two sites in a major cultivation area of red peppers in Korea, where the peppers were cultivated according to widely used conventional methods.

The last two pesticide applications were performed with backpack-type sprayers fitted with standard nozzles, 10 days apart at site 1, and 4 days apart at site 2, with double the authorised rates to obtain sufficient residue levels for the processing studies.

The residues were determined in fresh peppers and in the dried powder with three replicate analyses. The processing factors (pf=residue in dried/residue in fresh) obtained from the crops treated at the two sites were azinphos-methyl (1.9, 2.9), chlorfenapyr (5.1, 4.2), clothianidin (2.6, 3.1), diazinon (4.6, 3.0), diethofencarb (2.5, 2.5), EPN (1.8, 3.5), folpet (2.5, 4.6), imidacloprid (2.5, 1.7),

indoxacarb (2.4, 3.2), metalaxyl (3.1, 3.3), methomyl (3.4, 2.9), methoxyfenozide (3.1, 3.2), tetraconazole (2.8, 2.3), and vinclozolin (2.3, 1.7).

The processing factors derived from the Korean trials ranged from 1.7 to 5.1. The repeatability of the procedures, expressed as relative differences, varied between 2% (diethofencarb) and 61% (EPN) with a repeatability relative standard deviation of 23%.

The theoretical concentration factor (cf) calculated from the reported water content of fresh and dried peppers is 4.31. If all residues remained in the sample then the pf/cf should be 1. However, the experimental pf/pc values ranged from 0.39 to 1.2 indicating that a pesticide residue will disappear in a compound-specific way under the same drying processes. It should be noted that the experimental values are affected by the combined uncertainty of all the procedures.

Interpretation of the results

As the trials were performed at exaggerated rates, the residues obtained cannot be used for the estimation of maximum residue levels, however they are suitable for the calculation of processing factors.

In addition to the results of drying chilli peppers, as per the Korean Food Code procedure, information was collected on the water content of fresh chilli peppers, sweet peppers and dried peppers from Australia, New Zealand, Germany, Japan and the USA to provide the best estimate of concentration factors, resulting from the drying process.

The water content of fresh bell peppers is higher (91–94%) than in the chilli peppers (75–93%), depending upon the species. The dried ground powder made from chilli peppers shows a relatively larger variation (1.7–31%) in water content than in fresh peppers.

The ground red peppers obtained in the Korean trials contained much higher percentages of water (31%), than the levels found in other countries (1.7 to < 12%).

Taking into account all available information on the water content of the fresh chilli peppers and the dried chilli peppers prepared from them, the concentration factors are: 3.9, 4.3, 6.3, 6.5, 7.5, 7.7, 7.7, and 14. The median and average concentration factors are 7.0 and 7.2.

The concentration factors calculated from the national standards for situations where dry peppers are prepared from sweet peppers are: 10, 11, 12, 13, 14 and 15.

The 2004 JMPR took note of the similarity in distribution of concentration factors, and the results of an inter-comparison study performed by the Spice Industry indicating an average water content of 7.06% in ground chilli pepper samples taken from commercial commodities. The 2004 JMPR concluded that a rounded figure of 10 for dehydration/concentration factors, based on the average water content (7.06%)(determined from a large number of commercial products), would be an appropriate basis for estimating pesticide residue levels in dried chilli peppers, from MRLs established for peppers (2004 JMPR Evaluation p.1152). The present Meeting supports that conclusion.

The variations of compound specific processing factors provided by the Korean trials (1.7–5.1) indicate that ideally, processing studies representing the world-wide practices for drying chilli peppers would be needed for each pesticide as is the case for any major commodities. In view of the fact that chilli peppers are a very minor crop in most countries, the provision of sufficient representative processing studies, for the estimation of compound specific maximum residue levels for dried chilli peppers, cannot generally be expected. Consequently the decision of the CCPR allows the application of default concentration factors to be used in such cases.

Recommendation

Based on the available information, the Meeting recommends continuing the use of the concentration factor of 10 for the estimation of maximum residue levels of pesticides in dried chilli peppers from the HR values estimated for residues in or on sweet peppers.

Where the residues on fresh chilli peppers are available, the Meeting recommends that in the future a concentration factor of 7 should be used for the estimation of maximum residue levels in dried chilli peppers from maximum residue levels in or on fresh chilli peppers. The concentration factor should be applied to multiply the actual measured residue values in fresh chilli peppers, and estimate the maximum residue and median residue levels from the converted data set.

Where residue data, reflecting the GAP and representative processing studies on residues in or on chilli peppers are available, the maximum residue levels for dried chilli peppers shall be estimated based on the actual experimental data.

The present Meeting applied this principle, and the estimated residue levels are listed under the specific pesticides.

3. RESPONSE TO SPECIFIC CONCERNS RAISED BY THE CODEX COMMITTEE ON PESTICIDE RESIDUES

3.1 CARBENDAZIM (072)

Background

At the 38th Session of the CCPR,[16] the delegation of the European Community (EC) raised concerns regarding the ARfD established by the JMPR in 2005 on the basis of developmental effects (Annex 5, reference *104*). The ARfD established by the EC is different to that established by JMPR and the EC thus has requested harmonization.

Evaluation of carbendazim by the JMPR

In 2005, the Meeting established an ARfD of 0.1 mg/kg bw based on an overall NOAEL of 10 mg/kg bw per day for developmental toxicity from three studies in rats and a NOAEL of 10 mg/kg bw per day in one study in rabbits, and a safety factor of 100. The Meeting concluded that this ARfD applies only to women of childbearing age.

For the general population including children, the Meeting established an ARfD of 0.5 mg/kg bw based on the NOAEL of 50 mg/kg bw in the study of toxicity to the male reproductive system in rats and supported by the studies on micronucleus or aneuploidy induction in vivo, using a safety factor of 100.

An additional safety factor for the severity of the effects was considered to be unnecessary because the underlying mechanism is well understood and there is a clear threshold identified for these effects, both mechanistically and biologically.

Evaluation of carbendazim by the EC

In the EC, an ARfD of 0.02 mg/kg bw had been proposed for carbendazim based on the NOAEL of 10 mg/kg bw per day for developmental effects from studies in rats and rabbits, and using a safety factor of 500. An increased safety factor was used since carbendazim is classified for mutagenic and reproductive effects as “Category 2” (presumed human mutagenic and reproductive toxicants).

Comment by JMPR

JMPR and the EC identified the same overall NOAEL for development effects as the basis for the ARfD. The primary difference in the JMPR and EC assessments was that the EC applied an additional safety factor to address the hazard classifications. JMPR uses an overall weight-of-evidence approach that considers the severity of the effect, mode of action, and dose–response relationship in its assessment of the need for additional safety factors. As clearly stated in the JMPR report, an additional safety factor was not considered necessary because the mechanism causing reproductive toxicity and genotoxicity (aneuploidy) is well understood and has a clear threshold mechanism for which a NOAEL was identified.

3.2 BIFENAZATE (219)

Bifenazate was evaluated for the first time by JMPR in 2006 and an ADI of 0–0.01 mg/kg was established. An ARfD was determined to be unnecessary. MRLs were recommended for a number of crop and animal commodities.

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[16] Codex Alimentarius Commission. *Report of the 38th Session of the Codex Committee on Pesticide Residues, 3–8 April 2006, Fortaleza*, Brazil (ALINORM06/29/24).

CCPR at its 39th Session (2007) requested JMPR to address the USA concern relating to the animal dietary burden (ALINORM 07/30/24 – Rev 1, para 129).

The concern relates to the bifenazate high residue value of 18 mg/kg for cotton fodder, which was the main contributor to the cattle dietary burden. It was noted that bifenazate residues were not particularly stable in cotton commodities during frozen storage and that the cotton gin by-product samples were stored for a long interval prior to analysis, so it might be appropriate to adjust for residue loss during sample storage. With a high residue value above 18 mg/kg in cotton gin by-products, the increased animal dietary burden might necessitate an MRL of 0.1 mg/kg in place of the current recommendation of 0.05 mg/kg for mammalian meat (fat).

Clarification was also sought if 'cotton fodder' means 'cotton gin byproducts.'

In the Codex Commodity Classification, 'Cotton fodder, dry' is the only feed commodity listed for cotton. It has been taken to mean cotton gin by-products and cotton stubble.

JMPR compares the freezer-storage intervals for samples from supervised trials with the intervals demonstrating storage stability in the freezer-storage tests. If the storage interval in a supervised trial exceeds the interval for demonstrated stability (i.e. no more than approx 30% loss) the data from that trial are rejected. Residue data are not adjusted for estimated storage losses.

In the freezer storage tests on cotton gin trash approximately 50% of the residue had disappeared in 44 days. The interval between ginning and analysis for the trial with the highest residue (18 mg/kg) was 17 days (theoretically < 30% loss) and so the trial data were accepted as valid.

In 2007, JMPR adopted the OECD Harmonized Table of Livestock Feed Commodities[17] for animal dietary burden calculation.

In the revised tables, cotton gin byproducts now contribute a maximum of 5% to the diet of beef cattle (US–Canada) as compared with a maximum 20% listed in the previous tables from the US[18]. The revised tables also specify that the STMR should be used for calculation of dietary burden for residues in cotton gin byproducts.

In view of these developments, a recalculated dietary burden for bifenazate is unlikely to exceed the one calculated in 2006.

The Meeting recommended no change to the animal commodity MRLs proposed in 2006.

3.3 METHIOCARB (132)

At the 38th Session of the CCPR in April 2006, the German Delegation raised concerns regarding the MRL proposed by the JMPR in 2005 for Peppers, sweet. The EU submitted a concern form in June 2006 which indicated that the short-term dietary intake calculated using the German model for children (between the ages of 2 and 5 years old) for Peppers, sweet was 470% (with a variability factor of 7) or 200% (with a variability factor of 3) of the ARfD established by 2005 JMPR.

The CCPR noted that no intake concerns were identified by JMPR for this compound and advanced all proposed draft MRLs to Step 5/8 (ALINORM 06/29/24, para. 104). The 29th Session of Codex Alimentarius Commission in July 2006 adopted all the MRLs for methiocarb as proposed by the 38th CCPR as Codex MRLs.

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[17] OECD. 10-Oct-2006. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Series on Testing and Assessment. Number 64. Series on Pesticides. Number 32. Guidance Document on Overview of Residue Chemistry Studies. Document ENV/JM/MONO(2006)32.

[18] FAO. 2002. Submission and evaluation of pesticide residues data for the estimation of maximum residue levels in food and feed. Appendix IX. Maximum proportion of agricultural commodities in animal feed. FAO Plant Production and Protection Paper, 170.

No additional data became available for consideration by the current Meeting. The Meeting therefore confirmed its previous recommendation for Peppers, sweet.

3.4 QUINOXYFEN (223)

Livestock Dietary Burden Aspects

Quinoxyfen was reviewed for the first time by the 2006 JMPR. An ADI of 0–0.2 mg/kg bw was established, and it was determined that an ARfD was not necessary. Several MRL recommendations were made at the time.

The 39th CCPR requested JMPR to reconsider the dietary burden for cattle and its impact upon the proposed MRL for meat (ALINORM 07/30/24, Par. 133). It was noted that the dietary burden of beef cattle was utilized, whereas it might be appropriate to utilize the higher dietary burden of dairy cattle for the determination of the MRL for meat.

The maximum dietary burdens of quinoxyfen for beef cattle and dairy cattle are 0.66 ppm and 2.14 ppm, respectively. The STMR dietary burdens for beef cattle and dairy cattle are 0.25 ppm and 0.40 ppm, respectively. The Meeting confirmed that it is appropriate to use the higher dietary burden value for estimations involving cattle tissues. In this case, the values for dairy cattle would be used.

The augmented total residue calculations are summarized as follows, with new entries in bold:

Quinoxyfen total residues, mg/kg

Dietary burden (ppm) Feeding level [ppm]		Cream	Milk	Muscle		Liver		Kidney		Fat	
		Mean	Mean	Highest	Mean	Highest	Mean	Highest	Mean	Highest	Mean
MRL, beef cattle	(0.66) [0.6]			(< 0.01) [< 0.002]		(< 0.01) [< 0.01]		(< 0.01) [< 0.01]		(0.02) [0.02]	
MRL, dairy cattle	(2.1) [2]	(0.068) [0.068]	(0.0088) [0.0088]	**(< 0.01) [< 0.01]**		**(< 0.01) [< 0.01]**		**(< 0.01) [< 0.01]**		**(0.11) [0.10]**	
STMR beef cattle	(0.25) [0.2]				(0.002) [< 0.002]		(0.002) [< 0.002]		(< 0.01) [< 0.01]		(0.01) [0.01]
STMR dairy cattle	(0.40) [0.2/0.6]	(0.01) [0.003/ 0.016]	(0.002) [< 0.001/ 0.002]		**(0.002) [< 0.002/ < 0.002]**		**(0.006)[1] [< 002/ < 0.01]**		**(< 0.01) [< 0.01/ < 0.01]**		**(0.011) [0.01/ 0.012]**

[1] The kidney value (0.01 mg/kg) is used as the STMR for offal. Thus, 0.006 vs. 0.002 mg/kg had no effect.

The Meeting noted that the use of the dairy cattle diet as opposed to the beef cattle diet leads to an increase in the estimate of the maximum residue level for meat from "0.02 fat" mg/kg to "0.2 fat" mg/kg. No other cattle commodity MRLs are impacted and the STMR values as used in the human dietary risk assessment calculation are unchanged.

Definition of the residue

Plants and Animals

Definition of the residue (for compliance with MRL and estimation of dietary intake): quinoxyfen

The residue is fat soluble.

The Meeting estimated the maximum residue level of 0.2 (fat) mg/kg and STMR values of 0.011 (fat) and 0.002 muscle mg/kg to replace the previous recommendations. The maximum residue level is recommended for use as an MRL.

3.5 THIABENDAZOLE (065)

Thiabendazole is authorized as a post-harvest fungicide on citrus in many countries. It was evaluated for residues several times by the JMPR from 1970 to 2006. The 1997 JMPR reviewed the compound under the CCPR Periodic Re-evaluation Programme and proposed withdrawal of the existing CXL for citrus fruits of 10 mg/kg. The 2000 JMPR reviewed residue data from Spain on the basis of which an MRL of 3 mg/kg was proposed. At the CCPR Meeting in 2004, the MRL of citrus fruits was returned to Step 6 pending receipt of new residue data. The 2006 JMPR received new residue data on oranges and mandarins from Morocco and proposed for thiabendazole a maximum residue level of 5 mg/kg Po for citrus fruits.

At the 39th Session of the CCPR in 2007, the Committee noted the concerns expressed by Australia and the USA regarding the proposed MRL of 5 mg/kg Po of thiabendazole for citrus fruits. The delegations suggested, based on the reported HR of 5.2 mg/kg and statistical analysis, a more appropriate MRL would be 7 mg/kg. The Committee decided to advance the proposed draft MRL for citrus fruits of 5 mg/kg for adoption at Step 5 and to request JMPR to reconsider the statistical calculation used to derive the MRL.

Results of supervised trials on crops

Citrus fruits

The 2006 JMPR received information on supervised field trials on citrus fruits from trials conducted in Morocco during 2003 and 2004. Thiabendazole, formulated as a 500 SC, was applied to oranges (15 trials) and mandarins (eight trials) according to Moroccan GAP (post-harvest use, spray application mixed with wax, 0.375 kg ai/hL).

The residue levels in whole oranges treated according to the GAP were: 1.6, 1.6, 1.8, 1.8, 2.1, 2.2, 2.5, 3.3, 3.3, 3.4, 3.4, 3.8, 4.0, 4.2 and 5.2 mg/kg. The residue levels in whole mandarins treated according to the GAP were: 1.3, 2.4, 2.7, 2.7, 2.7, 2.8, 3.5 and 3.5 mg/kg. The 2006 JMPR decided to combine the data for whole oranges and mandarins to give a data set for whole citrus fruits (n=23): 1.3, 1.6, 1.6, 1.8, 1.8, 2.1, 2.2, 2.4, 2.5, 2.7, 2.7, 2.7, 2.8, 3.3, 3.3, 3.4, 3.4, 3.5, 3.5, 3.8, 4.0, 4.2 and 5.2 mg/kg.

The 2007 JMPR did not receive additional new information or data, but reconsidered the data reported by the 2006 JMPR because the proposed maximum residue level of 5 mg/kg for thiabendazole in citrus fruits fails to account for the value above 5 mg/kg.

The Meeting estimated a maximum residue level of 7 mg/kg Po for thiabendazole in citrus fruits to replace the previous recommendation of 5 mg/kg.

The HR value of 0.84 mg/kg and the STMR value of 0.045 mg/kg were derived by the 2006 JMPR from thiabendazole residues in the edible portion of the fruits from the same data set.

Dietary risk assessment

The MRL recommendation of 7 mg/kg Po for thiabendazole in citrus fruits will not alter the dietary intake estimates provided by the 2006 JMPR as these were based on the HR and the STMR values in the edible portion of the fruits from the same dataset. For long- and short-term intake estimations see the JMPR report 2006.

4. DIETARY RISK ASSESSMENT FOR PESTICIDE RESIDUES IN FOODS

Assessment of risk from long-term dietary intake

At the present Meeting risks associated with long-term dietary intake were assessed for compounds for which MRLs were recommended and STMRs estimated. International estimated daily intakes (IEDIs) were calculated by multiplying the concentrations of residues (STMRs and STMR-Ps) by the average daily per capita consumption estimated for each commodity on the basis of the 13 GEMS/Food Consumption cluster diets, available at http://www.who.int/foodsafety/chem/gems/en/index1.html. IEDIs are expressed as a percentage of the ADI for a 55 kg or 60 kg person, depending on the cluster diet.

The toxicological evaluation of atrazine was recommended by the WHO Drinking-water Guidelines programme and an ADI established. As the residues of this compound in food have not been considered by the JMPR, the long-term dietary risk assessment was not conducted by the Meeting.

The evaluation of bifenazate, captan, carbaryl, carbendazim, fenpyroximate, folpet, indoxacarb, methiocarb, thiabendazole and quinoxyfen performed at this Meeting do not affect the long-term dietary assessment conducted by the previous JMPR for these compounds.

Azinphos-methyl, lambda-cyhalothrin, procymidone, and profenofos were evaluated toxicologically at this Meeting under the Periodic Re-evaluation Programme and new ADIs were allocated. The long-term dietary risk assessment for these compounds will be considered during the periodic re-evaluation for residues at subsequent Meetings.

A summary of the long-term dietary risk assessments conducted by the present meeting is shown on Table 6. The detailed calculations of long-term dietary intakes are given in Annex 3. The percentages are rounded to one whole number up to 9 and to the nearest 10 above that. Percentages above 100 should not necessarily be interpreted as giving rise to a health concern because of the conservative assumptions used in the assessments. Calculations of dietary intake can be further refined at the national level by taking into account more detailed information, as described in the Guidelines for predicting intake of pesticide residues.[19]

Table 6. Summary of long-term dietary risk assessments conducted by the 2007 JMPR.

CCPR code	Compound Name	ADI (mg/kg bw)	Range of IEDI as % of maximum ADI
220	*Aminopyralid*	*0 - 0.9*	*0*
156	*Clofentezine*	*0 - 0.02*	*0 - 3*
157/228	*Cyfluthrin/Beta Cyfluthrin*	*0 - 0.04*	*0 - 2*
169	*Cyromazine*	*0 - 0.06*	*0 – 2*
224	*Difenoconazole*	*0 - 0.01*	*0 – 10*
225	*Dimethomorph*	*0 - 0.2*	*0 – 1*
037	*Fenitrothion*	*0 - 0.006*	*30 – 80*
165	*Flusilazole*	*0 - 0.007*	*2 – 10*
103	*Phosmet*	*0 - 0.01*	*2 – 90*
160	*Propiconazole*	*0 - 0.07*	*0 – 2*
226	*Pyrimethanil*	*0 - 0.2*	*0 – 5*
133/168	*Triadimefon/ Triadimenol*	*0 - 0.03*	*1– 4*
143	*Triazophos*	*0 - 0.001*	*0 – 20*
227	*Zoxamide*	*0 - 0.5*	*0*

[19] WHO (1997) Guidelines for predicting dietary intake of pesticide residues. 2nd revised edition, GEMS/Food Document WHO/FSF/FOS/97.7, Geneva

Assessment of risk from short-term dietary intake

Available consumption data was reviewed at the present Meeting to assess the risks associated with short term dietary intake for compounds with STMR and HR estimated values and established acute reference doses (ARfDs). The procedures for calculating the short-term intake were defined primarily in 1997 at an FAO/WHO Geneva Consultation,[20] refined at the International Conference on Pesticide Residues Variability and Acute Dietary Risk Assessment sponsored by the Pesticide Safety Directorate and at subsequent JMPR Meetings.

Data on the consumption of large portions were provided by the governments of Australia, France, The Netherlands, Japan, South Africa, Thailand, the UK and the USA. Data on unit weights and per cent edible portions were provided by the governments of France, Sweden, the UK and the USA. The body weights of adults and children aged ≤ 6 years were provided by the governments of Australia, France, the Netherlands, South Africa, the UK and the USA. The consumption, unit weight and body weight data used for the short-term intake calculation were compiled by GEMS/FOOD and are available at http://www.who.int/foodsafety/chem/acute_data/en/. The documents are dated May, 2007 (large portions and body weights) and May, 2003 (unit weights).

The procedures used for calculating the International estimated short-term intake (IESTI) are described in detail in Chapter 3 of the 2003 JMPR report. Detailed guidance on setting ARfD is described in Section 2.1 of the 2004 JMPR report.[21]

The toxicological evaluation of atrazine was recommended by the WHO Drinking-water Guidelines programme and an ARfD was established. As the residues of this compound in food have not been considered by the JMPR, the short-term dietary risk assessment was not conducted by the Meeting.

The evaluation of captan, carbendazim, folpet, methiocarb and thiabendazole performed at this Meeting do not affect the short-term dietary assessment conducted by the previous JMPR for these compounds.

Azinphos-methyl, lambda-cyhalothrin, procymidone, and profenofos were evaluated toxicologically at this Meeting under the Periodic Re-evaluation Programme, and new ARfDs allocated. The short-term dietary risk assessment for these compounds will be considered during the periodic re-evaluation for residues at subsequent Meetings.

On the basis of data received by the present or previous Meeting, the establishment of an ARfD for aminopyralid, bifenazate, clofentezine, pyrimethanil, quinoxyfen and zoxamide was considered to be unnecessary. The short-term intakes of these compounds were not estimated.

The short-term intakes as percentages of the ARfDs for the general population and for children are summarized in Table 7. The percentages are rounded to one whole number up to 9 and to nearest 10 above that. Percentages above 100 should not necessarily be interpreted as giving rise to a health concern because of the conservative assumptions used in the assessments.

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[20] WHO (1997) Food consumption and exposure assessment of chemicals. Report of a FAO/WHO Consultation. Geneva, Switzerland, 10-14 February 1997, Geneva

[21] Annex 5, reference 98.

Table 7. Summary of short-term dietary risk assessments conducted by the 2007 JMPR.

CCPR code	Compound Name	ARfD (mg/kg bw)	Commodity	Percentage of ARf D	
				General population	Children aged ≤ 6 years
008	*Carbaryl*	*0.2*	*Cranberry and dried chilli pepper*	*0*	*0 – 1*
157/ 228	*Cyfluthrin/ Beta Cyfluthrin*	*0.04*	*Broccoli*	*70*	*120*
			Cabbage, head	*100*	*240*
			Other commodities	*0 – 60*	*0 – 80*
169	*Cyromazine*	*0.1*	*Cabbage, head*	*120*	*280*
			Spinach	*130*	*390*
			Other commodities	*0 – 20*	*0 – 40*
224	*Difenoconazole*	*0.3*	*All commodities*	*0 – 7*	*0 – 10*
225	*Dimethomorph*	*0.6*	*All commodities*	*0 – 10*	*0 – 20*
037	*Fenitrothion**	*0.04*	*Wheat bran, unprocessed*	*70*	*110*
			Other commodities	*0 – 80*	*0 – 70*
193	*Fenpyroximate*	*0.02*	*Apple*	*20*	*60*
			Grape	*60*	*150*
165	*Flusilazole*	*0.02*	*All commodities*	*0 – 40*	*0 – 100*
216	*Indoxacarb*	*0.1*	*Cabbage, head*	*40*	*90*
103	*Phosmet*	*0.2*	*All commodities*	*1 – 50*	*0 – 100*
160	*Propiconazole*	*0.3*	*All commodities*	*0 – 1*	*0 – 3*
133/ 168	*Triadimefon/ Triadimenol*	*0.08*	*Grapes (excluding wine)*	*80*	*220*
			Other commodities	*0 – 20*	*0 – 60*
143	*Triazophos*	*0.001*	*Soya bean (immature)*	*140*	*230*
			Cotton seed oil	*2*	*5*

* Since unprocessed wheat bran is not an edible commodity and further processing is likely to reduce the level of residues, the Meeting assumed that the intake of fenitrothion from processed wheat bran would be below the ARfD. The Meeting concluded that the short-term intake of residues of fenitrothion from uses considered by the Meeting was unlikely to present a public health concern.

5. EVALUATION OF DATA FOR ACCEPTABLE DAILY INTAKE AND ACUTE DIETARY INTAKE FOR HUMANS, MAXIMUM RESIDUE LEVELS AND SUPERVISED TRIAL MEDIAN RESIDUE VALUES

5.1 AMINOPYRALID (220)

Toxicology

Aminopyralid is the International Organization for Standardization (ISO) approved name for 4-amino-3,6-dichloropyridine-2-carboxylic acid (Chemical Abstracts Service, CAS No. 150114-71-9). It is a post-emergent auxin-type herbicide for the control of a wide variety of broadleaf weed species. Most of the toxicological studies evaluated were performed with the aminopyralid acid. However, some studies were performed with the commercially available aqueous solution of aminopyralid triisopropylammonium (TIPA) salt, called GF-871, since products are also marketed in this form. Throughout the current evaluation, aminopyralid acid is termed aminopyralid and its TIPA salt is termed aminopyralid TIPA. GF-871 is a 41.3–41.9% aqueous solution of aminopyralid TIPA corresponding to approximately 21.7% aminopyralid. If not otherwise stated, doses are given as aminopyralid equivalents.

Aminopyralid has not been evaluated previously by the Meeting. The evaluation of aminopyralid was scheduled for the 2006 JMPR but, owing to incomplete submission of data, was postponed to the present Meeting. Aminopyralid was reviewed at the request of CCPR. All pivotal studies with aminopyralid and aminopyralid TIPA were certified as complying with GLP.

Biochemical aspects

In a study of the absorption, distribution, metabolism and excretion of radiolabelled aminopyralid administered by oral gavage, male rats were given single doses at 50 or 1000 mg/kg bw, or a single dose at 50 mg/kg bw after 14 days pre-treatment with unlabelled aminopyralid at a dose of 50 mg/kg bw per day. Male rats also received radiolabelled aminopyralid TIPA salt as a single oral gavage dose at 96 mg/kg bw (equal to aminopyralid at 50 mg/kg bw). The pharmacokinetic behaviour of aminopyralid and its TIPA salt was very similar. Of the administered dose, 42–59% was absorbed and rapidly excreted in the urine, most within the first 24 h. Excretion was biphasic, with half-lives of 3.0–3.8 h and 10.2–12.3 h. Biliary excretion was assumed to be negligible. After a depletion period of 168 h, virtually all tissue samples showed concentrations of radiolabel of less than 0.01% of the administered dose. Aminopyralid was not metabolized in rats; more than 95% of the radiolabel was accounted for. On the basis of lack of coordination in gait after exposure to aminopyralid and aminopyralid TIPA in rabbits but not in rats, an extensive study of the absorption, distribution, metabolism and excretion of aminopyralid in rabbits was performed. A group of non-pregnant rabbits received radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw. A group of pregnant rabbits (“late-stage”) received non-labelled aminopyralid on days 7–21 of gestation and then radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw on day 22 of gestation. An additional group of pregnant rabbits (“early-stage”) received radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw on day 7 of gestation, without pre-treatment with unlabelled aminopyralid. Using plasma from animals in these three groups and from a group of non-pregnant rats given radiolabelled aminopyralid as a single dose at 280–370 mg/kg bw, a study of plasma-protein binding was performed. In late-stage pregnant rabbits pre-treated with repeated doses of unlabelled aminopyralid, the absorption of aminopyralid (based on lower T_{max}, higher area under the curve for concentration–time [AUC] and increased renal excretion) was somewhat more rapid and greater than in non-pregnant and early-stage pregnant rabbits. Plasma-protein binding was lower (43–58%) in late-stage pregnant rabbits pre-treated with repeated doses of unlabelled aminopyralid than in non-

pregnant and early-stage pregnant rabbits (47–68%). The difference in bioavailability (expressed as unbound compound) of aminopyralid was at most twofold. However, interpretation of these results remains ambiguous because of the different dosing regimens used (single dose without pre-treatment in non-pregnant and early-stage pregnant rabbits and single dose after pre-treatment in late-stage pregnant rabbits). Based on renal excretion of radiolabel, absorption of aminopyralid in rabbits was close to 80% or greater, being 20–40% higher than in rats.

Toxicological data

Aminopyralid and aminopyralid TIPA have low acute toxicity in rats when administered orally, dermally or by inhalation. The oral and the dermal LD50s are both > 5000 mg/kg bw, and by inhalation, the LC50 is > 5.5 mg/L, the highest dose tested. Aminopyralid is clearly irritating to the eye, while aminopyralid TIPA is only slightly irritating. Aminopyralid is not a dermal irritant, while aminopyralid TIPA is a slightly irritant. In guinea-pigs, aminopyralid and aminopyralid TIPA produced no signs of skin-sensitizing potential, as tested by the Magnusson & Kligman method.

In short-term feeding studies in mice, rats and dogs and in a study of dermal exposure studying rats, animals received aminopyralid at doses of up to 1000 mg/kg bw per day. Body weight was reduced only in female dogs receiving aminopyralid at 967 mg/kg bw per day, the highest dose tested in a 1-year study. Males and females at this dose also showed a slight increase in relative liver weights accompanied by hepatocyte hypertrophy in two out of four animals per sex. In male and female rats at doses of 500 mg/kg bw per day and greater, reversibly increased absolute and relative weights of full and empty caeca were observed and slight mucosal hyperplasia of the caecum and the ileum was found in males at the highest dose. These changes were considered to be a consequence of physiological adaptation. Mucosal hyperplasia of the stomach was observed in all dogs at 967 mg/kg bw per day. Treatment-related clinical chemistry changes were restricted to the urine of rats at 500 mg/kg bw per day and greater, where decreased pH values and decreased concentrations of protein and ketone were found. Changes in the pH of the urine were most likely due to urinary excretion of the unchanged, acid parent compound. Generally, aminopyralid was well tolerated by mice, rats and dogs in short-term studies. The NOAEL for aminopyralid in mice was 1000 mg/kg bw per day (the highest dose tested), 1000 mg/kg bw per day in rats (the highest dose tested) and 93.2 mg/kg bw per day in dogs, on the basis of histopathological changes in the gastric mucosa at the highest dose tested. In a 13-week feeding study in rats with aminopyralid TIPA, the same effects on caecal weights and urine chemistry were observed as with aminopyralid. Based on the lack of other effects, the NOAEL for aminopyralid TIPA in rats was 2421 mg/kg bw per day as GF-871, equal to aminopyralid at 525 mg/kg bw per day, the highest dose tested.

In an 18-month study in mice and a 24-month feeding study in rats, diets adjusted to provide aminopyralid at maximal doses of 1000 mg/kg bw per day did not induce any increases in the incidence of neoplastic findings.

Mortality was increased in all groups of female mice receiving aminopyralid, but appeared to be compound-related only in animals at the highest dose. Animals that died showed an increased incidence of age-related nephropathy. Although the overall incidence of nephropathy was not increased in this or any treated group, exacerbation of the effects of age-related nephropathy in these animals may have been responsible for the increase in mortality. Other treatment-related signs of toxicity in this group e.g., pale kidneys, reduced body fat, haemolysed blood in the gastrointestinal tract, atelectasis of the lung and perineal soiling, were most likely a reflection of the moribund state of the animals. The NOAEL was 250 mg/kg bw per day on the basis of increased mortality in females at 1000 mg/kg bw per day. Although the final cumulative mortality in rats was comparable at all doses, the onset of mortality in males at 1000 mg/kg bw per day appeared earlier. As statistical significance and clear treatment-related causes for death were lacking, the Meeting did not consider this finding as being related to treatment.

Slightly reduced body-weight gain was observed in male rats at 500 mg/kg bw per day and above. Increased absolute and relative weights of full and empty caeca were observed in males and females at 500 mg/kg bw per day and above, accompanied by very slight mucosal hyperplasia of the caeca, which was statistically significant only in males at the highest dose. This finding is common to many substances and, due to the lack of further histological changes, was not considered to be an adverse finding. In males and females at 500 and 1000 mg/kg bw per day, increased urine volumes, decreased specific gravity and protein and ketone contents and reduced pH values were observed without any renal histopathological correlate. These changes were not considered to be toxicologically significant, the change in pH most likely reflecting urinary excretion of the acidic parent compound. The NOAEL in this feeding study in rats was 500 mg/kg bw per day on the basis of slight but statistically significant body-weight decreases in males at 1000 mg/kg bw per day.

Aminopyralid was not carcinogenic in mice or rats.

Aminopyralid and its TIPA salt were tested for genotoxicity in an adequate range of tests, including an assay for reverse mutation in *Salmonella typhimurium* and *E. coli*, an assay for forward mutation in HGPRT with Chinese hamster ovary cells, an assay for chromosomal aberration in rat lymphocytes in vitro and an assay for micronucleus formation in mouse bone marrow in vivo. In the assay for chromosomal aberration in rat lymphocytes in vitro, an increased frequency of chromosomal aberration was seen only after 24 h of treatment at clearly cytotoxic concentrations of aminopyralid and in the absence of metabolic activation. The results of all other assays showed no evidence for genotoxicity.

The Meeting concluded that aminopyralid and aminopyralid TIPA are unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in rats and mice, the Meeting concluded that aminopyralid is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproduction in rats given diets containing aminopyralid, only increases in the weight of full and empty caeca in parental animals treated with aminopyralid were observed and no changes in reproductive parameters were observed. The caecal changes were not considered to be toxicologically significant. The NOAEL for general toxicity and reproductive toxicity was 1000 mg/kg bw per day, the highest dose tested. In pregnant rats and rabbits treated with aminopyralid by gavage, even the highest doses tested, 1000 and 500 mg/kg bw per day, respectively, did not induce any treatment-related malformations in the offspring. When rats and rabbits were treated with aminopyralid TIPA, no treatment-related malformations occurred, but foetal body-weight reduction was observed in only in rabbits at high doses (aminopyralid equivalents, 526 mg/kg bw per day).

In rabbits treated with aminopyralid at doses of 500 mg/kg bw per day and greater, body-weight gain was reduced, and uncoordinated gait was evident immediately after dosing, lasting for approximately 2 h. Neither the severity nor the duration of uncoordinated gait increased as the study progressed. Additionally, two rabbits treated with aminopyralid at the highest dose were killed in a moribund condition on day 17 of gestation, being found to have pale kidneys, watery and dark caecal contents and erosions/ulcers in the glandular mucosa of the stomach. Owing to severe clinical signs observed at 750 mg/kg bw per day, all remaining animals in this study were killed at day 20 of gestation and were not available for evaluation of reproductive performance. Therefore, the NOAEL for maternal toxicity was 250 mg/kg bw per day. In pregnant rabbits treated with aminopyralid TIPA, maternal toxicity was evident as uncoordinated gait at a dose of 78 mg/kg bw per day as aminopyralid equivalents and greater. At higher doses, the clinical effects observed were similar to those seen with aminopyralid. The NOAEL for maternal toxicity was 26 mg/kg bw per day as aminopyralid equivalents.

In a study of acute toxicity and a 1-year study of neurotoxicity in rats treated with aminopyralid, no signs of behavioural changes and no histopathological findings suggesting neurotoxic potential were observed. Faecal and urine soiling at 2000 mg/kg bw in the study of acute

toxicity were most likely due to general toxicity rather than indicative of specific neurotoxicity. The NOAELs for neurotoxicity in these two studies were 2000 and 1000 mg/kg bw per day as aminopyralid, respectively, the highest doses tested.

To obtain further insight into the possible mode of action leading to uncoordinated gait in rabbits, pregnant and non-pregnant animals were treated with aminopyralid and aminopyralid TIPA. Again, uncoordinated gait was observed but without any histopathological correlate in the central or peripheral nervous system. Additionally, no changes in consciousness, muscle strength or autonomic and somatic control were observed. For the acute dietary risk assessment, the occurrence of unco-ordination after one or two doses in pregnant rabbits treated with aminopyralid may be the only relevant end-point. The NOAEL for aminopyralid for this effect was 250 mg/kg bw per day. In one study with aminopyralid TIPA, slight unco-ordination was seen in one animal after 1 day of treatment at a dose of 173.6 mg/kg bw per day as aminopyralid equivalents. In another two studies of developmental toxicity in rabbits receiving aminopyralid TIPA, a dose-related increase in the incidence of unco-ordination was found. However, at doses at which unco-ordination was observed, i.e., 78, 105, 263 and 525 mg/kg bw per day as aminopyralid equivalents, the effect occurred only after at least six exposures. The NOAEL was 26 mg/kg bw per day as aminopyralid equivalents. The Meeting concluded that the weight of evidence indicated that the NOAEL for acute unco-ordination was 250 mg/kg bw as aminopyralid. Effects observed in short-term studies in dogs were not considered relevant for establishing an acute reference dose.

The Meeting concluded that the existing data were adequate to characterize the potential hazard to foetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.9 mg/kg bw based on a NOAEL of 93.2 mg/kg bw per day identified on the basis of histological changes in the gastric mucosa at higher doses in a 1-year study in dogs, and a safety factor of 100.

The Meeting concluded that it was not necessary to establish an ARfD for aminopyralid. The only end-point that might be suitable as a basis for establishing an ARfD for aminopyralid was uncoordinated gait in the studies of developmental toxicity in rabbits. Although this effect was observed after repeated exposure at 78 mg/kg bw with a NOAEL of 26 mg/kg bw, it was observed after one or two exposures only at higher doses, with a NOAEL of 250 mg/kg bw. As this effect was dependent on C_{max} and in view of the kinetics of aminopyralid and the dynamics of this response, the Meeting considered it appropriate to adjust the safety factor. Applying a safety factor of 25 to the NOAEL of 250 mg/kg bw would result in a putative ARfD of 10 mg/kg bw which is greater than the JMPR-recommended cut-off value for an ARfD of 5 mg/kg bw.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Test material	Study	Effect	NOAEL[b]	LOAEL[b]
Mouse	Aminopyralid	Eighty-week study of toxicity and carcinogenicity[a]	Toxicity	250 mg/kg bw per day	1000 mg/kg bw per day
			Carcinogenicity	1000 mg/kg bw per day[c]	—
Rat	Aminopyralid	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 mg/kg bw per day	1000 mg/kg bw per day
			Carcinogenicity	1000 mg/kg bw per day[c]	—

	Aminopyralid	Two-generation study of reproductive toxicity[a]	Parental toxicity/	1000 mg/kg bw per day[c]	—
			Offspring toxicity	1000 mg/kg bw per day[c]	—
	Aminopyralid	Developmental toxicity[b]	Maternal toxicity	1000 mg/kg bw per day[c]	—
			Embryo/fetotoxicity	1000 mg/kg bw per day[c]	—
	Aminopyralid TIPA	Developmental toxicity[d]	Maternal toxicity	525 mg/kg bw per day[c]	—
			Embryo/fetotoxicity	525 mg/kg bw per day[c]	—
	Aminopyralid	Long-term study of neurotoxicity	Neurotoxicity	1000 mg/kg bw per day[c]	—
Rabbit	Aminopyralid	Developmental toxicity[d]	Maternal toxicity	250 mg/kg bw per day	500 mg/kg bw per day
			Embryo/fetotoxicity	500 mg/kg bw per day[c]	—
	Aminopyralid TIPA	Developmental toxicity[d]	Maternal toxicity	26 mg/kg bw per day	78 mg/kg bw per day
			Embryo/fetotoxicity	263 mg/kg bw per day	526 mg/kg bw per day
Dog	Aminopyralid	One-year study of toxicity[a]	Toxicity	93.2 mg/kg bw per day	967 mg/kg bw per day

TIPA, triisopropylammonium.

[a] Dietary administration.

[b] Values for aminopyralid TIPA are given as aminopyralid equivalents.

[c] Highest dose tested.

[d] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.9 mg/kg bw

Estimate of acute reference dose

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, 42–59%

Dermal absorption	No data
Distribution	Extensive
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid
Metabolism in animals	Minimal. No metabolites identified.
Toxicologically significant compounds in animals, plants and the environment	Aminopyralid
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 5000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.5 mg/L
Rabbit, dermal irritation	Aminopyralid is not an irritant, aminopyralid TIPA is a slight irritant.
Rabbit, ocular irritation	Aminopyralid is an irritant, aminopyralid TIPA is not an irritant.
Skin sensitization (test method used)	Not a sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Histopathological changes in stomach
Lowest relevant oral NOAEL	93.2 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	1000 mg/kg bw per day, the highest dose tested (4-week study in rats)
Lowest relevant inhalation NOAEC	No studies available
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Increased mortality in female mice
Lowest relevant NOAEL	250 mg/kg bw per day (18-month study in mice)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects in rats
Lowest relevant reproductive NOAEL	1000 mg/kg bw per day, the highest dose tested
Developmental target/critical effect	No developmental effects in rats and rabbits with aminopyralid; foetal body-weight changes with aminopyralid TIPA.
Lowest relevant developmental NOAEL	263 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	
	No neurotoxic effects in rats with aminopyralid and aminopyralid TIPA; uncoordinated gait in pregnant rabbits, with both aminopyralid and aminopyralid TIPA.
Lowest relevant NOAEL	26 mg/kg bw per day (repeated doses), 250 mg/kg bw (single dose)

Medical data

No data available

Summary

	Value	Study	Safety factor
ADI	0–0.9 mg/kg bw	Dog, 1-year study	100
ARfD	Unnecessary	—	—

TIPA, triisopropylammonium

RESIDUE AND ANALYTICAL ASPECTS

Aminopyralid was evaluated for residues and toxicology by the 2006 JMPR and a discussion of residue aspects included in the report of the 2006 JMPR. As the toxicological evaluation was not completed in 2006 a dietary risk assessment could not be conducted and recommendations for maximum residue levels were not made. The current Meeting has completed the evaluation of the toxicological data and, based on the evaluation of the 2006 JMPR, recommendations are now made for maximum residue levels in various commodities.

Recommendations

On the basis of the data from supervised trials and farm animal feeding studies reported by the 2006 JMPR, the Meeting concluded that the residue levels as listed in Annex 1 are appropriate for establishing maximum residue limits and for IEDI assessment.

Definition of the residue for plants and animals (for compliance with MRLs and estimation of dietary intake): aminopyralid and its conjugates that can be hydrolysed, expressed as aminopyralid.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of aminopyralid resulted in recommendations for MRLs and STMR values for raw and processed commodities. The International Estimated Daily Intakes (IEDI) of aminopyralid in the 13 GEMS/Food cluster diets, based on estimated STMRs were all < 1% of the maximum ADI of 0.9 mg/kg bw. The Meeting concluded that the long-term intake of residues of aminopyralid from uses that have been considered by the JMPR is 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 aminopyralid residues is unlikely to present a public health concern.

5.2 ATRAZINE

TOXICOLOGY

Atrazine, 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine (International Union of Pure and Applied Chemistry, IUPAC) (CAS No. 1912-24-9), is a selective systemic herbicide of the

chlorotriazine class, used for the control of annual broadleaf and grassy weeds. It acts as a photosynthetic electron transport inhibitor at the photosystem II receptor site. Atrazine and its chloro-*s*-triazine metabolites deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT) have been found in surface and ground water as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide. Hydroxyatrazine is more commonly detected in ground than in surface water. The relative order of concentrations in rural wells in the USA was generally as follows: atrazine ~DEA ~DACT > DIA > hydroxyatrazine. However, concentrations of DEA that are several-fold higher than those of the parent compound have been reported.

Atrazine was evaluated previously by WHO, a tolerable daily intake (TDI) of 0.0005 mg/kg bw being established in the 1993 Guidelines for Drinking-water Quality based on a NOAEL of 0.5 mg/kg bw per day in a study of carcinogenicity in rats and using a safety factor of 1000 (100 for inter- and intraspecies variation and 10 to reflect potential carcinogenic risk to humans).

Atrazine had not been previously evaluated by JMPR, and no ADI had been established. For that reason, the WHO Drinking-water Guidelines programme recommended that atrazine should be evaluated toxicologically by JMPR.

The database on atrazine was extensive, consisting of a comprehensive set of GLP-compliant guideline studies with atrazine and its four key metabolites, as well as a large number of published studies. The present Meeting did not aim to perform a review of the database de novo, but to summarize the key studies focusing on the issues of carcinogenicity, endocrine disruption (especially its neuroendocrine mode of action) and immunotoxicity. Reference was made to a number of reviews made by national and international agencies and organizations in recent years.

Biochemical aspects

After oral administration to rats, [^{14}C] labelled atrazine was rapidly and almost completely absorbed, independent of dose and sex. Radioactivity was widely distributed throughout the body. Excretion was more than 93% of the administered dose within 7 days, primarily via the urine (approximately 73%) and to a lesser extent via the faeces (approximately 20%; approximately 7% via bile), with more than 50% being excreted within the first 24 h. The elimination half-life of radiolabel from the whole body was 31.3 h in rats; this prolonged half-life was caused by covalent binding of atrazine to cysteine sulfhydryl groups in the β-chain of rodent haemoglobin. Seven days after administration of a single low dose (1 mg/kg bw), tissue residues represented 6.5–7.5% of the dose, with the highest concentrations in erythrocytes (≤ 0.63 ppm), liver (≤ 0.50 ppm) and kidneys (≤ 0.26 ppm). Atrazine was extensively metabolized; more than 25 metabolites have been identified in rats. The major metabolic pathways were stepwise dealkylation via either deisopropyl-atrazine (DIA) or deethyl-atrazine (DEA) to diaminochlorotriazine (DACT), the major metabolite. Dechlorination involving conjugation with glutathione was a minor pathway. The biotransformation of atrazine in rats and humans was qualitatively similar.

Toxicological data

Atrazine was of low acute toxicity in rats exposed orally (LD_{50}, 1870–3090 mg/kg bw), dermally (LD_{50}, > 2000 mg/kg bw) or by inhalation (LC_{50}, > 5.8 mg/L). Atrazine was not a skin irritant or an eye irritant in rabbits. Although spray dilutions of atrazine did not appear to be sensitizing in humans, atrazine was a skin sensitizer in tests in guinea-pigs (Magnusson & Kligman, Maurer optimization test).

In short-term studies of toxicity in rats, dogs and rabbits, the consistent toxic effects noted across species included reduced body-weight gain and food intake and a slight decrease in erythrocyte parameters. Also in rats, liver weights and splenic haemosiderin deposition were increased, while in dogs there was marked cardiac toxicity.

In a 90-day study of toxicity in rats, the NOAEL was 50 ppm, equal to 3.3 mg/kg bw per day, on the basis of decreased body-weight gain and increased splenic haemosiderin deposition at 500 ppm.

In a 52-week study of toxicity in dogs, the NOAEL was 150 ppm, equal to 5 mg/kg bw per day, on the basis of decreased body-weight gain and marked cardiac toxicity at 1000 ppm, equal to 33.7 mg/kg bw per day.

In a 25-day study in rabbits treated dermally, the NOAEL for systemic toxicity was 100 mg/kg bw per day on the basis of decreased body-weight gain and food intake, a slight reduction in erythrocyte parameters and increased spleen weight at 1000 mg/kg bw per day.

Atrazine was tested for genotoxicity in a large number of studies covering an adequate range of end-points, including assays for gene mutation in bacteria and eukaryotic cells in vitro, for DNA damage and repair in bacteria and mammalian cells (rat hepatocytes, human fibroblasts) in vitro, and for chromosomal aberration in vitro and in somatic and germ cells in vivo. Mostly negative results were obtained in standard assays. In a few published studies, positive responses were reported. However, a number of reviews by national and international agencies (United States Environmental Protection Agency, European Union, International Agency for Research on Cancer) have concluded that, based on the weight of evidence, atrazine is not genotoxic.

The Meeting agreed that it is unlikely that atrazine is genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. As in short-term studies, reduced body weight gain and food intake and a decrease in erythrocyte parameters were noted consistently. Additionally, reduced survival of females and cardiovascular effects (atrial thrombi) in both sexes were observed in mice at high doses.

In three studies of carcinogenicity in mice, no treatment-related carcinogenic effects were observed at dietary concentrations of up to 3000 ppm, equal to about 386 and 483 mg/kg bw per day in males and females, respectively. Overall, the NOAEL was 10 ppm, equal to 1.2 mg/kg bw per day, on the basis of lower body weight/body-weight gain at 300 ppm, equal to 38.4 mg/kg bw per day, and greater.

In two studies of carcinogenicity in Fischer-344 (F344) rats fed diets containing atrazine at concentrations of up to 400 ppm, equivalent to about 20 mg/kg bw per day, there was no effect at any dose on the onset or incidence of tumours. The NOAEL was 70 ppm, equivalent to about 3.5 mg/kg bw per day, on the basis of decreased body weight at concentrations of 200 ppm and greater. In a non-guideline study of carcinogenicity in F344 rats, there was a significant increase in the incidence of benign mammary tumours in males and in uterine adenocarcinomas in females at the highest dose of 750 ppm, equivalent to about 38 mg/kg bw per day; however, interpretation of the result was limited by increased survival at the highest dose, and a survival-adjusted analysis of tumour prevalence did not indicate any significant increase in the incidence of benign, malignant or combined mammary tumours.

In seven studies of carcinogenicity in Sprague-Dawley rats fed diets containing atrazine at concentrations of up to 1000 ppm (equal to about 42 and 65 mg/kg bw per day in males and females, respectively), an increased incidence of mammary tumours (adenomas, carcinomas, fibroadenomas) with or without an earlier onset (relative to controls) was observed in four studies, while in two studies there was an earlier onset of mammary tumours without any increase in their overall lifetime incidence. An earlier onset of pituitary tumours was also observed in one study, with no increase in incidence at term. Overall, the NOAEL for mammary carcinogenicity was 25 ppm, equal to 1.5 mg/kg bw per day, on the basis of a statistically significant increased incidence in mammary tumours at 50 ppm, equal to 3.1 mg/kg bw per day.

In a study of carcinogenicity in ovariectomized Sprague-Dawley rats, neither increases in mammary-gland proliferative changes nor mammary tumours were seen at dietary concentrations of

up to 400 ppm (equal to about 21 mg/kg bw per day), suggesting that the carcinogenic mode of action of atrazine in Sprague-Dawley rats is related to ovarian function.

In a mechanistic 6-month study in Sprague-Dawley rats, attenuation of the luteinizing hormone (LH) surge and subsequent disruption of the oestrous cycle (characterized by an increase in days in oestrous) were observed at ≥ 50 ppm (equal to 3.65 mg/kg bw per day), with a NOAEL of 25 ppm (equal to 1.8 mg/kg bw per day). The NOAEL and LOAEL for these effects were comparable to those found in the studies of carcinogenicity. The effects on the LH surge and disruption of the oestrous cycle were further supported by a number of short-term mechanistic studies. Additional experiments suggested that the effects of atrazine on LH and prolactin secretion are mediated via a hypothalamic site of action.

The postulated mode of action for atrazine-induced mammary tumours in female Sprague-Dawley rats involved disruption of the hypothalamic–pituitary–ovary axis. Atrazine modifies catecholamine function and the regulation of gonadotropin-releasing hormone (GnRH) pulsatility in the rat hypothalamus, with the consequence that the pulse of LH released from the pituitary gland is of insufficient amplitude or duration to trigger the ovulation. The failure to ovulate results in persistent secretion of oestrogen, which provides a feedback to the pituitary leading to increased secretion of prolactin. As a result, atrazine accelerates the normal reproductive ageing process in female Sprague-Dawley rats whereby reproductive senescence is characterized by persistent exposure to oestrogen and prolactin. In contrast, women respond to reduced levels of LH by reductions in levels of oestrogen. Thus, the Meeting considered that the mode of carcinogenic action in certain susceptible rat strains is not relevant for human risk assessment.

Investigations of other modes of action did not provide any evidence that atrazine had intrinsic estrogenic activity or that it increased aromatase activity in vivo.

The Meeting concluded that atrazine is not likely to pose a carcinogenic risk to humans.

Although carcinogenicity in humans was not a concern owing to the rat-specific mode of action, alterations in neurotransmitter and neuropeptide function regulating LH and secretion of prolactin may potentially induce adverse effects during critical periods of development (as found in special studies showing pregnancy loss, delayed puberty in males and females, and decreased suckling-induced prolactin release in lactating dams). Unlike the carcinogenic effects, the developmental effects do not appear to be specific to certain strains of rats and the Meeting therefore considered these effects to be relevant for risk assessment in humans.

In special studies of reproductive toxicity, exposure of rats during early pregnancy (i.e., the LH-dependent period) caused increased pre- or post-implantation losses, including full-litter resorptions. Effects were seen at doses of ≥ 50 mg/kg bw per day after treatment on days 6–10 of gestation, with a NOAEL of 25 mg/kg bw per day. In contrast, exposure on days 11–15 of gestation (after the LH-dependent period of pregnancy) at a dose of 200 mg/kg bw per day did not induce full-litter resorptions.

Suppression of the suckling-induced prolactin release in lactating rats was seen with atrazine at doses of ≥ 25 mg/kg bw per day, with a NOAEL of 12.5 mg/kg bw per day. Treatment of lactating rats on postnatal days 1–4 affected the development of tuberoinfundibular dopaminergic neurons in the pups (presumably due to the lack of prolactin derived from the dam’s milk), with the consequence of impaired regulation of prolactin secretion, hyperprolactinaemia prior to puberty and prostatitis in the adult male offspring.

A delay in sexual development was observed in female rats after exposure on postnatal days 21–46 at doses ≥ 30 mg/kg bw per day, with a NOAEL of 10 mg/kg bw per day, and in male rats after exposure on postnatal days 23–53 at doses ≥ 12.5 mg/kg bw per day, with a NOAEL of 6.25 mg/kg bw per day.

In a standard two-generation study of reproduction (conducted according to earlier guidelines, which did not include end-points such as oestrous cyclicity and sexual development) in rats, there was

no effect on fertility at 500 ppm, the highest dose tested. The NOAEL for parental toxicity was 50 ppm, equal to 3.6 mg/kg bw per day, on the basis of decreased body-weight gains and food consumption at 500 ppm, equal to 36.1 mg/kg bw per day. The NOAEL for reproductive toxicity was 50 ppm on the basis of decreased body weights of male pups at postnatal day 21 at 500 ppm.

In two studies of prenatal developmental toxicity in rats given atrazine on days 6–15 of gestation, the NOAELs for maternal toxicity were 10 or 25 mg/kg bw per day on the basis of decreased body-weight gain and food intake at 70 or 100 mg/kg bw per day, respectively. The NOAELs for developmental toxicity were 10 or 25 mg/kg bw per day on the basis of incomplete ossification at several sites at 70 or 100 mg/kg bw per day, respectively. In a study of prenatal developmental toxicity in rabbits given atrazine on days 7–19 gestation, the NOAEL for maternal toxicity was 5 mg/kg bw per day on the basis of clinical signs, abortion and decreased food intake and body-weight gain at 75 mg/kg bw per day. The NOAEL for developmental toxicity was 5 mg/kg bw per day on the basis of increased resorptions, reduced litter size and incomplete ossification at 75 mg/kg bw per day. In rats and rabbits, the developmental effects were observed only at maternally toxic doses.

The Meeting concluded that atrazine was not teratogenic.

Studies using a variety of test systems in vitro and in vivo indicated that modulation of the immune system occurs after exposure to atrazine. However, effects suggestive of impaired function of the immune system were only observed at doses greater than those shown to affect neuroendocrine function, leading to disruption of the oestrous cycle or developmental effects.

A range of epidemiological studies (including cohort studies, case–control studies, and ecological or correlational studies) assessed possible relationships between atrazine or other triazine herbicides and cancer in humans. For some cancer types, such as prostate or ovarian cancer and non-Hodgkin lymphoma, the increased risks reported in single studies could either be explained by the methodology used or had not been confirmed in more reliable studies. Thus, the weight of evidence from the epidemiological studies did not support a causal association between exposure to atrazine and the occurrence of cancer in humans.

The Meeting concluded that the existing database on atrazine is adequate to characterize the potential hazards to foetuses, infants and children.

Metabolites of atrazine

The toxicity profiles and mode of action of the chloro-*s*-triazine metabolites were similar to those of atrazine; the potency of these metabolites appeared to be similar to that of the parent compound with regard to their neuroendocrine-disrupting properties.

Like atrazine, the chloro-*s*-triazine metabolites were of moderate or low acute oral toxicity in rats; LD_{50}s were 1110, 1240 and 2310–5460 mg/kg bw for DEA, DIA and DACT, respectively.

Like atrazine, its chloro-*s*-triazine metabolites delayed sexual development of male rats exposed on postnatal days 23–53 at atrazine molar equivalent doses of ≥ 25 (DEA, DIA) and ≥ 12.5 mg/kg bw per day (DACT), with NOAELs of 12.5 and 6.25 mg/kg bw per day, respectively. Exposure of female rats to DACT on postnatal days 22–41 delayed sexual development at atrazine molar equivalent doses of ≥ 50 mg/kg bw per day, and the NOAEL was 25 mg/kg bw per day. Doses at which these effects occurred were similar to those observed for parent atrazine.

In short-term feeding studies in rats, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body-weight gain and decreased erythrocyte parameters, and also for DACT-induced disruption of the oestrous cycle. The NOAELs were 50 ppm (equal to 3.2 mg/kg bw per day) for DEA and DIA, and 100 ppm (equal to 7.6 mg/kg bw per day) for DACT.

In a 29/52-week study with DACT in Sprague-Dawley rats, effects comparable to those observed with atrazine (attenuation of the LH surge, increased incidences of mammary tumours) were

seen at 270 ppm; the NOAEL was 48 ppm, equal to 3.4 mg/kg bw per day. No long-term studies were performed with DEA or DIA.

In short-term feeding studies in dogs, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body-weight gain and decreased erythrocyte parameters, while DEA and DACT showed cardiac toxicity. The NOAELs were 100 ppm, equal to 3.7, 3.8 and 3.5 mg/kg bw per day, for DEA, DIA and DACT, respectively.

DEA, DIA and DACT did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In studies of prenatal developmental toxicity in rats, the chlorinated metabolites induced increased incidences of fused sternebrae and/or incomplete ossification at doses of 25 to 100 mg/kg bw per day; the NOAELs for developmental toxicity were 25, 5 and 2.5 mg/kg bw per day for DEA, DIA and DACT, respectively. The effects were seen only at doses that also produced maternal toxicity.

The metabolite hydroxyatrazine does not have the same mode of action or toxicity profile as atrazine and its chlorometabolites. The main effect of hydroxyatrazine was kidney toxicity (owing to its low solubility in water, resulting in crystal formation and a subsequent inflammatory response), and there was no evidence that hydroxyatrazine has neuroendocrine-disrupting properties. Also, the acute oral toxicity of hydroxyatrazine in rats (LD_{50}, > 5050 mg/kg bw) was lower than that of atrazine or its chlorometabolites.

In short-term feeding studies, the main effects of hydroxyatrazine in rats included reduced body-weight gain, increased water consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions. The overall NOAEL was 100 ppm, equal to 6.3 mg/kg bw per day. In dogs, effects included reduced body-weight gain and food consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions; the NOAEL was 150 ppm, equal to 5.8 mg/kg bw per day.

In a 2-year study of toxicity and carcinogenicity in rats, the effects of hydroxyatrazine included clinical signs and increased mortality, reduced body-weight gain and food consumption, increased water consumption, changes in haematological, clinical chemistry and urine analysis parameters, and kidney lesions. The NOAEL was 25 ppm, equal to 1.0 mg/kg bw per day. There was no evidence of carcinogenicity.

Hydroxyatrazine did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In a study of prenatal developmental toxicity in rats, the effects of hydroxyatrazine consisted of reduced food consumption and body-weight gain in dams and increased incidences of incomplete and absent ossification in foetuses at 125 mg/kg bw per day; the NOAEL was 25 mg/kg bw per day for maternal and developmental toxicity. Exposure of female rats on postnatal days 22–41 at atrazine molar equivalent doses of up to 200 mg/kg bw per day did not delay sexual development.

Toxicological evaluation

Drinking-water may contain metabolites of atrazine as well as atrazine itself. The chloro-*s*-triazine metabolites DEA, DIA and DACT share the same mode of action as atrazine and have a similar toxicological profile and hence the Meeting decided to establish a group ADI and ARfD. Hydroxyatrazine, the plant and soil degradate, was not included because its mode of action and toxicological profile are different to those of atrazine and its chloro-*s*-triazine metabolites.

The Meeting established a group ADI of 0–0.02 mg/kg bw based on the NOAEL for atrazine of 1.8 mg/kg bw per day identified on the basis of LH surge suppression and subsequent disruption of the oestrous cycle seen at 3.6 mg/kg bw per day in a 6-month study in rats, and using a safety factor of 100. The Meeting considered that this NOAEL is protective for the consequences of

neuroendocrine and other adverse effects caused by prolonged exposure to atrazine and its chloro-*s*-triazine metabolites.

The Meeting established a group ARfD of 0.1 mg/kg bw based on the NOAEL for atrazine of 12.5 mg/kg bw per day identified on the basis of impaired suckling-induced prolactin secretion in dams and subsequent alterations in development of the central nervous system and prolactin regulation in male offspring in a special 4-day study in rats, and using a safety factor of 100. This ARfD was supported by the results of other studies of developmental toxicity with atrazine and its chlorometabolites, from which overall NOAELs/LOAELs of 25/50 mg/kg bw per day in rats and 5/75 mg/kg bw per day in rabbits were identified on the basis of effects that might occur after a single exposure (i.e., post-implantation loss, fused sternebrae). The study in rabbits (in which there was a 15-fold difference between NOAEL and LOAEL) was not selected as the basis for the ARfD, because examination of the studies in rats indicated that the dose selected for the ARfD would be adequately protective for these end-points in rabbits.

For hydroxyatrazine, the Meeting established an ADI of 0–0.04 mg/kg bw based on the NOAEL of 1.0 mg/kg bw per day identified on the basis of kidney toxicity (caused by low solubility in water resulting in crystal formation and a subsequent inflammatory response) at 7.8 mg/kg bw per day in a 24-month study in rats, and using safety factor of 25. A modified safety factor based on kinetic considerations was deemed appropriate since the critical effect of hydroxyatrazine is dependent on its physicochemical properties and the interspecies variability for such effects is lower than for AUC-dependent effects.

The Meeting concluded that it was not necessary to establish an ARfD for hydroxyatrazine in view of its low acute toxicity, the absence of relevant developmental toxicity that could be a consequence of acute exposure, and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

A toxicological monograph was prepared.

Levels relevant to risk assessment

(a) Atrazine

Species	Study	Effect	NOAEL	LOAEL
Mouse	Long-term studies of carcinogenicity[a,d]	Toxicity	10 ppm, equal to 1.2 mg/kg bw per day	300 ppm, equal to 38.4 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 385.7 mg/kg bw per day[c]	—
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.3 mg/kg bw per day	500 ppm, equal to 34.0 mg/kg bw per day
	Two-year studies of toxicity and carcinogenicity[a,d] (Sprague-Dawley rats)	Toxicity	70 ppm, equal to 2.6 mg/kg bw per day	500 ppm, equal to 19.9 mg/kg bw per day
		Carcinogenicity	25 ppm, equal to 1.5 mg/kg bw per day	50 ppm, equal to 3.1 mg/kg bw per day[e]
	Two-year studies of toxicity and carcinogenicity[a,d] (Fischer 344 rats)	Toxicity	70 ppm, equal to 3.5 mg/kg bw per day	200 ppm, equal to 10 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 20 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Fertility	500 ppm, equal to 36.1 mg/kg bw per day[c]	—

Species	Study	Effect	NOAEL	LOAEL
		Parental toxicity	50 ppm, equal to 3.6 mg/kg bw per day	500 ppm, equal to 36.1 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 3.6 mg/kg bw per day	500 ppm, equal to 36.1 mg/kg bw per day
	Developmental toxicity[b,d]	Maternal toxicity	10 mg/kg bw per day	70 mg/kg bw per day
		Embryo/fetotoxicity	10 mg/kg bw per day	70 mg/kg bw per day
	Special 6-month study[a]	Endocrine disruption (luteinizing hormone surge)	25 ppm, equal to 1.8 mg/kg bw per day	50 ppm, equal to 3.65 mg/kg bw per day
	Special 4-day study[b]	Endocrine disruption (prolactin release)	12.5 mg/kg bw per day	25 mg/kg bw per day
	Special 5-day study[b]	Post-implantation loss	25 mg/kg bw per day	50 mg/kg bw per day
	Special 25-day study[b]	Female pubertal delay	10 mg/kg bw per day	30 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	6.25 mg/kg bw per day	12.5 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	75 mg/kg bw per day
		Embryo/fetotoxicity	5 mg/kg bw per day	75 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	150 ppm, equal to 5 mg/kg bw per day	1000 ppm, equal to 33.7 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] Results of two or more studies combined.

[e] Mammary gland tumours—not relevant to humans.

(b) Deethyl-atrazine (DEA)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	500 ppm, equal to 35.2 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	25 mg/kg bw per day
		Embryo- and fetotoxicity	25 mg/kg bw per day	100 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	12.5 mg/kg bw per day[c]	25 mg/kg bw per day[c]
Dog	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.7 mg/kg bw per day	1000 ppm, equal to 28.9 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Atrazine molar equivalent dose.

(c) Deisopropyl-atrazine (DIA)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	500 ppm, equal to 34.9 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	25 mg/kg bw per day

		Embryo/fetotoxicity	5 mg/kg bw per day	25 mg/kg bw per day
	Special 30-day study[b]	Male pubertal delay	12.5 mg/kg bw per day[c]	25 mg/kg bw per day[c]
Dog	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.8 mg/kg bw per day	500 ppm, equal to 18.0 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Atrazine molar equivalent dose.

(d) Diaminochlorotriazine (DACT)

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Endocrine disruption (oestrous cycle)	100 ppm, equal to 7.6 mg/kg bw per day	250 ppm, equal to 19.7 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	2.5 mg/kg bw per day	25 mg/kg bw per day
		Embryo/fetotoxicity	2.5 mg/kg bw per day	25 mg/kg bw per day
	Special study, 29/52-weeks[a]	Endocrine disruption (LH surge)	48 ppm, equal to 3.4 mg/kg bw per day	270 ppm, equal to 18.8 mg/kg bw per day
	Special 19-day study[b]	Female pubertal delay	25 mg/kg bw per day[c]	50 mg/kg bw per day[c]
	Special 30-day study[b]	Male pubertal delay	6.25 mg/kg bw per day[c]	12.5 mg/kg bw per day[c]
Dog	Thirteen-/52-week study of toxicity[a]	Toxicity	100 ppm, equal to 3.5 mg/kg bw per day	1500/750 ppm, equal to 23.8 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Atrazine molar equivalent dose.

(e) Hydroxyatrazine

Species	Study	Effect	NOAEL	LOAEL
Rat	Thirteen-week study of toxicity[a]	Toxicity	100 ppm, equal to 6.3 mg/kg bw per day	300 ppm, equal to 18.9 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a] (Sprague-Dawley rats)	Toxicity	25 ppm, equal to 1.0 mg/kg bw per day	200 ppm, equal to 7.8 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 17.4 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	125 mg/kg bw per day
		Embryo/fetotoxicity	25 mg/kg bw per day	125 mg/kg bw per day
	Special 19-day study[b]	Female pubertal delay	200 mg/kg bw per day[c,d]	—
Dog	Thirteen-/52-week study of toxicity[a]	Toxicity	150 ppm, equal to 5.8 mg/kg bw per day	1500 ppm, equal to 59.6 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] Atrazine molar equivalent dose.

Estimate of acceptable daily intake for humans

Group ADI for atrazine, deethyl-atrazine, deisopropyl-atrazine and diaminochlorotriazine
0–0.02 mg/kg bw
Hydroxyatrazine
0–0.04 mg/kg bw

Estimate of acute reference dose

Group ARfD for atrazine, deethyl-atrazine, deisopropyl-atrazine and diaminochlorotriazine

0.1 mg/kg bw

Hydroxyatrazine

Unnecessary

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

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

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

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, > 80% in rats
Distribution	Widely distributed
Rate and extent of excretion	> 50% in 24 h and > 93% within 7 days; approximately 73% via urine, approximately 20% via faeces (approximately 7% via bile)
Potential for accumulation	Low; binding to rat haemoglobin, not relevant to humans
Metabolism in mammals	Extensive (> 95%) to at least 25 metabolites; major pathway is *N*-dealkylation
Toxicologically significant compounds in animals, plants and the environment	Parent compound, chloro-s-triazine metabolites DEA, DIA, DACT (animals, environment), hydroxyatrazine (plants, environment)
Acute toxicity	
Rat, LD_{50}, oral	1870–3090 mg/kg bw
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, $LC_{50,}$ inhalation	> 5.8 mg/L
Rabbit, skin irritation	Not an irritant
Rabbit, eye irritation	Not an irritant
Guinea-pig, skin sensitization	Sensitizer (Magnusson & Kligman; Maurer optimization test)
Short-term studies of toxicity	
Target/critical effect	Reduced body-weight gain, ovaries (inhibition of ovulation), cardiotoxicity (in dogs only)
Lowest relevant oral NOAEL	3.3 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (25-day study in rabbits)

Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Unlikely to be genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Ovaries (inhibition of ovulation) and related endocrine changes
Lowest relevant NOAEL	1.8 mg/kg bw per day (6-month luteinizing-hormone surge study in Sprague-Dawley rats)
Carcinogenicity	No relevant carcinogenicity
Reproductive toxicity	
Reproductive target/critical effect	Reduced body weight gain in pups at parentally toxic doses
Lowest relevant reproductive NOAEL	3.6 mg/kg bw per day
Developmental target/critical effect	Increased resorptions and incomplete ossification at maternally toxic doses; delayed sexual development
Lowest relevant developmental NOAEL	6.25 mg/kg bw per day (rat; male pubertal development)
	5 mg/kg bw per day (rabbit)
Neurotoxicity	
	No evidence of neurotoxicity in standard toxicity tests; however, neuroendocrine mode of action has been established for atrazine and its chloro-s-triazine metabolites
Other toxicological studies	
Studies on metabolites	DEA, DIA, DACT have the same neuroendocrine mode of action and similar potency to atrazine
	Hydroxyatrazine has a different mode of action and toxicity profile to atrazine
Mode of neuroendocrine action	Atrazine and its chlorometabolites modify hypothalamic catecholamine function and gonadotrophin-releasing hormone (GnRH) regulation, leading to alterations in pituitary luteinizing hormone (LH) and prolactin secretion
Mode of carcinogenic action	The postulated mode of carcinogenic action in female Sprague-Dawley rats involves acceleration of the reproductive ageing process (suppression of LH surge, subsequent oestrous cycle disruption), which is not relevant to humans
Direct estrogenic activity	Atrazine has no intrinsic estrogenic activity
Aromatase expression	No effect on aromatase expression in rats
Effects on sexual development	Evidence of delayed sexual development in male and/or female rats by atrazine, DEA, DIA and DACT
Effects on neuronal development	Evidence of impaired post-natal CNS development (and subsequent alterations in prolactin regulation)
Immunotoxicity	Evidence for immune system modulation at doses above LOAELs for neuroendocrine disruption or reproductive and developmental effects
Medical data	
	No evidence of atrazine causing effects in manufacturing plant

		personnel. Epidemiology studies do not support a causal association between exposure to atrazine and cancer in humans.	
Summary			
Atrazine			
	Value	**Study**	**Safety factor**
Group ADI[a]	0–0.02 mg/kg bw	Sprague-Dawleys rat; 6-month study of LH surge/oestrous cycle disruption	100
Group ARfD[a]	0.1 mg/kg bw	Rat; special 4-day study of prolactin release, supported by studies of developmental toxicity in rats and rabbits	100
Hydroxyatrazine			
ADI	0–0.04 mg/kg bw	Sprague-Dawley rats; 2-year study	25
ARfD	Unnecessary	—	—

[a] Group ADI or ARfD for atrazine, deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT)

5.3 AZINPHOS METHYL (002)

TOXICOLOGY

Azinphos-methyl is the ISO approved common name for *S*-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl *O*,*O*-dimethyl phosphorodithioate (IUPAC) or *O*,*O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate (CAS; CAS No. 86-50-0). It is a broad-spectrum organophosphorus pesticide. Its toxicity was first evaluated by the 1965 JMPR, when an ADI of 0–0.0025 mg/kg bw was established based on a NOAEL of 0.25 mg/kg bw per day for inhibition of cholinesterase activity in serum and erythrocytes in a repeat-dose study in rats. The 1968 JMPR considered a number of additional studies that were not available to the Meeting in 1965. The ADI established in 1965 was confirmed. The 1973 JMPR considered new studies that involved human volunteers but, owing to the absence of sufficient information on the conduct of these studies in humans, the existing ADI was re-affirmed. In 1991, the ADI was increased to 0–0.005 mg/kg bw on the basis of reduced body-weight gain and fertility observed in the multigeneration study in rats. Azinphos-methyl was reviewed by the present Meeting within the Periodic Re-evaluation Programme of the CCPR. All studies previously submitted to JMPR were available for consideration by the present Meeting. Several new studies, including two double-blind clinical studies in human volunteers, were also considered by the present Meeting.

Most studies, excluding some described in previous JMPR monographs, were certified as having been performed in compliance with good laboratory practice (GLP) and in accordance with the relevant Organization for Economic Co-operation and Development (OECD) test guidelines. The studies in humans were conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki, or equivalent statements prepared for use by national and/or multinational authorities.

Biochemical aspects

After oral administration of radiolabelled azinphos-methyl, the radiolabel was rapidly and completely (90–100% of the administered dose) absorbed from the mammalian intestinal tract, widely distributed throughout the organs, and eliminated in the urine (60–70% of the administered dose) and faeces via bile (25–35% of the administered dose) within 48 h. The maximum concentration in blood was reached within 2–3 h after administration. Azinphos-methyl was rapidly cleared from the blood and tissues, and consequently there is negligible potential for accumulation. In rats, the initial steps of metabolism involved the formation of the highly reactive oxon metabolite and mercaptomethylbenzazimide by cytochrome P450. Glutathionyl methylbenzazimide and desmethyl isoazinphos-methyl were formed via glutathione transferase. Subsequent hydrolysis of glutathionyl methyl-benzazimide resulted in the formation of cysteinyl-methylbenzazimide, which was then oxidized to form its corresponding sulfoxide and sulfone.

Toxicological data

Azinphos-methyl was highly acutely toxic (LD_{50} range, 4.4–26 mg/kg bw) when administered orally in an aqueous or non-aqueous vehicle to rats, and its profile of clinical signs was similar to those of other cholinesterase-inhibiting organophosphorus pesticides. Clinical signs observed in experimental animals after acute exposure were salivation, lacrimation, vomiting, diarrhoea, anorexia, reduced locomotor activity, piloerection, staggering gait and muscular tremors. These signs were generally evident within 5–20 min after dosing. There was very little difference in the sensitivity of male and female rats to the acute effects of azinphos-methyl.

The main toxicological findings in repeat-dose studies in rodents and dogs were inhibition of cholinesterase activity and, at higher doses, reduced body-weight gain and signs of neurotoxicity. In short-term studies of toxicity of less than 12 months duration, the NOAEL for inhibition of erythrocyte acetylcholinesterase activity was 0.2 mg/kg bw per day in rats and dogs. The NOAEL for inhibition of brain cholinesterase activity was 0.9 mg/kg bw per day in rats, and 0.7 mg/kg bw per day in dogs. Toxicity observed in rats and dogs was limited to the characteristic muscarinic signs (diarrhoea, salivation) and reduced body-weight gain. The effect doses for these clinical signs in short-term studies correlated with the high levels of inhibition of brain cholinesterase activity (> 80%) in rats and dogs.

Azinphos-methyl was tested in an adequate range of studies of genotoxicity in vitro and in vivo and showed no evidence of genotoxicity. The Meeting concluded that azinphos-methyl is unlikely to be genotoxic.

In long-term studies of toxicity, inhibition of cholinesterase activity was again the main toxicological finding in mice and rats. In mice, erythrocyte and brain cholinesterase activities were inhibited at 3.8 mg/kg bw per day, with a NOAEL of 0.9 mg/kg bw per day. Reduced body-weight gain and clinical signs involving hyperactivity and convulsions were observed in mice at higher doses (6.25 mg/kg bw per day). At equivalent doses in rats, body tremors and deaths were reported, although reduced body-weight gain was observed at 2.7 mg/kg bw per day. In rats, the NOAEL for inhibition of erythrocyte acetylcholinesterase activity was 0.3 mg/kg bw per day, while for brain cholinesterase activity it was 0.9 mg/kg bw per day and the NOAEL for a reduction in body-weight gain was 0.9 mg/kg bw per day. There was no evidence of carcinogenicity with azinphos-methyl at dietary concentrations of up to 40 ppm (equal to 12.8 mg/kg bw per day) in mice and up to 45 ppm (equal to 2.7 mg/kg bw per day) in rats; these were the highest doses tested.

In the absence of any carcinogenic potential in rodents and the lack of genotoxic potential in vitro and in vivo, the Meeting concluded that azinphos-methyl is unlikely to pose a carcinogenic risk to humans.

In multigeneration studies of reproductive toxicity in rats, the treatment-related effects of azinphos-methyl were cholinergic signs at high doses, reductions in body-weight gain and inhibition

of cholinesterase activity. These effects were consistent with those seen in short- and long-term studies of toxicity. However, there was also evidence of reduced pup viability at 4.8 mg/kg bw per day. The NOAEL for inhibition of brain cholinesterase activity in dams was 5 ppm, equal to 0.5 mg/kg bw per day. The NOAEL for inhibition of brain cholinesterase activity in pups was 15 ppm, equal to 1.5 mg/kg bw per day.

In studies of developmental toxicity with azinphos-methyl in mice, rats and rabbits, teratogenicity was not observed at doses of up to 2, 3.6 and 15 mg/kg bw per day respectively. The only developmental effect noted in any of these studies was delayed ossification in rat and rabbit foetuses at doses that also caused inhibition of brain and erythrocyte cholinesterase activity in the dams. The NOAEL for developmental effects in foetuses was 2 mg/kg bw per day in rats and 1.5 mg/kg bw per day in rabbits. Inhibition of brain cholinesterase activity was not observed in rats at doses of 1 mg/kg bw per day or in rabbits at doses of 2.5 mg/kg bw per day.

In studies of delayed neurotoxicity, azinphos-methyl was administered to chickens either as a single dose at up to 330 mg/kg bw or as repeated doses of up to 225 mg/kg bw per day in the feed for 30 days; there was no evidence of delayed neuropathy.

In rats given azinphos-methyl as a single dose at up to 12 mg/kg bw by gavage or as repeated doses of up to 7.4 mg/kg bw per day in the diet for 13 weeks, cholinergic signs and significant inhibition of erythrocyte and brain cholinesterase activity were seen at a number of doses. In these studies, which included a functional observational battery (FOB) of tests, clinical signs of intoxication (perianal stain, red lacrimation, increased reactivity, uncoordinated gait, tremor) were observed. However, cholinergic signs were observed only when brain cholinesterase activity was inhibited by more than 70% or when erythrocyte acetylcholinesterase activity was inhibited by approximately 65–80%. This occurred after repeated doses in excess of 3.2 mg/kg bw per day or after a single dose of 6 mg/kg bw. The NOAEL for inhibition of cholinesterase activity in the brain after a single dose was 2 mg/kg bw or 1 mg/kg bw per day after repeat dosing.

In a randomized double-blind study in human volunteers (seven of each sex) given ascending single oral doses, azinphos-methyl did not induce cholinergic signs or changes in acetylcholinesterase activity in erythrocytes at doses of up to 1 mg/kg bw in males and up to 0.75 mg/kg bw in females; these were the highest doses tested.

When eight male volunteers were given azinphos-methyl as a daily oral dose at 0.25 mg/kg bw per day for 28 days, there were no cholinergic signs and erythrocyte acetylcholinesterase activity was not significantly inhibited. In another study, two groups of five male volunteers were given azinphos-methyl at a dose of 0.26 or 0.29 mg/kg bw per day for 30 days did not induce cholinergic signs or changes in cholinesterase activity in erythrocytes or plasma. In a third study, a similar outcome was reported when two male volunteers were given azinphos-methyl orally at a dose of 0.23 mg/kg bw per day for 30 days.

Regular medical examinations of workers involved in formulating products containing azinphos-methyl had revealed no effects, except for one case of possible dermatosis resulting in sensitive dry skin.

The Meeting concluded that the existing database on azinphos-methyl was adequate to characterize the potential hazards to foetuses, infants, and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw per day based on a NOAEL of 0.29 mg/kg bw per day for the absence of inhibition of erythrocyte acetylcholinesterase activity in a 30-day study of toxicity in male volunteers and a safety factor of 10. Since the database indicated that rodents and dogs of each sex and humans had similar NOAEL values for the most sensitive end-point, namely inhibition of acetylcholinesterase activity in erythrocytes, the NOAELs identified in the studies in humans were considered to be protective for the entire population. The Meeting also considered the

ADI to be protective for other, non-neurotoxic effects of azinphos-methyl observed in short- and long-term studies with repeated doses, and in studies of reproductive and developmental toxicity, where the use of a safety factor of 100 would be appropriate. The effect of azinphos-methyl on body-weight gain and fertility in dams at 15 ppm (0.5 mg/kg bw per day) in multigeneration studies of reproduction in rats was reconsidered. The Meeting concluded that it was a marginal effect that could not be directly attributed to treatment.

The Meeting established an ARfD of 0.1 mg/kg bw based on the NOAEL of 1 mg/kg bw and using a safety factor of 10. The NOAEL observed in a study of single doses in volunteers was the highest tested dose in males. Although the maximum dose given to females was only 0.75 mg/kg bw, there was no apparent observed difference in sensitivity between the sexes, so the NOAEL observed in males was also considered to be protective of effects in females. In a study of acute neurotoxicity in rats, the NOAEL was 2 mg/kg bw on the basis of inhibition of cholinesterase activity in the brain. At a dose of 2 mg/kg bw, significant inhibition of acetylcholinesterase activity in erythrocytes of male rats was observed, but not at 1 mg/kg bw in female rats. In rats, pup deaths as a result of inhibition of cholinesterase activity were observed at 6 mg/kg bw in females and at 12 mg/kg bw in males, suggesting a steep dose–response effect. Based on the median oral LD_{50} value of 13 mg/kg bw (range, 4.4–26 mg/kg bw) in all available studies in rats, there would be about a 130-fold margin between the ARfD and the LD_{50} in rats.

A toxicological monograph was prepared.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity[a]	Inhibition of brain cholinesterase activity	2.0 mg/kg bw	3.0 mg/kg bw
Human	Single dose[b,d]	No adverse effects	1.0 mg/kg bw per day	—
Mouse	Two-year study of toxicity and carcinogenicity[c]	Inhibition of erythrocyte and brain cholinesterase activity	5 ppm, equal to 0.9 mg/kg bw per day	20 ppm, equal to 3.8 mg/kg bw per day
		Carcinogenicity[d]	40 ppm, equal to 12.8 mg/kg bw per day	—
Rat	Three-month study of toxicity[c]	Inhibition of brain cholinesterase activity	0.9 mg/kg bw per day	3.4 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[c]	Reduced body-weight gain and inhibition of brain cholinesterase activity	15 ppm, equal to 0.9 mg/kg bw per day	45 ppm, equal to 2.7 mg/kg bw per day
		Carcinogenicity[d]	45 ppm, equal to 2.7 mg/kg bw per day	—
	Multigeneration study of reproductive toxicity[c,e]	Parental toxicity	5 ppm, equal to 0.5 mg/kg bw per day	15 ppm, equal to 1.5 mg/kg bw per day
		Offspring toxicity	15 ppm, equal to 1.5 mg/kg bw per day	45 ppm equal to 4.8 mg/kg bw per day
	Developmental toxicity[a,e]	Maternal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
		Embryo/fetotoxicity	2.0 mg/kg bw per day	3.6 mg/kg bw per day
	Three-month study of neurotoxicity[c]	Inhibition of brain cholinesterase activity	15 ppm, equal to 1 mg/kg bw per day	45 ppm, equal to 3 mg/kg bw per day
Rabbit	Developmental toxicity[b,c]	Maternal toxicity	2.5 mg/kg bw per day	6.0 mg/kg bw per day
		Embryo/fetotoxicity	1.5 mg/kg bw per day	4.75 mg/kg bw per day
Dog	One-year study of toxicity[a]	Reduced body-weight gain and inhibition of brain cholinesterase activity	25 ppm, equal to 0.7 mg/kg bw per day	125 ppm, equal to 4.1 mg/kg bw per day
Human	Clinical 30-day study[b,d,e]	No adverse effects	0.29 mg/kg bw per day	—

[a] Gavage administration.

[b] Capsule administration.

[c] Dietary administration.

[d] Highest tested dose.

[e] Two or more studies combined

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

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

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

Critical end-points for setting guidance values of exposure for azinphos-methyl

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Almost complete absorption. Maximum plasma concentration 2–3 h after dosing.
Dermal absorption	See previous azinphos-methyl monographs
Distribution	Extensive
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Largely complete within 48 h; approximately 95% excreted in urine and bile.
Metabolism in animals	Extensive; two major urinary metabolites and six other products. Five faecal metabolites (10–12% of the administered dose).
Toxicologically significant compounds in	Azinphos methyl, azinphos-methyl oxon

animals, plants and the environment	
Acute toxicity	
Rat, LD_{50}, oral	4.4–26 mg/kg bw
Rat, LD_{50}, dermal	See previous azinphos-methyl monographs
Rat, LC_{50}, inhalation	See previous azinphos-methyl monographs
Skin sensitization (test method used)	See previous azinphos-methyl monographs
Short-term studies of toxicity	
Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant oral NOAEL	0.7 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	See previous azinphos-methyl monographs
Lowest relevant inhalation NOAEC	See previous azinphos-methyl monographs
Genotoxicity	
	Unlikely to pose a genotoxic risk in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant NOAEL	0.9 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in rats and mice
Reproductive toxicity	
Reproduction target/critical effect	Inhibition of brain cholinesterase activity in dams
Lowest relevant reproductive NOAEL	0.5 mg/kg bw per day (rats)
Developmental target/critical effect	Delayed ossification at maternally toxic doses (rats, rabbits)
Lowest relevant developmental NOAEL	1.0 mg/kg bw per day (rats); 2.5 mg/kg bw per day (rabbits)
Neurotoxicity/delayed neurotoxicity	No evidence of delayed neuropathy observed in hens
	NOAEL: 1 mg/kg bw in a repeat-dose study of neurotoxicity in rats
Medical data	
	Medical examinations of workers involved in formulating azinphos-methyl products revealed no effects, except for one case of possible dermatosis resulting in sensitive dry skin.

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw per day	Humans, 30-day oral-dosing study	10
ARfD	0.1 mg/kg bw	Humans, study of acute toxicity	10

5.4 CAPTAN (007)

TOXICOLOGY

Evaluation for an acute reference dose

Captan, the ISO approved name for *N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide, is a fungicide (CAS No. 133-06-2) registered for the control of fungal diseases in crops. The toxicology of captan was evaluated by the JMPR in 1963, 1965, 1969, 1973, 1978, 1982, 1984, 1990, 1995 and 2004. Toxicological monographs were prepared by the Meeting in 1963, 1965 and 1969, and monograph addenda were prepared in 1973, 1977, 1978, 1982, 1984, 1990, 1995 and 2004. In 1984, an ADI of 0–0.1 mg/kg bw was established based on a NOAEL of 12.5 mg/kg bw per day in studies of reproductive toxicity in rats and monkeys. This ADI was confirmed by JMPR in 1995. In 2004, the Meeting established an ARfD of 0.3 mg/kg bw, for women of childbearing age only, based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in the study in rabbits and a safety factor of 100.

The Meeting concluded that the database was insufficient, particularly with regard to information about the possible developmental effects of the metabolite 1,2,3,6-tetrahydrophthalimide (THPI), to establish the mode of action by which the increased incidences of intrauterine deaths and foetuses with malformations were induced.

The sponsor conducted a study of developmental toxicity with THPI, and studies to evaluate the potential effects of captan and THPI on the intestinal flora of the rabbit. It was known that the rabbit is dependent on the presence of caecotrophs for adequate nutrition. The sponsor suggested that disruption of the intestinal flora might result in maternal malnutrition, with possible consequent adverse effects on foetal development.

At the request of the CCPR at its 39th Session,[22] the present Meeting reconsidered the ARfD on the basis of new data.

All pivotal studies with captan and THPI were certified as being compliant with GLP

Toxicological data

Data previously evaluated by the Meeting in 2004

With respect to the developmental toxicity of captan, the following description is quoted from JMPR 2004 (Annex 5, reference *101*, *103*):

> In a study from the published literature, the teratogenic effects of a number of phthalimide derivatives, including captan, were tested in pregnant golden hamsters. The Meeting noted that this study has major limitations (e.g., small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to captan, albeit at maternally toxic doses.
>
> In a study of developmental toxicity in rats treated by gavage, captan was not teratogenic. The NOAEL for maternal toxicity was 18 mg/kg bw per day on the basis of a reduction in body weight and food consumption. The NOAEL for offspring toxicity was 90 mg/kg bw per day on the basis of the reduction in foetal body weight and an increased incidence of skeletal variations.
>
> In a study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of a markedly reduced body-weight gain and reduced food consumption at 30 mg/kg bw per day. The NOAEL for embryo- and fetotoxicity was 10 mg/kg bw per day on the basis of

[22] Codex Alimentarius Commission. *Report of the 39th Session of the Codex Committee on Pesticide Residues, 7–12 May 2007, Beijing, China* (ALINORM07/30/24).

increases in skeletal variations at 30 and 100 mg/kg bw per day. At 100 mg/kg bw per day, increased incidences of early and late intra-uterine deaths were observed, as were increased incidences of several malformations. The NOAEL for these effects was 30 mg/kg bw per day. Multiple malformations observed in two foetuses in the group receiving the intermediate dose were considered to be incidental. In another study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption at 40 mg/kg bw per day. On the basis of the increase in post implantation losses and the increase in incidence of minor skeletal variations at 160 mg/kg bw per day, the NOAEL for embryo- and fetotoxicity was 40 mg/kg bw per day. In a third study in rabbits treated by gavage, the NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of reductions in body-weight gain during the initial phase of treatment. The NOAEL for embryo- and fetotoxicity was 25 mg/kg bw per day on the basis of a reduction in foetal body weight at 60 mg/kg bw per day. The Meeting considered that maternal toxicity and the associated increases in skeletal variations and foetal body-weight reductions observed were likely to be caused by high local concentrations of captan produced by administration by gavage, and are not considered to be relevant to dietary exposure.

Toxicokinetics

In a study evaluated by the JMPR in 2004, in mice given captan at a dose of 400 or 3000 ppm (equivalent to 57 and 429 mg/kg bw per day) no captan was found in mouse duodenal tissue or its contents (extracts of 5 cm sections of the duodenum were analysed; limit of quantitation, 0.5–3 nmoles, i.e., 0.150–1 µg), indicating that in mice, captan is largely, if not completely, degraded to THPI in the stomach.

In a toxicokinetic study in rats (previously evaluated by the JMPR in 1995), concentrations of captan and its metabolites were measured in the faeces and urine. In order to establish whether captan reached the distal parts of the gastrointestinal tract in rats, the study was re-evaluated by the present Meeting.

Rats were given [^{14}C] labelled captan at a dose of 10 or 500 mg/kg bw by gavage. Extracts of urine and faeces were qualitatively analysed by thin-layer chromatography (TLC) through comparison with reference compounds. Quantification of metabolites was performed by linear plate scanner and autoradiography. Subsequently, some metabolites isolated by TLC were further identified by gas chromatography/mass spectrometry (GC/MS). Several compounds were identified in urine and faeces, including THPI, 3- and 5-hydroxylated THPI, THPI-diol, THPI-epoxide, cyclohexene acid amide and hydroxycyclohexene acid amide and parent captan. The presence of captan in extracts of urine and faeces was identified by TLC only (i.e., by comparison with the retention factor, Rf, of captan). No further identification was attempted. Toxicokinetic data are presented in Table 8.

Table 8. Toxicokinetic data for rats given [^{14}C] labelled captan by gavage.

Dose	Radioactivity excreted (% of administered dose)		Captan in urine (% of radioactivity)	Captan in faeces	
	Urine	Faeces		% of radioactivity (males/females)	% of total administered dose
10 mg/kg bw, single dose	81	8–9	ND	6.5/16.8	0.5–1.5
10 mg/kg bw, repeated dose	88–91	7–9	ND/0.7 (males/females)	ND/4.6	ND/0.4
500 mg/kg bw, single dose	69–73	23–25	1.3–1.6	44.1/40.9	± 12 (males and females)

From Lappin & Havell (1990). ND, not detected

After a single dose at 10 mg/kg bw, 81% and 8–9% of the administered radioactivity was recovered from urine and faeces, respectively. At this single dose, no captan was detected in the urine. Between 0.5% and 1.5% of the total dose recovered in the faeces was captan.

After 14 consecutive doses at 10 mg/kg bw, 88–91% and 7–9% of the administered radioactivity was recovered from the urine and faeces, respectively. At this repeated low dose, 0.7% of urinary radioactivity was detected as captan in the urine of females (not detected in males). Of the radioactivity recovered from the faeces up to 4.6% was captan.

At 500 mg/kg bw, about 71% and 24% of the administered radioactivity was recovered from the urine and faeces, respectively. At this dose, 1–2% of urinary radioactivity was provisionally identified as intact captan.[23]

Developmental toxicity

In a study of developmental toxicity, groups of 25 time-mated female New Zealand White rabbits received THPI (purity, 98.4%) at a dose of 0, 5, 10 or 22.5 mg/kg bw per day by gavage from days 6 to 28 after mating. The vehicle was water containing 0.5% w/v Tween 80 and 0.7% w/v carboxymethylcellulose. In view of the relative molecular masses of captan (300.6) and THPI (151.2), a dose of THPI of 22.5 mg/kg bw per day would be equimolar to a dose of captan of about 45 mg/kg bw per day. All animals were examined twice per day for clinical signs. Body weight was recorded daily from the day of mating until day 29 of gestation, when the animals were killed. Food intake was recorded daily from the first day after mating until day 29 of gestation. On day 29 of gestation, the animals were killed and examined macroscopically. In females of the control group and at the highest dose, the duodenum and sphincter of Oddi (hepatopancreatic sphincter) were examined microscopically. The ovaries and uterus were removed and the foetuses were weighed and examined for visceral and skeletal abnormalities.

One female in the control group died on day 12 of gestation. The cause of death could not be established. One animal at 10 mg/kg bw per day was killed in extremis on day 14 of gestation. At necropsy, in the control group and in the groups at 5, 10 and 22.5 mg/kg bw per day, six, one, three and one female respectively did not appear to be pregnant. In the dams, no treatment-related clinical signs or effects on body weight and food consumption, and no macroscopic or microscopic abnormalities were observed. In the foetuses, no treatment-related embryo/fetotoxicity or effects on visceral and skeletal parameters were observed. The NOAELs for maternal and embryo/fetotoxicity were 22.5 mg/kg bw per day i.e., the highest dose tested.[24]

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[23] Lappin, G.J. & Havell, M.L. (1990) Captan: biotransformation study in the rat. Unpublished report No CTL/P/2951 from ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

[24] Blee, M.A.B. (2006) Tetrahydrophthalimide. Prenatal toxicity study in the rabbit by oral gavage administration. Unpublished report No R-18202, MAK 864/053232 from Huntingdon Life Sciences Limited, Woolley Road, Alconbury, Huntingdon, Cambridgeshire. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Inhibition of microbial activity in vitro

A study was performed to determine minimum inhibitory concentrations (MIC) of captan (purity, 95.1%) against two bacterial species (*Bacteroides* sp. and *Enterococcus faecalis*) and one species of yeast (*Candida albicans*). These bacteria were considered to be representative of anaerobic bacteria in the rabbit gut. *Candida albicans* was considered to be representative of yeast that may occur in the rabbit gut. The MIC values for *Bacteroides* sp., *Enterococcus faecalis* and *Candida albicans* were 20–50, 50–500 and 2–5 µg/mL, respectively. The Meeting concluded that captan demonstrates antimicrobial activity against organisms considered representative of rabbit gut flora.[25]

In a study to determine MIC of THPI (purity, 98.4%), MIC values for *Bacteroides* sp., *Enterococcus faecalis* and *Candida Albicans* were all > 1000 µg/mL. The Meeting concluded that THPI demonstrates no antimicrobial activity against organisms considered representative of rabbit gut flora.[26]

The Meeting concluded that the existing database (i.e., the new available studies and the previously evaluated studies), was adequate to characterize the potential hazards of captan to foetuses, infants and children.

Toxicological evaluation

In 2004, the Meeting established an ARfD of 0.3 mg/kg bw for captan for women of childbearing age, based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in a study in rabbits and using a safety factor of 100.

The new study of developmental toxicity with THPI did not elucidate the mode of action by which the increased incidences of intrauterine deaths and of foetuses with malformations (observed at 100 mg/kg bw per day expressed as captan) were induced. Because the maximum dose was similar to the NOAEL in the study of developmental toxicity with captan, it was not possible to use this study to determine the contribution of THPI to the developmental toxicity of captan.

Studies in vitro showed that captan, but not its metabolite THPI, has antimicrobial action on gut flora.

A toxicokinetic study in mice indicated that an oral dose of captan is largely, if not completely, metabolized in the stomach in this species. Toxicokinetic studies in rats given captan by gavage indicated that, at a high dose (500 mg/kg bw), a considerable amount of captan reaches the distal part of the gastrointestinal tract. At this dose, intact captan was also present in rat urine. At a single low dose of 10 mg/kg bw given by gavage, captan also reached the distal part of the gastrointestinal tract. It was not detected in urine after a single dose at 10 mg/kg bw, but was detected in the urine after 14 daily doses. No toxicokinetic data in rabbits, the species showing the developmental effect of concern, were provided.

There was some evidence that THPI can reach the caecum and that the developmental effects of captan in rabbits might be secondary to an effect on the gut flora of this species. The doses achieved in the study with THPI did not exclude a systemic role for this compound (and its further metabolites) in the developmental effects seen with captan in rabbits. Furthermore, it could not be excluded that the effects observed in a study of developmental toxicity in rabbits were a result of

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[25] Akhurst, L.C (2005a) Captan: determination of minimum inhibitory concentration against selected micro-organisms representative of rabbit gut microflora. Unpublished report No. R-18666, MAK 0888/052848 from Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

[26] Akhurst, L.C (2005b) THPI: determination of minimum inhibitory concentration against selected micro-organisms representative of rabbit gut microflora. Unpublished report No. R-18735, MAK 0890/053252 from Huntingdon Life Sciences Limited, Huntingdon, Cambridgeshire, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

direct action by captan or the thiocarbonyl chloride moiety released after metabolism to THPI. The presence of captan in the urine indicated that a certain amount of captan is available systemically.

In view of these considerations, the Meeting reconfirmed the ARfD of 0.3 mg/kg bw based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in the study in rabbits and using a safety factor of 100. This ARfD applies to women of childbearing age. The Meeting concluded that it was unnecessary to establish an ARfD for the rest of the general population.

An addendum to the toxicological monograph was not prepared.

Estimate of acute reference dose

0.3 mg/kg bw for women of childbearing age

Unnecessary for the rest of the general population

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

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

5.5 CARBARYL (008)

RESIDUE AND ANALYTICAL ASPECTS

The carbaryl was last evaluated for residues by the 2002 JMPR. Residue data on cranberries and chilli peppers were evaluated by the current Meeting for estimation of maximum residue levels.

Carbaryl is approved for the control of a range of insect pests in cranberries such as Cranberry fireworm, Cranberry fruitworm and Cranberry twig girdler as well various larvae and bugs in chilli peppers.

Results of supervised trials on crops

Supervised trials were carried out following the maximum registered dosage rate in cranberries in the USA and in chilli peppers in Thailand. The residues were determined with HPLC after post-column derivatisation in all trials. The limit of quantification was 0.02 mg/kg. The recoveries ranged between 81% and 110%.

Cranberries

The US GAP permits a maximum of 5 applications at 7 day intervals with a dosage rate of 1.68–2.24 kg ai/ha. Three replicate samples were taken from each plot. The highest residues derived from maximum application rates 7 days (PHI) after the last application was: 0.52, 0.94, 1.85, 2.95 mg/kg.

Taking into account that cranberry is a minor crop, the Meeting considered that four trials performed at maximum GAP were sufficient, and estimated a maximum residue level of 5 mg/kg, an STMR of 1.40 mg/kg and an HR of 2.95 mg/kg.

Chilli peppers

Residues in mature chilli peppers treated according to maximum GAP (0.425–0.6375 kg ai/ha at 7–10 day intervals with a PHI of 14 days) were: 0.05, 0.5, 0.09, 0.09, 0.10, 0.25 mg/kg.

The Meeting estimated a maximum residue level of 0.5 mg/kg, an STMR of 0.09 mg/kg and an HR of 0.25 mg/kg for fresh chilli peppers.

Based on the concentration factor of 7 (for explanation and rational see report section on Chilli peppers), the Meeting estimated an STMR of 0.63 (7 × 0.09) mg/kg and a maximum residue level of 2 (7 × 0.25=1.75) mg/kg to replace its previous recommendation of 50 mg/kg for dried chilli pepper, which was based on an MRL of 5 for sweet peppers, and the default concentration factor of 10.

DIETARY RISK ASSESSMENT

Long- term intake

Using the consumption figures for chilli peppers and the STMR value of 0.63 for dried chilli peppers, the long term intake from use of carbaryl on chilli peppers and cranberries amounts to 0–2% of the ADI (0-0.008 mg/kg bw) in the 13 regional diets.

The Meeting concluded that the long-term intake of residues derived from carbaryl use on cranberries and chilli peppers that have been considered by the present JMPR will not, in practical terms, change the total intake of residues from other uses considered by the 2002 JMPR.

Short-term intake

The rounded cranberry short term intake is 0% of the ARfD (0.2 mg/kg bw) for both children and for adults. The short term intake derived from the consumption of dried chilli pepper is 0% for adults and 1% for children.

The Meeting concluded that the short-term intake estimate derived from residues of carbaryl use on cranberries and chilli peppers that has been considered by the JMPR is unlikely to present a public health problem.

5.6 CLOFENTEZINE (156)

RESIDUE AND ANALYTICAL ASPECTS

Clofentezine, an acaricide first evaluated by the JMPR in 1986 and re-evaluated for residues several times up to 1992. A toxicological review was conducted in 2005, when an ADI of 0-0.02 mg/kg bw was established. The 2005 JMPR concluded that an ARfD was not necessary. At the 37th session of the CCPR, clofentezine was scheduled for Periodic Re-evaluation of residues by the 2007 JMPR.

The manufacturer supplied information on identity; metabolism and environmental fate; residue analysis; use patterns; residues resulting from supervised trials on citrus, pome fruits, stone fruits, grapes, strawberries, currants, melons, tree nuts, tomatoes and cucumbers; and the fate of residues on apple, peach, almond and animal tissues during storage and orange, apple, grape and strawberries in processing. GAP information and enforcement methods were supplied by the manufacturer and the governments of the Netherlands and Australia.

Animal metabolism

The Meeting received animal metabolism studies with clofentezine in lactating cows, goats and laying hens. Clofentezine [^{14}C] labelled in the tetrazine ring was used in the animal metabolism studies.

The metabolism of clofentezine in rat, mouse, rabbit, calf and cow, dog, baboon and hen was qualitatively similar (details on laboratory animal metabolism are given in the toxicology report), with

hydroxylation of the phenyl ring and/or replacement of chlorine with a methylthio group being the 2 major pathways.

A lactating cow was orally dosed with [^{14}C] labelled clofentezine for 5 consecutive days at about 0.27 mg/kg bw per day. Residues in milk reached a plateau level of approximately 0.007 mg/L on the second day. Residues were highest in bile (1.1 mg/kg) and liver (0.09 mg/kg). Heart, muscle and fat contained residues less than 0.01 mg/kg.

A lactating cow was orally administered with [^{14}C] labelled clofentezine for 3 consecutive days at an exaggerated rate (2.2 mg/kg bw per day) in order to produce quantifiable residues. Levels of radioactivity in milk were shown to plateau at a level of 0.17 mg/kg clofentezine equivalents on day 3 after treatment. The major component of the residue in milk was 4-OH clofentezine (75 % TRR, total radioactive residues). The [^{14}C] residue was higher in the liver (0.76 mg/kg) than in other tissues, of which at least 67 % was identified as 4-OH clofentezine. In renal fat, 90% of the TRR (0.24 mg equivalents/kg) was confirmed to be 4-OH clofentezine. Residues in kidney were also found to be composed predominantly of 4-OH clofentezine (83% TRR, 0.30 mg equivalents/kg). The remaining components appeared to be hydrolysis products of 4-OH clofentezine.

A lactating goat was given a single oral dose (0.63 mg/kg bw per day) of [^{14}C]clofentezine. The results showed [^{14}C] in all tissues at levels below 0.05 mg/kg, with all the [^{14}C] being excreted within 72 hours of dosing. Highest TRR was in goat milk at a level of 0.049 mg/L clofentezine equivalents at a time of 24 h after dosing.

Another study at an exaggerated rate (2.2 mg/kg bw per day) was undertaken for 7 consecutive days, in which a plateau for TRR in milk was reached at days 3 or 4 of the test, with a maximum residue of 0.2 mg/L being obtained. Over 95% of the TRR was confirmed as 4-OH clofentezine.

In summary, most of the [^{14}C]clofentezine fed to ruminants was excreted within 72 h. Liver was the target tissue and the major part of the residue was 4-OH clofentezine. There are no qualitative differences in the comparative metabolism studies of rodents (rats) and ruminants (goats and cattle).

Laying hens were administered [^{14}C]clofentezine orally for 3 consecutive days at a dose level of 17 mg clofentezine/kg bodyweight/day. By far the greatest [^{14}C] residue was found in fat (3.0 mg/kg equivalents). The majority of each daily dose of clofentezine (71–79%) was excreted by hens during the subsequent 24 hour period. The majority of the identified component of the residue found in all tissue samples, was the parent clofentezine (fat: 70%, muscle: 34%, unlaid developing eggs: 32% and skin: 7.0%), with varying quantities of both 3 and 4-OH clofentezine. The remaining residue most likely consisted of conjugates of the 3 and 4-OH clofentezine metabolites.

Plant metabolism

The Meeting received plant metabolism studies with clofentezine on lemon, apple, peach and grapes.

In plants, parent clofentezine was the major component of the residue at shorter and longer intervals with lower and higher application rates. Levels of metabolites were usually much lower than parent clofentezine. Residues were mostly found as a surface residue.

The metabolism of [^{14}C]clofentezine (applied at 0.3 kg ai/ha) on lemon leaves was studied. Parent clofentezine was the main residue component and 2-chlorobenzonitrile was also present, at levels of 88% TRR and 8.1% TRR 25 days post-treatment, and 77% TRR and 6.8% TRR 103 days post-treatment, respectively.

The metabolism of [^{14}C]clofentezine in apple foliage was studied following application at a nominal dosage of 0.5 kg ai/ha. The main component of the residue found 25 days after treatment was parent clofentezine present amounting to 87% of TRR. Levels of parent clofentezine decreased over time to a level of 66% of TRR, with an increase of fibre bound residue. The metabolite NC 22505 (3, 6-bis(2-chlorophenyl)-1,2-dehydro -1,2,4,5-tetrazine) was present 10 and 100 days post-treatment but

was not found at intermediate time points. Other single metabolites appeared in concentrations less than 1% of TRR.

The metabolism of [^{14}C]clofentezine was investigated in apples treated at a field spray concentration of 0.03 kg ai/hL and the exaggerated spray concentration of 0.76 kg ai/hL. Residues in mature apple fruit 72 days post-treatment consisted predominantly of parent clofentezine and peel fibre bound residues. However, both residues were at levels of 0.012 mg/kg or less at a rate of 0.03 kg ai/hL. At the exaggerated spray concentration, the same components were present, however the level of the parent clofentezine was much higher, i.e., 82% of TRR (0.81 mg/kg).

Further investigation was made into the components of the fibre bound residue, with apples being treated at spray concentrations of 0.06 kg ai/hL and 0.48 kg ai/hL. Samples were taken at 25 and 64 days post-treatment. Only limited quantities (approximately 0.01 mg/kg) of fibre bound radioactivity was recovered in this trial. Base and enzyme hydrolysis revealed that approximately 50% of bound residue was likely to be unchanged clofentezine, with the remainder consisting of 2-chlorobenzoic acid, 2-chlorobenzylacohol and 2-chlorobenzaldehyde.

Peach trees in a glasshouse were treated with [^{14}C]clofentezine at spray concentrations of 0.01 and 0.1 kg ai/hL and peaches were harvested for analysis 62 days post-treatment. Following the application at 0.01 kg ai/hL the overall TRR was only 0.047 mg/kg, of which 0.036 mg/kg was parent clofentezine. At the higher treatment rate, the TRR was 0.70 mg/kg consisting of 0.63 mg/kg parent clofentezine and 0.038 mg/kg 2-chlorobenzonitrile.

Grape vines were treated in a glasshouse with [^{14}C]clofentezine at spray concentrations of 0.01 and 0.1 kg ai/hL (equivalent to application rates of 0.1 and 1.0 kg ai/ha). Grape samples were collected for analysis 25 and 46 days post-treatment. At 25 days post-treatment, the total radioactive residue found at the 0.1 kg ai/ha rate was 0.38 mg/kg and 2.5 mg/kg at the 1.0 kg ai/ha rate. At the lower rate, the majority of the residue was found to be parent clofentezine (0.29 mg/kg) followed by 2-chlorobenzonitrile (0.04 mg/kg), the remainder of the residue comprised of polar materials (< 0.01 mg/kg). At 46 DAT the overall residue levels were much lower (0.11 mg/kg at 0.1 kg ai/ha and 0.45 mg/kg at 1.0 kg ai/ha), and the parent clofentezine remained the prevalent component (69% of TRR or 0.31 mg/kg).

Environmental fate in soil

The Meeting received information on the environmental fate of clofentezine in soil, including studies on aerobic soil metabolism, field dissipation and crop rotational studies.

The environmental fate of clofentezine was investigated in a number of laboratory studies using either unlabelled or [^{14}C] tetrazine ring labelled material under aerobic conditions for various durations. The degradation rates were not strongly affected by soil organic carbon content, but greatly influenced by the soil pH, with the faster degradation at higher soil pH. The aerobic soil metabolism half-lives for clofentezine ranged from approximately 2 to 12 weeks. After one year, in the loamy sand, clay and clay loam, approximately 56%, 38% and 25% of the applied radioactivity respectively had been mineralized to [^{14}C]O_2, and 30–40% of the initial dose was extractable residue in the loamy sand, clay and clay loam respectively.

During the study period, the maximum concentrations of the major metabolites, expressed as the percentage of the initial dose, were: 13% of AEC 593600 [2-chlorobenzoic (2-chlorobenzylidene) hydrazide], 1.6% of AEC 512898 [N, N'-bis-(2-chlorobenzoyl)-hydrazine], 0.8% of AEF 092117 [2-chlorobenzamide] and 6.2% of AEC 500233 [2-chlorobenzoic acid].

Very little of the applied clofentezine moved below the top 15 cm of the soil during field dissipation trials of up to 8 months' duration in several different soils. Clofentezine concentrations declined to half of their initial values within 14 days to approximately 6 months. In orchard soil residue decline trials, quantifiable residues of clofentezine were mostly detected in the top soil layer and declined below the limit of determination within 60 days.

The low water solubility and relatively high octanol/water partition coefficient of clofentezine lessen the uptake of clofentezine residues from soil into following crops. A crop uptake study with orange and apple trees grown under glasshouse conditions indicated that the potential for uptake of clofentezine residues from the previously treated soil was low.

Methods of analysis

The Meeting received analytical methods descriptions and validation data for residues of clofentezine in a number of crops, and residues of clofentezine or 4-OH clofentezine or total residues of all compounds containing the 2-chlorobenzoyl moiety in animal tissues, milk and eggs.

Methods rely on HPLC-UV, HPLC-DAD, GC-ECD and GC-MSD for analysis of clofentezine or 4-OH clofentezine and all compounds containing the 2-chlorobenzoyl moiety in the various matrices. Several multi-residue methods with HPLC-DAD and GC-MSD were suitable for enforcement for plant and animal commodities (LOQ values 0.01–0.05 mg/kg).

In summary, numerous recovery data on a wide range of substrates showed that the methods for data collection and enforcement were valid over the relevant concentration ranges.

Stability of residues in stored analytical samples

The Meeting received information on the freezer storage stability of residues of clofentezine in apple, peach, almond (hulls and nutmeats), muscle, liver, fat and milk.

Residues were stable in apple, almond/nutmeat and peach samples for a period of at least one year when stored frozen.

After 6 months storage, the mean percentage of clofentezine fortified had fallen to 38% (muscle), 72% (liver), 50% peritoneal fat), and 50% (milk). The percentage of clofentezine-derived residues (all metabolites containing the 2-chlorobenzoyl moiety) determined by derivation to 2-chlorobenzoic acid was more than 90% of the original residue in muscle, liver and fat, and approximately 84% in milk after 15 months storage. Parent clofentezine is relatively unstable in products of animal origin, but the total residue of all metabolites containing the 2-chlorobenzoyl moiety was stable in animal products for at least 15 months.

Definition of the residue

The main residues in fruit crops were the parent clofentezine, and metabolite 2-chlorobenzonitrile. The levels of 2-chlorobenzonitrile found were < 0.05 mg/kg, which was approximately a tenth of those of the parent residue. Other metabolites identified were present only at low levels and these metabolites were not considered to be of toxicological significance. Therefore the parent compound is only included in the residue definition for plant matrices.

The metabolism data submitted for clofentezine in animal products showed the vast majority of the residue in cattle and goat tissues is 4-OH clofentezine. Poultry studies however, showed more significant quantities of parent clofentezine, in addition to 3 and 4-OH clofentezine. Quantities of 3 and 4-OH clofentezine were not separated in the poultry study, but quantities of 3-OH clofentezine are much smaller than the combined totals of parent and 4-OH clofentezine.

In the cow metabolism study, the TRRs in subcutaneous fat and muscle were about 0.02 mg/kg, but TRR in renal fat was about 16 times as high as that in muscle. The main component of the residue in poultry commodities is the parent clofentezine and the TRR in fat was approximately 22 times higher than in the muscle. Based on the above results and the octanol-water partition coefficient of clofentezine (log P_{OW}=4.1), clofentezine is considered as fat-soluble.

Based on the available comparative animal and plant metabolism studies, the Meeting recommended a residue definition for clofentezine as follows:

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities*: clofentezine.*

Definition of the residue (for compliance with the MRL and estimation of dietary intake) for animal commodities: *sum of clofentezine, and* a*ll metabolites containing the 2-chlorobenzoyl moiety, expressed as clofentezine.*

The Meeting decided that residue is fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for clofentezine uses on orange, lemon, mandarin, apple, pear, peach, apricot, cherry, nectarine, plum, grapes, strawberry, currant, gherkin, cucumber, melon, tomato, walnut and almond. Residue data were also provided on almond hulls.

Labels (or translations of labels) were available to the Meeting from Argentina, Australia, Belgium, Canada, France, Germany, Greece, Italy, the Netherlands, Portugal, South Africa, Spain, Switzerland, UK, and USA describing the relevant GAP for evaluation of clofentezine.

Citrus fruits

Supervised trials were conducted on orange trees in Greece (citrus GAP 0.015 kg ai/hL, one application, 30-day PHI), Italy (citrus GAP 0.015–0.02 kg/hL, one application, 30-day PHI) and Spain (citrus GAP 0.005–0.01 kg/hL, 21-day PHI) in 1984, 1990 and 2001.

In two orange trials from Greece with application conditions in line with GAP, clofentezine residues were 0.10 and 0.18 mg/kg, with residues in flesh at 0.02 and 0.03 mg/kg.

In six orange trials from Spain and two trials from Italy with application conditions matching Spanish GAP, clofentezine residues were: 0.06, 0.07, 0.09(3), 0.10, 0.12 and 0.14 mg/kg, with some residues in flesh at < 0.01, 0.01, 0.02(3) and 0.03 mg/kg.

In one trial on lemons from Greece with application conditions in line with GAP, clofentezine residue was 0.15 mg/kg, with residue in flesh at 0.03 mg/kg.

In one trial on lemons from Italy matching Spanish GAP, clofentezine residue in lemon was 0.09 mg/kg, with residue in flesh at 0.02 mg/kg.

In one trial on tangerines from Italy matching Spanish GAP, clofentezine residue was 0.24 mg/kg, with residue in flesh at 0.03 mg/kg.

In six mandarin trials from Spain with application conditions in line with GAP, clofentezine residues were 0.08(3), 0.15, 0.17 and 0.18 mg/kg, with residues in flesh for two trials at 0.02 and 0.17 mg/kg.

The Meeting noted that the residue data populations for orange, lemon, tangerine and mandarin were from similar populations and can be combined. The residues in ranked order on citrus fruits were: 0.06, 0.07, 0.08(3), 0.09(4), <u>0.10</u> (2), 0.12, 0.14, 0.15(2), 0.17, 0.18(2) and 0.24 mg/kg (n=19). A similar situation exists for the flesh and the ranked order of concentrations in flesh was: < 0.01, 0.01, <u>0.02</u>(5), 0.03(3) and 0.17 mg/kg (n=11).

The Meeting estimated a maximum residue level for citrus fruits and an STMR value for flesh of 0.5 and 0.02 mg/kg respectively. The Meeting also estimated an STMR value for clofentezine in whole citrus fruits of 0.10 mg/kg (for estimating STMR-R value in orange juice). The recommendation for a maximum residue level of 0.5 mg/kg for citrus fruits confirms the previous recommendation of 0.5 mg/kg.

Pome fruits

Supervised trials were conducted on apple trees in Australia (pome fruit GAP 0.015 kg/hL, one application, 21-day PHI), Canada (GAP 0.15–0.30 kg ai/ha, one application, 45-day PHI), UK (GAP 0.2 kg ai/ha, one application, 28-day PHI), France (GAP 0.02 kg ai/hL, one application, 42-day PHI), Greece (GAP 0.015 kg ai/hL, one application, 45-day PHI), Germany (pome fruit GAP 0.02 kg ai/hL, one application per year, 35-day PHI), Belgium (GAP 0.13 kg ai/ha, one application, no PHI), South Africa (GAP 0.02 kg ai/hL, 30-day PHI), USA (GAP 0.12–0.24 kg ai/ha, one application, 45-day PHI) annually from 1980 to 1987 and then 1992 and 1993.

In one trial from Canada with application conditions in line with GAP, the residue of clofentezine found in apple was 0.04 mg/kg.

In fifteen apple trials from France with application conditions in line with GAP, clofentezine residues were < 0.01(3), 0.01, 0.03, 0.05(2), 0.06, 0.07(3), 0.08, 0.10, 0.11 and 0.22 mg/kg.

In one apple trial from Greece with application conditions in line with GAP, clofentezine residue was 0.04 mg/kg.

In two apple trials from South Africa with application conditions in line with GAP, clofentezine residues were 0.09(2) mg/kg.

In sixteen apple trials from USA with application conditions in line with GAP, clofentezine residues were 0.01(2), 0.02(3), 0.04(3), 0.05(3), 0.07(2), 0.11 and 0.12(2) mg/kg.

In five apple trials from Germany with application conditions in line with GAP, clofentezine residues were 0.02, 0.03, 0.04, 0.05 and 0.09 mg/kg.

In four apple trials from UK with application conditions matching French GAP, clofentezine residues were 0.10, 0.16, 0.17 and 0.24 mg/kg.

The Meeting noted that the residues in apples from the above countries were from similar populations and could be combined. The ranked order of residues were: < 0.01(3), 0.01(3), 0.02(4), 0.03(2), 0.04(6), 0.05(6), 0.06, 0.07(5), 0.08, 0.09(3), 0.10(2), 0.11(2), 0.12(2), 0.16, 0.17, 0.22 and 0.24 mg/kg (n=44).

Supervised trials were conducted on pear trees in Australia (pome fruit GAP 0.015 kg/hL, one application, 21-day PHI), Canada (GAP 0.15-0.30 kg ai/ha, one application, 21-day PHI), Italy (GAP 0.02 kg ai/hL, one application, 30-day PHI), South Africa (GAP 0.02 kg ai/hL, 30-day PHI), USA (GAP 0.12-0.24 kg ai/ha, one application, 21-day PHI) in 1982, 1984, 1985, 1986, 1987, 1988 and 1993.

In one pear trial from Australia with application conditions in line with GAP, clofentezine residue was 0.02 mg/kg.

In four pear trials from Canada with application conditions in line with GAP, clofentezine residues were 0.12, 0.13, 0.14 and 0.19 mg/kg.

In one pear trial from Italy with application conditions in line with GAP, clofentezine residue was 0.04 mg/kg.

In two pear trials from South Africa with application conditions in line with GAP, clofentezine residues were 0.20 and 0.22 mg/kg.

In thirteen pear trials from USA with application conditions in line with GAP, clofentezine residues were 0.04, 0.05, 0.06(3), 0.08(2), 0.09(3), 0.15, 0.18 and 0.20 mg/kg.

The Meeting noted that the residues in pears from the above countries were from similar populations and could be combined. The residues in ranked order were: 0.02, 0.04(2), 0.05, 0.06(3), 0.08(2), 0.09(3), 0.12, 0.13, 0.14, 0.15, 0.18, 0.19, 0.20(2) and 0.22 mg/kg.

The Mann-Whitney test indicated the residue data populations for apple and pear were not significantly different and could be combined to support a pome fruit MRL. The ranked order of concentrations, median underlined, were < 0.01(3), 0.01(3),0.02(5), 0.03(2), 0.04(8), 0.05(7), 0.06(4), 0.07(5), 0.08(3), 0.09(6), 0.10(2), 0.11(2), 0.12(3), 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20(2), 0.22(2) and 0.24 mg/kg (n=65). The Meeting estimated a maximum residue level and an STMR value for clofentezine in pome fruits of 0.5 and 0.05 mg/kg respectively. The recommendation for a maximum residue level of 0.5 mg/kg for pome fruits confirms the previous recommendation.

Stone fruits

Supervised trials were conducted on peach in Australia (stone fruit GAP 0.015 kg ai/hL, one application, 21-day PHI), in Italy (no GAP), and USA (GAP 0.059–0.24 kg ai/ha, one application, 21-day PHI) in 1984, 1986, 1987, 1988, 1991, 1993 and 2002.

In two peach trials from Australia with application conditions in line with GAP, clofentezine residues in flesh were 0.06 and 0.13 mg/kg.

In eighteen peach trials conducted at GAP in USA, clofentezine residues were 0.02, 0.03, 0.04, 0.05, 0.06, 0.08(2), 0.09(2), 0.11, 0.12, 0.13, 0.14(2), 0.18(2), 0.24 and 0.35 mg/kg.

The Meeting noted that the residue data populations from Australia and USA for peach were from similar populations and should be combined. The residues in ranked order were: 0.02, 0.03, 0.04, 0.05, 0.06(2), 0.08(2), 0.09(2), 0.11, 0.12, 0.13(2), 0.14(2), 0.18(2), 0.24 and 0.35 mg/kg.

Supervised trials were conducted on apricot in Greece (GAP 0.015 kg ai/hL, one application, 45-day PHI) and USA (GAP 0.059-0.24 kg ai/ha, one application, 21-day PHI) in 1987, 1989 and 1993.

In one apricot trial conducted at GAP in Greece, clofentezine residue was 0.16 mg/kg.

In two apricot trials conducted at GAP in USA, clofentezine residues were 0.13 and 0.14 mg/kg.

Combined residues from Greek and USA apricot trials were 0.13, 0.14 and 0.16 mg/kg.

Supervised trials were conducted on cherries in UK (0.2 kg ai/ha, one application, 56-day PHI) in 1983.

In two cherry trials conducted in accordance with British GAP, clofentezine residues were 0.01 and 0.02 mg/kg.

Supervised trials were conducted on nectarines in USA (GAP 0.059–0.24 kg ai/ha, one application, 21-day PHI) in 1986 and 1987.

In three nectarine trials conducted in accordance with USA GAP, clofentezine residues were 0.04, 0.11 and 0.17 mg/kg.

Supervised trials were conducted on plums in Germany (GAP 0.02 kg ai/hL, one application limited by growth stage of the crop, no PHI) in 1985 and 1986. The last application was made during fruit development, which is not in accordance with German GAP, as a result the residue data could not be used for the evaluation.

The Meeting combined the residue data in peach, apricot, cherry and nectarine. The residues in ranked order were: 0.01, 0.02(2), 0.03, 0.04(2), 0.05, 0.06(2), 0.08(2), 0.09(2), 0.11(2), 0.12, 0.13(3), 0.14(3), 0.16, 0.17, 0.18(2), 0.24 and 0.35 mg/kg (n=28).

The Meeting estimated a maximum residue level and an STMR value for clofentezine in stone fruits of 0.5 and 0.11 mg/kg respectively. The recommendation for a maximum residue level of 0.5 mg/kg for stone fruits replaces the previous recommendation of 0.2 mg/kg.

Grapes

Supervised trials were conducted on grapes in France (GAP 0.2 kg ai/ha, one application, 42-day PHI), in Greece (no GAP, use that of Spain), in Germany (GAP 0.06–0.24 kg ai/ha, one application, 35-day PHI), in Italy (GAP 0.01-0.015 kg ai/hL, one application, 30-day PHI) and in Spain (GAP 0.01-0.03 kg ai/hL, 30-day PHI) in 1984, 1985, 1986, 1987, 1991, 1992 and 2001.

In twelve trials conducted in line with German GAP, clofentezine residues were 0.09, 0.12, 0.14, 0.20, 0.22, 0.23, 0.39, 0.61, 0.69, 0.73, 0.79 and 0.89 mg/kg.

In one trial conducted in accordance with Italian GAP, clofentezine residue was 0.35 mg/kg.

In two trials conducted in accordance with Spanish GAP, clofentezine residues were 0.25 and 0.27 mg/kg.

In one trial in Greece conducted in accordance with Spanish GAP, clofentezine residue was 0.67 mg/kg.

In one trial in Italy conducted in accordance with Spanish GAP, clofentezine residue was 0.12 mg/kg.

In two trials in France conducted in accordance with Spanish GAP, clofentezine residues were 0.09 and 0.11 mg/kg.

The Meeting noted that the residues in grapes were from similar populations and could be combined. The residues in ranked order were: 0.09(2), 0.11, 0.12(2), 0.14, 0.20, 0.22, 0.23, 0.25, 0.27, 0.35, 0.39, 0.61, 0.67, 0.69, 0.73, 0.79 and 0.89 mg/kg (n=19).

The Meeting estimated a maximum residue level and an STMR value for clofentezine in grapes of 2 and 0.25 mg/kg respectively. The recommendation for a maximum residue level of 2 mg/kg for grapes replaces the previous recommendation of 1 mg/kg.

Strawberries

Supervised trials were conducted on strawberries in France (GAP 0.2 kg ai/ha, 3-day PHI), in Germany (GAP 0.3 kg ai/ha, one application, no PHI, growth-stage restriction), in the Netherlands (GAP 0.075–0.15 kg ai/ha, one to two applications, no PHI, growth-stage restriction) and in Spain (GAP 0.01–0.02 kg ai/hL, 3-day PHI) in 1989, 1990, 1992, 2000 and 2001.

In eight outdoor trials in France conducted in line with French GAP, clofentezine residues were 0.08(2), 0.09, 0.13, 0.19, 0.20 and 0.24(2) mg/kg.

In two outdoor trials in the Netherlands conducted in accordance with Dutch GAP, clofentezine residues were 0.08 and 0.13 mg/kg.

In nine outdoor trials in Spain conducted in accordance with Spanish GAP, clofentezine residues were 0.50, 0.56, 0.60, 0.70, 0.72, 0.73, 0.75, 0.81 and 1.10 mg/kg.

In five outdoor trials in Germany conducted in line with French GAP, clofentezine residues were 0.09(2), 0.16, 0.18 and 0.23 mg/kg.

The data populations from France and those from the Netherlands and Germany were not significantly different and should be combined. The data from Spain were significantly different from those from France, Germany and the Netherlands in strawberries on a Mann-Whitney test.

Based on data from Spain, the Meeting estimated a maximum residue level and an STMR value for clofentezine in strawberries of 2 and 0.72 mg/kg respectively. The recommendation for a maximum residue level of 2 mg/kg for strawberry confirms the previous recommendation of 2 mg/kg.

Black, red and white Currants

Four supervised trials were conducted on blackcurrants in France (maximum GAP: 0.2 kg ai/ha, 45-day PHI) in 2001. The residues in ranked order on currants were: < 0.04(3) and 0.09 mg/kg.

The Meeting agreed to extrapolate from blackcurrants to red and white currants and estimated a maximum residue level and an STMR value for clofentezine in currants of 0.2 and 0.04 mg/kg respectively. The recommendation for a maximum residue level of 0.2 mg/kg for currants replaces the previous recommendation of 0.05 mg/kg.

Cucurbits

Supervised trials were conducted on cucumbers in France (cucurbits GAP 0.2 kg ai/ha, 3-day PHI), in Greece (cucumber GAP 0.015 kg ai/hL, one application, 4-day PHI) and in Switzerland (cucumber GAP 0.02 kg ai/hL, one application, 14-day PHI) in 1985, 1987, 1988 and 1991.

In one trial in France conducted on cucumber in accordance with French GAP, clofentezine residue was 0.07 mg/kg.

In four trials in Greece conducted on cucumber in accordance with Greek GAP, clofentezine residues were 0.12(2), 0.14 and 0.16 mg/kg.

In one trial in Switzerland conducted on cucumber in accordance with French GAP, clofentezine residue was 0.13 mg/kg.

The Meeting noted that the residue data in cucumber were from similar populations and could be combined. The residues in ranked order were: 0.07, 0.12(2), 0.13, 0.14 and 0.16 mg/kg (n=6).

The Meeting estimated a maximum residue level and an STMR value for clofentezine in cucumber of 0.5and 0.125 mg/kg respectively. The recommendation for a maximum residue level of 0.5 mg/kg for cucumber replaces the previous recommendation of 1 mg/kg.

Melons

Supervised trials were conducted on melons in France (GAP 0.2 kg ai/ha, 3-day PHI), in Greece (no GAP, use that of France), in Italy (GAP 0.015–0.02 kg ai/hL, one application, 15-day PHI), in Portugal (no GAP, use that of France) and in Spain (GAP 0.01–0.02 kg ai/hL, 3-day PHI) in 1999 and 2000.

In two trials in France conducted in line with French GAP, clofentezine residues were < 0.05 and 0.05 mg/kg, with no detectable residue in pulp.

In one trial in Greece conducted in accordance with Greek GAP, clofentezine residue was 0.03 mg/kg, with no detectable residue in pulp.

In two trials in Portugal conducted in accordance with Portuguese GAP, clofentezine residues were < 0.05 mg/kg, with no detectable residue in pulp.

In two trials in Spain conducted in accordance with Spanish GAP, clofentezine residues were < 0.01 and < 0.05 mg/kg, with no detectable residue in pulp.

In two trials in Italy conducted in accordance with French GAP, clofentezine residues were 0.03 and 0.06 mg/kg, with no detectable residue in pulp.

The Meeting noted that the residues in melons were from similar populations and could be combined. The residues in ranked order were: < 0.01, 0.03(2), < 0.05(4), 0.05 and 0.06 mg/kg (n=9). The residues in all pulp samples were below the limit of quantification (n=9).

The Meeting estimated a maximum residue level of 0.1 mg/kg. Taking into account that the parent compound practically did not translocate in plants, the Meeting estimated an STMR value of 0 mg/kg for clofentezine in melons.

Tomato

Supervised trials were conducted on tomato in France (no GAP, use that of the Netherlands), in Germany (no GAP, use that of the Netherlands), in Greece (no GAP, use that of the Netherlands), in Italy (GAP 0.02-0.03 kg ai/hL, one application, 15-day PHI), in the Netherlands (GAP 0.075–0.23 kg ai/ha, 1-2 applications, 3-day PHI) and in Spain (0.01-0.02 kg ai/hL, one application, 3-day PHI) in 1986, 1992, 1999, 2000 and 2005.

In seven trials in Italy conducted in accordance with Italian GAP, clofentezine residues were 0.01, 0.03, 0.05(2), 0.06, 0.07 and 0.10 mg/kg.

In seven glasshouse trials in the Netherlands conducted in accordance with Dutch GAP, clofentezine residues were < 0.05, 0.09, 0.10, 0.11, 0.12, 0.16 and 0.18 mg/kg.

In four trials in France conducted matching Dutch GAP, clofentezine residues were < 0.05, 0.05, 0.06 and 0.09 mg/kg.

In three trials in Germany conducted matching Dutch GAP, clofentezine residues were < 0.05, 0.06 and 0.11 mg/kg.

In one trial in Greece conducted matching Dutch GAP, clofentezine residue was < 0.05 mg/kg.

In one trial in Italy conducted matching Dutch GAP, clofentezine residue was < 0.05 mg/kg.

In one trial in Spain conducted matching Dutch GAP, clofentezine residue was 0.09 mg/kg.

The Meeting noted that the residues from France, Germany and the Netherlands, in line with Dutch GAP, were from similar populations and could be combined. The data populations from France, Germany and the Netherlands and from Greece, Italy and Spain were not from similar populations based on the Mann-Whitney test, and could not be combined. The residues in ranked order based on France, Germany and the Netherlands were: < 0.05(3), 0.05, 0.06(2), 0.09(2), 0.10, 0.11(2), 0.12, 0.16 and 0.18 mg/kg (n=14).

The Meeting estimated a maximum residue level and an STMR value for clofentezine in tomato of 0.5 and 0.09 mg/kg respectively.

Tree nuts

Eight supervised trials were conducted on walnut in USA (maximum GAP: 0.24 kg ai/ha, one application, 30-day PHI) in 1987 and 1988. The ranked order of concentrations on walnut was: < 0.02(8) mg/kg.

Thirty four supervised trials were conducted on almond in USA (maximum GAP: 0.24 kg ai/ha, 30-day PHI) in 1985, 1987, 1993, 1998 and 2002. The ranked order of concentrations on almond was: < 0.01(9), < 0.02(3), < 0.05(13), 0.10(3), 0.20(2), and 0.30(4) mg/kg.

The residue data for walnut and almond were from similar populations and could be combined. The residues in ranked order on tree nuts were: < 0.01(9), < 0.02(11), < 0.05(13), 0.10(3), 0.20(2), and 0.30(4) mg/kg (n=42).

The Meeting estimated a maximum residue level and an STMR value for clofentezine in tree nuts of 0.5 and 0.05 mg/kg respectively.

Animal feedstuffs

Almond hull

In 34 supervised trials on almond compliant with US GAP, residues of clofentezine in almond hulls in rank order, median and highest residue underlined, were: 0.06, 0.10, 0.11, 0.12(2), 0.20(2), 0.30,

0.40(2), 0.50(2), 0.60(2), 0.70(2), 0.90, 0.91, 1.00, 1.10(2), 1.20, 1.40(2), 1.50(2), 1.60, 1.70, 1.80, 2.00, 2.20, 2.30, 2.50 and 2.70 mg/kg (fresh weight) (n=34).

The meeting estimated an STMR value of 0.91 mg/kg and a highest residue of 2.70 mg/kg for clofentezine in almond hulls (fresh weight).

Allowing for the standard 90% dry matter for almond hulls (*FAO Manual*, p. 147) the residues in almond hulls were: 0.07, 0.11, 0.12, 0.13(2), 0.22(2), 0.33, 0.44(2), 0.56(2), 0.67(2), 0.78(2), 1.00, 1.01, 1.11, 1.22(2), 1.33, 1.56(2), 1.67(2), 1.78, 1.89, 2.00, 2.22, 2.44, 2.56, 2.78 and 3.00 mg/kg. The Meeting estimated a maximum residue level of 5 mg/kg and an STMR of 1.01 mg/kg for almond hulls (dry weight). A highest residue level of 3.00 mg/kg was estimated for calculating the dietary burden of farm animals.

Fate of residues during processing

The Meeting received information on the fate of clofentezine residues during aqueous hydrolysis under conditions representing pasteurisation, baking, brewing, boiling and sterilisation. Information was also provided on the fate of clofentezine residues during the food processing of citrus, apples, grapes and strawberries.

Clofentezine was stable at pH 4 for 20 minutes at 90 °C with no degradation products formed, and moderately stable at pH 5 at 100 °C for 60 minutes. In this latter instance, clofentezine degraded slightly (by approximately 10%) to form metabolite 2-chlorobenzoic (2-chlorobenzylidene) hydrazide (AEC 593600). The parent clofentezine was rapidly hydrolysed at pH 6 and 120 °C and it was not detected after 20 minutes. The major reaction products as a percentage of the applied radioactivity were 2-chlorobenzoic (2-chlorobenzylidene) hydrazide (78%), 2-chlorobenzonitrile (4.9%) and 2-chlorobenzamide (17%).

The processing was carried out to produce apple sauce from samples spiked with clofentezine in the laboratory. The time of cooking and pasteurizing was approximately 15 minutes at a temperature of over 97 °C. The treated apples, the apple sauce and the processing by-products (washed apple, apple cores, peeled apples, wash water), were analysed for the potential degradation products of clofentezine (AEC 593600, 2-chlorobenzonitrile and 2-chlorobenzamide), but none of the compounds was present in quantifiable concentration in any of the samples. One uncooked apple peel sample contained 0.02 mg/kg for 2-chlorobenzonitrile. The clofentezine residue in the pasteurized apple sauce was reduced from 0.40 mg/kg in the apple in one study, and from 0.41 mg/kg to < 0.02 mg/kg in three samples in a follow up test, where the three degradation products were not detectable. The Meeting noted that the 3 degradation products found in the above hydrolysis study, performed at pH 6 and 120 °C, were not present in apple sauce prepared following a normal processing procedure.

Processing studies for the conversion of oranges, apples, grapes and strawberries to various processed products were reported from Germany, Italy, Spain and USA. In most cases, the raw agricultural commodities had quantifiable field incurred clofentezine residue. Calculated processing factors and the mean or best estimate for the processing factors are summarized in the following table.

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors. [1/]	Median or best estimate
Orange	Juice	< 0.08, < 0.11, < 0.14, 0.14, < 0.17(3), < 0.20, < 0.25(2), < 0.33(2)	0.14
Apples	Wet pomace	< 0.50, 1.20, 1.50 (2), 2.00 (4), 2.11, 2.40, 3.00, 3.44, 5.50, 5.69, 5.79, 6.00	2.06
	Juice	0.016, 0.11, 0.20, < 0.5 (3)	0.11
Grapes	Raisins	0.22, 0.28, 0.64, < 0.67, 1.09, 1.12, 1.70, 2.33, 2.92	1.11
	Juice	nd	0
	Wet pomace	1.88, 1.89	1.89
	White wine making	< 0.042, < 0.50 (2)	< 0.042

[1/] 'Less-than' (<) values are derived from cases where residues were not detected in the processed commodity. The 'less-than' processing factor is then calculated from the LOQ of the analyte in the processed commodity and the residue in the raw agricultural commodity.

The processing factor for orange juice (0.14) was applied to the estimated STMR for orange (0.10 mg/kg) to produce STMR-P values for orange juice (0.014 mg/kg).

The processing factors for wet apple pomace (2.06) and apple juice (0.11) were applied to the estimated STMR for apple (0.05 mg/kg) to produce STMR-P values for wet apple pomace (0.103 mg/kg) and apple juice (0.0055 mg/kg).

The processing factors for grapes to raisins (1.11), grape juice (0), white wine (0.042) and wet pomace (1.89) were applied to the estimated STMR for grapes (0.25 mg/kg) to produce an STMR-P value for dried grapes (0.28 mg/kg), grape juice (0), white wine (0.011 mg/kg) and wet pomace (0.47 mg/kg). The processing factors for raisins (1.09) were applied to the grape residue data (highest value 0.89 mg/kg) to produce estimated highest values for dried grapes (0.99 mg/kg).

The Meeting estimated a maximum residue level for clofentezine in dried grapes of 2 mg/kg.

Residues in animal commodities

Farm animal feeding studies

The Meeting received feeding studies on lactating dairy cows, calves and laying hens, which provided information on likely residues appearing in animal tissues, milk and eggs from residues in the animals' diet.

Lactating Friesian cows were dosed with clofentezine at the equivalent of 10 (1), 30 (3 ×) and 100 (10 ×) ppm in the dry-weight diet using an average feed consumption of 20 kg for 28 consecutive days. Milk was collected twice daily for analysis. Animals were sacrificed at 1 or 3 days after the final dosing.

No residues (total clofentezine=clofentezine and metabolites hydrolysable to 2-chlorbenzoic acid and expressed as clofentezine) were detected in milk samples taken from the control and the 1 × dose groups. From day 7, total residues between < 0.05 and 0.14 mg/kg were detected in the milk of cows from the 3 × dose group. In the 10 × dose group, residues occurred regularly from day 7 in the concentration range of 0.11 to 0.27 mg/kg.

The total residue was below the LOQ (0.05 mg/kg) in all tissue samples except liver from the treatment rate of 1 ×. No residue was detectable in heart, muscle and fat samples at any dose level. The average residues were present in liver (0.26, 1.15, 2.20 mg/kg) and in kidney (< 0.05, 0.18, 0.40 mg/kg) at the dose rates of 1 ×, 3 × and 10 ×, respectively.

Calves were dosed at a rate equivalent to 0.5 ppm feed (dry weight) using an average feed consumption of 3.5 kg for 28 days. Animals were sacrificed at 19 h after the final dosing. The total

clofentezine residues were below the limit of quantification (0.05 mg/kg clofentezine equivalents) in the liver and kidney samples.

Laying hens were fed clofentezine at 0.05, 0.15, 0.50 and 6.0 ppm in the diet (dry weight) for 28 days. Egg samples were taken daily during the study and kept frozen. Birds were sacrificed 1 day after the final dosing. Only one egg sample obtained from the highest dose rate group contained quantifiable residues (0.06 mg/kg). From the dose groups of 0.05, 0.15, 0.50 ppm, no residue was present above the LOQ (0.05 mg/kg) in any tissue samples. Quantifiable residues were only present at the exaggerated 6.0 ppm dosage rate: liver (0.08 mg/kg), kidney (0.06 mg/kg) and abdominal and subcutaneous fat (0.13, 0.09 mg/kg).

Livestock dietary burden

The Meeting estimated the dietary burden of clofentezine in farm animals on the basis of the diets listed in the Annex 6 of the 2006 JMPR Report. Calculation from highest residue and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle and turkey are provided in Annex 6. The calculations were made according to the animal diets from USA, Canada, EU and Australia in the OECD Table (Annex 6 of the 2006 JMPR Report).

The calculations are then summarized and the highest dietary burdens (underlined) are selected for MRL and STMR estimates on animal commodities.

	Animal dietary burden, clofentezine, ppm of dry matter diet					
	USA-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.35	0.15	0.05	0.05	<u>0.98</u> [1/]	<u>0.78</u> [2/]
Dairy cattle	0.33	0.13	0.03	0.03	<u>0.95</u> [3/]	<u>0.75</u> [4/]
Poultry - layer	0	0	0	0	0	0
Poultry - layer	0	0	0	0	0	0

[1/] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat.

[2/] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[3/] Highest maximum dairy cattle dietary burden suitable for MRL estimates for milk.

[4/] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

The dietary burden for both beef and dairy cattle was 0.93 mg/kg, below the lowest feeding level (10 ppm in the feed). Therefore, the resulting residues in milk and cattle tissues were calculated by applying the respective transfer factors (transfer factor=residue level in tissue or milk ÷ residue level in feed) to the estimated dietary burden. In the feeding study the highest residue levels in tissues were used to calculate the highest likely mammal commodity residue levels and mean residue levels in milk and tissues were used to estimate the mammal commodity STMRs. In the table below, dietary burdens and the corresponding estimated residues in brackets are indicated with Italic fonts.

	Feeding level (mg/kg) actual	Clofentezine residues, mg/kg									
		Milk		Muscle		Fat		Liver		Kidney	
		Highest	Mean	Highest	Mean	Highest	Mean	Highest	Mean	Highest	Mean
MRL	*0.98*			*(< 0.005)*		*(< 0.005)*		*(0.031)*		*(< 0.005)*	
beef cattle	10			< 0.05		< 0.05		0.33		< 0.05	

MRL	*0.95*		*(< 0.005)*								
dairy cow	10		< 0.05								
STMR	*0.78*				*(< 0.004)*		*(< 0.004)*		*(0.019)*		*(< 0.004)*
beef cattle	10				< 0.05		< 0.05		0.26		< 0.05
STMR	*0.75*		*(< 0.004)*								
dairy cow	10		< 0.05								

The dietary burden for laying hens was 0 mg/kg, therefore the table of calculation of MRLs and STMRs for poultry meat and eggs is not necessary.

The Meeting estimated maximum residue levels of 0.05 (*) mg/kg for mammalian meat (fat), mammalian edible offal, and milks to replace the present recommendations of 0.05 (*) mg/kg for cattle meat, 0.01 (*) mg/kg and 0.1 mg/kg for edible offal of cattle. The Meeting also estimated the following STMR values: muscle 0 mg/kg, fat 0 mg/kg, edible offal 0.05 mg/kg, and whole milk 0 mg/kg.

The Meeting estimated maximum residue levels of 0.05 (*) mg/kg for eggs, poultry meat (fat), and poultry edible offal, based on the limit of quantification for poultry commodities to confirm the present recommendation of 0.05 (*) mg/kg. Also estimated were STMRs of 0 mg/kg for eggs, meat, and edible offal of poultry.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDIs) of clofentezine, based on the STMRs estimated for sixteen commodities, were 0–3% of the maximum ADI of 0.02 mg/kg bw for the thirteen GEMS/Food regional diets. The Meeting concluded that the long-term intake of residues of clofentezine resulting from its uses that have been considered by JMPR is unlikely to present a public health concern.

Short-term intake

The 2005 JMPR decided that an ARfD was unnecessary. The Meeting therefore concluded that the short-term intake of clofentezine residues is unlikely to present a public health concern.

5.7 CYFLUTHRIN (157)/ BETA-CYFLUTHRIN (228)

RESIDUE AND ANALYTICAL ASPECTS

Cyfluthrin was identified as a priority compound under the Periodic Re-evaluation Programme at the 37th Session of the CCPR. The Meeting received information on cyfluthrin metabolism and environmental fate, methods of residue analysis, freezer storage stability, national registered use patterns, supervised residue trials, farm animal feeding studies, fate of residues in processing and national MRLs. The Meeting also received information on beta-cyfluthrin methods of residue analysis, freezer storage stability, national registered use patterns and supervised residue trials. The metabolism and environmental fate, transfer from feeds to farm animals and fate of residues in processing provided for cyfluthrin are used to support both pesticides.

Cyfluthrin was evaluated by the 48th JECFA for residues in animal commodities arising from direct animal treatment. In the case of animal commodities, the maximum residue limit recommendations of the 48th JECFA for cattle are fat 0.2 mg/kg, muscle, liver and kidney 0.02 mg/kg and milk 0.04 mg/kg. The residue definition (marker residue) chosen by JECFA was cyfluthrin.

The 2006 JMPR established common ADIs and ARfDs for beta-cyfluthrin and cyfluthrin of 0-0.04 mg/kg bw per day and 0.04 mg/kg bw respectively.

Cyfluthrin is a mixture of 8 stereoisomeric esters derived from esterification of the dichloro analogue of chrysanthemic acid, (2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylic acid, DCVA) with α -cyano-3-phenoxy-4-fluorobenzyl alcohol. Beta-cyfluthrin is an enriched isomeric form of the 2 biologically active diastereoisomeric pairs of isomers.

Conclusions reached in discussing cyfluthrin equally apply to beta-cyfluthrin. In the presence of water and other protic solvents, the isomer composition of beta-cyfluthrin changes through epimerisation such that with sufficient time the isomer ratio becomes the same as cyfluthrin.

The following abbreviations are used for the metabolites discussed below:

DCVA	2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylic acid
FPBald	4-fluoro-3-phenoxybenzaldehyde
FPBacid	4-fluoro-3-phenoxybenzoic acid
FPBalc	3-phenoxy-4-fluorobenzyl alcohol
OH-FPBacid	3-(4'-hydroxyphenoxy)-4-fluorobenzoic acid
Me-FPBacid	methyl 4-fluoro-3-phenoxybenzoate
FPBamide	4-fluoro-3-phenoxybenzamide
FPB	1-fluoro-2-phenoxybenzene
COOH-cyfluthrin	α-[[[3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropyl]carbonyl]oxy]-4-fluoro-3-phenoxybenzeneacetic acid

Animal metabolism

Two radiolabelled cyfluthrin preparations separately [^{14}C] labelled at the phenyl-UL-^{14}C- and fluorophenyl-UL positions, were used in the metabolism and environmental studies. The metabolism of laboratory animals was qualitatively the same as for farm animals. The proposed major route of cyfluthrin metabolism in livestock is via hydrolysis of the ester linkage. Hydrolysis gives initially DCVA and the unstable cyanohydrin which rapidly breaks down to the aldehyde (FPBald) and is oxidized to the corresponding acid/or hydroxylated acids (FPBacid, OH-FPBacid). A very minor route was observed in which a small amount of FPBald was converted to its corresponding alcohol, FPBalc.

Lactating cows were orally dosed with [phenyl-UL-^{14}C]-cyfluthrin at 0.5 mg/kg bw for 5 consecutive days. Cyfluthrin was the major identifiable product in milk (98% TRR, 0.039–0.079 mg/kg). The radiocarbon content of tissues, reported in cyfluthrin equivalents, was highest in liver (0.62 mg/kg), kidney (0.19 mg/kg), and fat (0.12–0.23 mg/kg) with low levels (< 0.05 mg/kg) present in other tissues. Cyfluthrin was the main component of the [^{14}C] in liver and kidney (56–86%) and in muscle and fat (93–100%). Hydrolysis products, formed from hydrolysis of the ester and oxidation, were only detected in liver (14% FPBald), kidney (43% FPBalc) and heart (29%, FPBalc).

Laying hens were orally dosed with [phenyl-UL-^{14}C]-cyfluthrin for 3 consecutive days at 5 mg/kg bw/hen/day. Radioactive residues in eggs collected in the 24 hour period prior to slaughter were 0.05 mg/kg cyfluthrin equivalents. Radioactive residues in tissues of birds slaughtered at 2 h after the last dose were highest in kidney (4.7 mg/kg cyfluthrin equivalents) and liver (3.0 mg/kg) with low levels observed in other tissues. Residues of [^{14}C] in fat were 0.1–0.2 mg/kg while those in muscle were 0.2–0.3 mg/kg, both expressed in cyfluthrin equivalents. The major radiolabelled fraction identified in eggs (56%), fat (75%) and muscle (21–39%) was cyfluthrin. Significant metabolites were FPBacid (12% liver, 11% kidney, 15–21% muscle) and OH-FPBacid (10% liver, 12% kidney, 11-20% muscle).

Plant metabolism

The Meeting received information on the fate of [phenyl-UL-^{14}C]cyfluthrin after foliar application to on apple, cotton, potato, soya bean and wheat and of [cyclopropyl-1-^{14}C]cyfluthrin on wheat. Studies were also available on the fate of [fluorophenyl-UL-^{14}C]cyfluthrin on tomatoes and stored wheat grain.

The metabolism of [^{14}C]cyfluthrin was studied in apples. The majority of the [^{14}C] was associated with the fruit surface (rinses and peel) with 4% or less associated with pulp. Cyfluthrin was the major component of the radioactivity detected at 0 to 28 days after application accounting for 91–84% of the [^{14}C] in rinse solutions and peel. Several minor components, principally FPBald and FPBacid, were present at ≤ 2% of the [^{14}C].

Unchanged cyfluthrin accounted for > 90% of the TRR present tomato fruit and leaves from 1 to 35 days after application to both fruit and leaves.

Potato plants were treated in a greenhouse study with [phenyl-UL-^{14}C]cyfluthrin as a foliar treatment. There was limited translocation of [^{14}C] to potato tubers. Residues in leaves sampled at 0–98 days after application mostly comprised unchanged cyfluthrin (70–95%) together with a number of free and conjugated metabolites (FPBald, FPBacid, OH-FPBacid, FPB) each present at < 5%TRR.

Cyfluthrin was also the major component of [^{14}C] residues following application of [phenyl-UL-^{14}C]cyfluthrin to soya bean plants raised in a greenhouse (43–92% TRR at 4–88 days after application). Individual metabolites identified in whole plants, leaves and stalks were present in both free and conjugated forms and were all individually < 10% TRR (FPBald, OH-FPBacid, FPBacid, FPBalc, Me-FPBacid, FPBamide, FPB). There was limited translocation of [^{14}C] to pods. Incubation of [phenyl-UL-^{14}C]cyfluthrin with soya bean tissue cultures produced a similar range of metabolites.

Cotton plants were treated with [phenyl-UL-^{14}C]cyfluthrin as a foliar treatment and exposed to natural sunlight or grown in a greenhouse. Cyfluthrin was the major component of the [^{14}C] accounting for 61–99% of the TRR at 0–63 days after application. All metabolites identified were present at levels ≤10% TRR in free and conjugated forms (FPBald, FPBalc, FPBacid, Me-FPBacid, OH-FPBacid). Little translocation was observed when individual leaves or bolls were treated. Degradation of cyfluthrin was greater for plants exposed to field conditions than those grown in the glasshouse.

The metabolism of [phenyl-UL-^{14}C]cyfluthrin was also studied in wheat. In forage, straw and heads at up to 21 days after application, 51–69% of [^{14}C] residues were identified as cyfluthrin. Minor metabolites were present at ≤ 5% TRR and occurred in both free and conjugated forms (COOH-cyfluthrin, FPBacid, OH-FPBacid). In a study where [cyclopropyl-1-^{14}C]cyfluthrin and [phenyl-UL-^{14}C]cyfluthrin were applied to wheat plants as seven applications with harvest one day after the last application, cyfluthrin was the major component of [^{14}C] in both heads and straw (77–86%). Metabolites were individually < 10% of TRR as would be expected with application of the last spray so close to harvest. Metabolites identified were DCVA, COOH-cyfluthrin, FPBald, FPBacid, FPBalc and OH-FPBacid.

In a study of the degradation of cyfluthrin on stored wheat grain treated with [fluorophenyl-UL-^{14}C]cyfluthrin at 0.3–0.8 mg/kg, the majority of the radioactivity was located on the grain surface and released into rinse solutions. Unchanged cyfluthrin was the major component identified. After 9 months of storage, 79% of the TRR was cyfluthrin with a further 1.9% identified as FPBald and 0.8% as FPBalc.

Metabolism studies in apples, tomato, cotton, potatoes, soya beans and wheat demonstrated that cyfluthrin was slowly degraded and that the degradation pattern was similar in all crops. The major identified products of cyfluthrin metabolism in plants are analogous to those in mammals. The proposed degradation pathway consists of epimerisation, hydrolysis, ester cleavage, reduction, oxidation and hydroxylation. Cyfluthrin is not systemic, with only limited translocation in plants.

Environmental fate in soil

The half-life for degradation of cyfluthrin in soil is estimated to be < 6 months. Degradation occurred via ester hydrolysis followed by oxidation and mineralisation to $[^{14}C]O_2$.

Photodegradation on soil surfaces is fast with half-lives for degradation of cyfluthrin that are < 20 days. During irradiation with artificial or natural sunlight cyfluthrin in soils underwent ester hydrolysis with FPBald, FPBacid and DCVA identified as degradates.

Hydrolysis in water is pH dependent. Cyfluthrin is considered stable at pH 4 and 7 but is rapidly hydrolysed at pH 9 with a half-life of < 2 days. Two degradation products were identified, FPBald and traces of DCVA, presumably formed from cyfluthrin on hydrolysis of the ester. Abiotic hydrolysis is unlikely to contribute significantly to the degradation of cyfluthrin residues in aquatic systems unless the pH is high.

In confined and field rotational crop studies, no significant residues of cyfluthrin (< 0.01 mg/kg) were found in any crop material. It is concluded that succeeding or rotational crops are unlikely to contain significant residues of cyfluthrin.

Analytical methods

Several different analytical methods have been reported for the analysis of cyfluthrin (and isomers) in plant material and animal commodities. The basic approach involves extraction by homogenisation with an organic solvent mixture incorporating varying proportions of polar and non-polar solvents depending upon the nature of the matrix being extracted and its water content. In general, a primary liquid – liquid partition follows extraction to transfer cyfluthrin residues to less polar solvents prior to column clean-up. Residues are finally determined by gas chromatography with electron capture or mass spectra detectors. In a small number of the methods the four pairs of diastereoisomeric enantiomers that make up cyfluthrin were resolved.

The methods for cyfluthrin and beta-cyfluthrin have been extensively validated with numerous recoveries on a wide range of substrates with LOQs typically in the range 0.01 to 0.05 mg/kg.

Stability of pesticide residues in stored analytical samples

Freezer storage stability was tested for a range of representative substrates. Residues of cyfluthrin were generally stable in crops and their processed products.

Cyfluthrin was stable in homogenized samples fortified at 1 mg/kg and stored frozen for at least 1118 days for apple, 1145 days for cantaloupe, 1130 days for corn, 1145 days for corn oil, 1125 days for corn starch, 1145 days for cucumber, 739 days for oranges, 1145 days for orange juice, 739 days for orange pulp, 1145 days for peanut shells, 1126 days for potatoes, 1130 days for potato chips, 1126 days for potato granules, 95 days for potato peel (wet), 1146 days for potato peel (dry), 102 days for rice, 207 days for rice hulls, 1155 for sugar cane stalks, 1125 days for molasses, 1151 days for tomatoes, 1130 days for wheat and for wheat bran, 1118 days for wheat flour and 1126 days for wheat dust. Incurred cyfluthrin residues were stable in bovine muscle, fat, milk and kidney tissues for at least 6 months and liver for at least 1 month. Fortified liver samples were stable on freezer storage for at least 1 year.

Residue definition

The residue following use of cyfluthrin on crops is predominantly cyfluthrin. Methods are available that can measure cyfluthrin however they do not generally resolve the individual diastereoisomers.

The ratio of cyfluthrin to major metabolites differed in the lactating cow metabolism and feeding studies. In the feeding study, cyfluthrin is the major component of the residue in edible animal commodities, tissues, milk and eggs. Major metabolites derived from hydrolysis of the ester are

DCVA and FPBald, FPBacid and FPBalc. None of these metabolites are unique to cyfluthrin. DCVA is a metabolite common to permethrin, cypermethrin and cyfluthrin while metabolites derived from FPBald are common to cyfluthrin and flumethrin. Separate methods are required for measuring the metabolites. The metabolites were not identified in the evaluation of the toxicological data for cyfluthrin and cypermethrin by the 2006 JMPR as being of toxicological concern.

No metabolism studies were available that specifically used beta-cyfluthrin however the data for cyfluthrin can be used to support beta-cyfluthrin. The residue following its use on crops is predominantly cyfluthrin. Methods are available that can measure both cyfluthrin and beta-cyfluthrin however they do not generally resolve the individual diastereoisomers. Epimerisation of beta-cyfluthrin leads to a change in isomer composition.

Based on the actual residue measured, the Meeting recommended that the residue definition for plant and animal commodities for compliance with MRLs and for estimation of dietary intake should be cyfluthrin. The log K_{ow} of cyfluthrin (pH 6) and the animal metabolism and feeding studies suggest that cyfluthrin should be described as fat-soluble. In the lactating cow metabolism study cyfluthrin residues were approximately 10 times greater in fat than muscle.

Definition of the residue (for compliance with MRL and for estimation of dietary intake) for plant and animal commodities: cyfluthrin (sum of isomers).

The residue is fat-soluble.

Results of supervised residue trials on crops

Supervised trials were available for the use of cyfluthrin on numerous crops: apples, pears, Brassica vegetables (broccoli, Brussels sprouts, cabbage, cauliflower and Chinese cabbage), cotton, oranges, grapefruit, lemons, peppers, potatoes, rape, soya beans, sunflower, sweet corn, mangoes and tomatoes. Specific supervised trials based on unvalidated analytical data (from Craven Laboratories) could not be considered further for sweet corn and soy beans.

Supervised trials were also available for the use of beta-cyfluthrin on several crops: mango, cabbage, cotton, soya beans and rape.

Trial data or relevant GAP was not submitted for maize for which there is a current recommendation for a maximum residue level. The Meeting agreed to withdraw its previous maximum residue level recommendation of 0.05 mg/kg for maize.

Citrus (cyfluthrin)

Cyfluthrin is registered in the USA for use on citrus fruits at 28–112 g ai/ha, PHI 0 days with a maximum seasonal application of 112 g ai/ha and no more than 112 g ai/ha to be applied in a seven day period. Trials conducted in the USA that approximated GAP were often conducted such that more than one plot was treated per trial location. Often treatments involved high and low spray volumes and in some cases different formulations (EC and WP). The Meeting decided that for the purposes of estimation of maximum residue levels that only one result per trial location be used. Seven trials from the USA on grapefruit were selected as complying with US GAP. Residues in whole fruit (n=7) were 0.02, 0.02, 0.03, 0.04, 0.04, 0.07 and 0.11 mg/kg. Residues in oranges from US trials conducted according to GAP (n=7) were 0.03, 0.05, 0.05, 0.05, 0.06, 0.06 and 0.2 mg/kg. Residues in lemons from US trials conducted according to GAP (n=5) were 0.08, 0.08, 0.10, 0.10 and 0.11 mg/kg.

The Meeting decided to combine the trials in the various citrus fruit for the purposes of estimating a maximum residue level and STMR. Residues in rank order are: 0.02, 0.02, 0.03, 0.03, 0.04, 0.04, 0.05, 0.05, 0.05, 0.06, 0.06, 0.07, 0.08, 0.08, 0.10, 0.10, 0.11, 0.11 and 0.2 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in citrus whole fruit of 0.3, 0.06 and 0.2 mg/kg respectively.

Apples and pears (cyfluthrin)

Data were available from supervised trials on apples in the USA (GAP: 25–49 g ai/ha, PHI 7 days with a maximum seasonal application of 49 g ai/ha and no more than 49 g ai/ha to be applied in a fourteen day period). Residues of cyfluthrin from twelve trials in USA at 49 g ai/ha with a PHI of 7 days were 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.03, 0.03, 0.03, 0.04 and 0.06 mg/kg.

Data were available from supervised trials on pears in the USA (GAP: 25–49 g ai/ha, PHI 7 days with a maximum seasonal application of 49 g ai/ha and no more than 49 g ai/ha to be applied in a fourteen day period). Residues of cyfluthrin from six trials in USA at 49 g ai/ha with a PHI of 7 days were 0.02, 0.02, 0.02, 0.02, 0.04 and 0.05 mg/kg.

The Meeting noted that the use patterns for apple and pears in the USA were the same and that the residues populations for each crop could be used to support the other. Therefore the Meeting decided to combine the data for apples and pears to increase the database for the purposes of estimating a maximum residue level, STMR and HR but to make separate recommendations as a general pome fruit use pattern does not exist in the USA.

Residues in rank order (n=18), median underlined, were: 0.01, 0.01, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.02, 0.03, 0.03, 0.03, 0.04, 0.04, 0.05 and 0.06 mg/kg.

The Meeting estimated maximum residue levels, STMR values and HR values for cyfluthrin in apples and pears of 0.1, 0.02 and 0.06 mg/kg respectively. The Meeting agreed to withdraw its previous recommendation of 0.5 mg/kg for apples.

Mangoes - cyfluthrin

Results from three supervised trials on mangoes conducted in the Philippines were made available to the Meeting. One trial was conducted using cyfluthrin and two with beta-cyfluthrin.

One cyfluthrin trial matched GAP for the Philippines (2.5 g ai/hL, PHI 14 days) with residues of 0.02 mg/kg in whole fruit. The Meeting considered a single trial insufficient to estimate a maximum residue level for cyfluthrin in mangoes.

Mangoes (beta-cyfluthrin)

Results from two supervised trials on mangoes conducted in the Philippines were made available to the Meeting. One beta-cyfluthrin residue trial matched GAP of the Philippines (10 g ai/hL, PHI 28 days) with residues in fruit of < 0.01 mg/kg. The Meeting considered one trial insufficient to estimate a maximum residue level.

Brassica vegetables (cyfluthrin)

Cyfluthrin is registered in the USA for use on Brassica vegetables at 15–56 g ai/ha, PHI of 0 days and a maximum application per season of 224 g ai/ha and a maximum of 56 g ai/ha in a 7 day period.

Trials were available from USA of Brussels sprouts approximating GAP with residues of 0.39 and 0.44 mg/kg. The Meeting considered two trials are not sufficient to recommend a maximum residue level.

Thirteen trials approximating GAP were available for broccoli: 0.04, 0.05, 0.19, 0.19, 0.19, 0.19, 0.20, 0.26, 0.28, 0.29, 0.30, 0.46 and 1.5 mg/kg. The Meeting estimated a maximum residue level of 2 mg/kg, STMP of 0.20 mg/kg and an HR of 1.5 mg/kg for residues of cyfluthrin in broccoli.

Six trials on cauliflower that matched GAP of the USA were: < 0.01, 0.11, 0.17, 0.31, 0.32 and 0.91 mg/kg. The Meeting estimated a maximum residue level of 2 mg/kg, STMR of 0.24 mg/kg and HR of 0.91 mg/kg for cauliflower.

In eighteen trials on cabbage from the USA that matched GAP residues were: 0.01, 0.03, 0.03, 0.06, 0.07, 0.10, 0.10, 0.18, 0.24, 0.25, 0.33, 0.42, 0.58, 0.62, 1.0, 1.2, 1.3 and 2.1 mg/kg for cabbage. The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in cabbages of 4, 0.25 and 2.1 mg/kg respectively.

Cabbage (beta-cyfluthrin)

Results from four supervised trials on cabbage conducted in Germany (no GAP) were made available to the Meeting. The Meeting decided to evaluate the German trials against the GAP of Sweden (10 g ai/ha, PHI 7 days). Four trials matched the GAP of Sweden with beta-cyfluthrin residues of < 0.01, < 0.01, 0.06 and 0.08 mg/kg.

Tomatoes (cyfluthrin)

Trials on tomatoes were reported from the USA (GAP: 28–49 g ai/ha, PHI of 0 days and a maximum application per season of 295 g ai/ha and a maximum of 49 g ai/ha in a 7 day period). All trials were for field grown tomatoes with no data for tomatoes grown under protective cover.

Cyfluthrin residues in eleven trials from the USA matching GAP in rank order were (median underlined): < 0.01, 0.01, 0.02, 0.06, 0.07, 0.07, 0.07, 0.08, 0.08, 0.09 and 0.10 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in tomatoes of 0.2, 0.07 and 0.10 mg/kg respectively. The recommendation replaces the previous recommendation of 0.5 mg/kg for tomatoes.

Peppers (cyfluthrin)

Trials on peppers were reported from the USA (GAP: 28–49 g ai/ha, PHI of 7 days and a maximum application per season of 295 g ai/ha and a maximum of 49 g ai/ha in a 7 day period). All trials were for field grown peppers (including chilli) with no data for peppers grown under protective cover.

The Meeting agreed to combine the three trials on chilli peppers (0.06, 0.08, 0.08 mg/kg) with the six trials on sweet peppers (0.01, 0.01, 0.05, 0.06, 0.12 and 0.12 mg/kg) matching GAP in the USA. Residues matching GAP in rank order were (median underlined): 0.01, 0.01, 0.05, 0.06, 0.06, 0.08, 0.08, 0.12 and 0.12 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in peppers of 0.2, 0.06 and 0.12 mg/kg respectively. The recommendation for peppers replaces the previous recommendation of 0.2 mg/kg for peppers sweet.

Egg plant (cyfluthrin)

The Meeting noted that the registered use of cyfluthrin in the USA also includes egg plant (GAP: 28–49 g ai/ha, PHI of 7 days and a maximum application per season of 295 g ai/ha and a maximum of 49 g ai/ha in a 7 day period). The meeting considered the results from the trials conducted on peppers and tomatoes that comply with GAP for egg plants could be extrapolated to egg plants for the purposes of estimating maximum residue, STMR and HR levels. Residues on tomatoes that matched GAP for egg plants were < 0.01, 0.01, 0.02, 0.02, 0.03, 0.03, 0.04, 0.04, 0.04, 0.05, 0.05, 0.05, 0.05, 0.05, 0.06, 0.08 and 0.09 mg/kg. Residues on peppers that matched GAP for egg plants were 0.01, 0.01, 0.05, 0.06, 0.06, 0.08, 0.08, 0.12 and 0.12 mg/kg. The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in egg plant of 0.2, 0.05 and 0.12 mg/kg respectively.

Sweet corn (cyfluthrin)

Trials on sweet corn were reported from the USA (GAP: 15–49 g ai/ha, PHI of 0 days and a maximum application per season of 493 g ai/ha and a maximum of 49 g ai/ha in a 2 day period).

Cyfluthrin residues in three trials from the USA matching GAP in rank order were (median underlined): < 0.01 (2) and 0.01 mg/kg.

The Meeting considered three trials insufficient to estimate a maximum residue level for cyfluthrin in sweet corn.

Potatoes (cyfluthrin)

Trials on potatoes were reported from Canada (no GAP) and the USA (GAP: 15–49 g ai/ha, PHI of 0 days and a maximum application per season of 295 g ai/ha and a maximum of 49 g ai/ha in a 7 day period).

Cyfluthrin residues in seventeen trials from the USA matching GAP in rank order were (median underlined): < 0.01 (17) mg/kg. Residues were not detected in residue trials and metabolism results on plants including potatoes confirm that cyfluthrin is not translocated by plants. The Meeting considered detectable residues in potato tubers to be unlikely.

The Meeting estimated a maximum residue level, an STMR value and an HR value for cyfluthrin in potatoes of 0.01*, 0 and 0 mg/kg respectively.

Soya beans (cyfluthrin)

Trials on soya beans were reported from the USA (GAP: 15–49 g ai/ha, PHI of 45 days and a maximum application per season of 196 g ai/ha and a maximum of 49 g ai/ha in a 7 day period).

Cyfluthrin residues in five trials from the USA matching GAP in rank order were (median underlined): < 0.01 (5) mg/kg. In addition, residues ranging from < 0.01 to 0.02 mg/kg were reported in unvalidated trials.

The Meeting considered five trials insufficient to estimate a maximum residue level for cyfluthrin in soya beans (dry).

Soya beans (beta-cyfluthrin)

Four trials on soya beans employing beta-cyfluthrin were reported from Brazil (12.5 g ai/ha, PHI 21 days) that complied with GAP for Brazil. Residues were < 0.01 and < 0.05 (3) mg/kg. The Meeting considered four trials on soya beans insufficient to estimate a maximum residue level for residues arising from the use of beta-cyfluthrin in soya beans.

Cotton seed (cyfluthrin)

Trials on cotton were reported from the USA (GAP: 15–49 g ai/ha, PHI of 0 days and a maximum application per season of 560 g ai/ha and a maximum of 56 g ai/ha in a 3 day period).

Cyfluthrin residues in seven trials from the USA matching GAP in rank order were (median underlined): < 0.01, 0.02, 0.03, < 0.1, < 0.1, 0.1 and 0.52 mg/kg.

The Meeting estimated a maximum residue level and an STMR value for cyfluthrin in cotton seed of 0.7 and 0.1 mg/kg respectively. The recommendation for cotton seed replaces the previous recommendation of 0.05 mg/kg.

Cotton seed (beta-cyfluthrin)

Beta-cyfluthrin trials on cotton were reported from the USA (GAP: 7–28 g ai/ha, PHI of 0 days and a maximum application per season of 280 g ai/ha and a maximum of 28 g ai/ha in a 3 day period). Beta-cyfluthrin residues in three trials from the USA matching GAP in rank order were: < 0.1, < 0.1 and 0.38 mg/kg.

Rape seed (cyfluthrin)

Cyfluthrin trials on rape were reported from Germany (no GAP). The Meeting decided to assess the German trials against the GAP of Belgium (15 g ai/ha, application according to growth stage, maximum 2 applications per crop, one spray from seed to 3 leaf BBCH 10–13, one at bud development BBCH 50–59 and one at pod development BBCH 70-75). Seven trials matched GAP of Belgium with residues of < 0.05 (6) and 0.05 mg/kg.

The Meeting estimated a maximum residue level and an STMR value for cyfluthrin in rape seed of 0.07, 0.05 and < 0.05 mg/kg respectively. The recommendation for rape seed replaces the previous recommendation of 0.05 mg/kg.

Rape seed (beta-cyfluthrin)

Trials conducted on rape using beta-cyfluthrin trials were reported from the Germany (GAP: 5.2–7.7 g ai/ha, 0.2-0.3 g ai/hL, PHI of 56 days). Beta-cyfluthrin residues in nine trials from the Germany matching GAP in rank order were (median underlined): < 0.01, < 0.01, 0.01, < 0.02 (4), < 0.05, and < 0.05 mg/kg.

Sunflower seed (cyfluthrin)

Trials on sunflower were reported from Canada (no GAP) and the USA (GAP: 15–49 g ai/ha, PHI of 30 days and a maximum application per season of 147 g ai/ha and a maximum of 49 g ai/ha in a 7 day period).

Cyfluthrin residues in five trials from Canada and the USA matching GAP of the USA in rank order were (median underlined): < 0.01 (3) and 0.01 (2) mg/kg.

The Meeting considered five trials insufficient to estimate a maximum residue level for cyfluthrin in sunflower seed.

Animal feedstuffs

Sweet corn forage (cyfluthrin)

Field trials on sweet corn were made available to the Meeting from the USA (GAP: 15–49 g ai/ha, PHI of 0 days and a maximum application per season of 493 g ai/ha and a maximum of 49 g ai/ha in a 2 day period).

Residues on sweet corn forage were 3.7, 3.7 and 7.7 mg/kg (fresh weight basis).

Residues on sweet corn cannery waste were 0.20, 0.43 and 0.90 mg/kg (fresh weight basis).

The Meeting considered three trials insufficient to estimate median and high residues for sweet corn livestock feeds.

Cotton gin-trash (cyfluthrin)

Cyfluthrin field trials on cotton were made available to the Meeting from the USA (GAP: 15–49 g ai/ha, PHI of 0 days and a maximum application per season of 560 g ai/ha and a maximum of 56 g ai/ha in a 3 day period; Do not graze treated fields).

Cyfluthrin residues on cotton gin-trash were 2.4, 2.8 and 9.2 mg/kg (fresh weight basis). The Meeting considered three trials insufficient to estimate median residues for cotton gin-trash as a livestock feed.

Cotton gin-trash (beta-cyfluthrin)

Beta-cyfluthrin field trials on cotton were made available to the Meeting from the USA (GAP: 7–28 g ai/ha, PHI of 0 days and a maximum application per season of 280 g ai/ha and a maximum of 28 g ai/ha in a 3 day period; Do not graze treated fields).

Beta-cyfluthrin residues on cotton gin-trash were 2.3, 2.6, 2.9 mg/kg (fresh weight basis). The Meeting considered three trials insufficient to estimate median residues for cotton gin-trash as a livestock feed.

Rape forage and straw (cyfluthrin)

Field trials on rape seed were made available to the Meeting from the Germany (GAP: 15 g ai/ha, application according to growth stage, maximum 2 applications per crop, one spray from seed to 3 leaf BBCH 10–13, one at bud development BBCH 50-59 and one at pod development BBCH 70–75).

Residues on rape straw were < 0.02, < 0.02, < 0.02, < 0.02, < 0.02 and 0.06 mg/kg. The Meeting estimated an STMR and a high residue value for cyfluthrin in rape straw of < 0.02 and 0.06 mg/kg, respectively, both on an as received basis.

As the registered use pattern for rape in Germany does not restrict grazing, the Meeting assumed that according to GAP in Germany rape could be grazed at the earliest time after application. Residues on rape forage were 0.13, 0.15, 0.18, 0.20, 0.21, 0.27, 0.32 and 0.34 mg/kg (fresh weight basis). The Meeting estimated an STMR and a high residue value for cyfluthrin in forage of 0.205 and 0.34 mg/kg, respectively, both on a fresh weight basis.

Rape fodder (beta-cyfluthrin)

For beta-cyfluthrin trials on rape were reported from Germany (GAP: 5.2–7.7 g ai/ha, 0.2–0.3 g ai/hL, PHI of 56 days). Beta-cyfluthrin residues from rape straw in seven trials from Germany matching GAP in rank order were (median underlined): < 0.05, < 0.05, < 0.05, 0.02, 0.06, 0.07, 0.08 mg/kg (fresh weight basis). As the directions for use in Germany do not provide specific guidance for livestock feeding it is assumed forage rape can be grazed without restriction anytime after application. Residues in rape forage at 0 days after application were: < 0.05, 0.08, 0.16, 0.17, 0.19, 0.24, 0.26, 0.27 and 0.33 mg/kg. The Meeting estimated an STMR and a high residue value for cyfluthrin in rape forage of 0.19 and 0.33 mg/kg, respectively, both on a fresh weight basis.

Soya bean forage and vines (cyfluthrin)

Field trials on soya beans were made available to the Meeting from the USA (GAP: 15–49 g ai/ha, PHI of 45 days and a maximum application per season of 196 g ai/ha and a maximum of 49 g ai/ha in a 7 day period; dry vines and green forage may be fed 45 and 15 days, respectively after last application).

Residues on soya bean forage were 0.10, 0.26, 0.33, 0.34, 0.38, 0.45, 0.96 and 3.3 mg/kg (fresh weight basis). The Meeting estimated an STMR and a high residue value for cyfluthrin in soya bean forage of 0.36 and 3.3 mg/kg, respectively, both on a fresh weight basis.

Residues on soya bean dry vines were 0.01, 0.09, 0.21, 0.31 and 2.66 mg/kg (fresh weight basis). The Meeting considered five trials insufficient to estimate median and high residues for soya vines that may be used as livestock feed.

Sunflower fodder (cyfluthrin)

Trials on sunflowers were reported from Canada (no GAP) and the USA (GAP: 15–49 g ai/ha, PHI of 30 days and a maximum application per season of 147 g ai/ha and a maximum of 49 g ai/ha in a 7 day period; pre-grazing or foraging interval, 30 days).

Cyfluthrin residues in sunflower fodder in five trials from Canada and the USA matching GAP of the USA were 0.04, 0.13, 0.30, 0.33 and 0.63 mg/kg (fresh weight basis). The Meeting estimated an STMR and a high residue value for cyfluthrin in sunflower fodder of 0.30 and 0.63 mg/kg, respectively, both on a fresh weight basis.

Fate of residues during processing

The fate of cyfluthrin residues has been examined in potato, cabbage, tomato, citrus fruit, apples and oil seed crops processing studies. Processing of tomatoes into pulp and paste showed a slight increase of cyfluthrin residues in the processed commodities compared to the RAC. Whilst there was a decrease in residues found in the corresponding juice, ketchup and purée. Citrus and apples also both showed a decrease in residues found in the juice, but a slight increase in pomace and/or oil and molasses. There was a concentration into the oil of cottonseed and sunflower. Processing studies on potatoes, cabbages, soya bean and rape seed did not show any indication regarding the fate of beta-cyfluthrin/cyfluthrin residues during processing as residues in the RAC or processed products were all below the LOQ. Estimated processing factors, HRs and STMRs are summarized below.

Summary of processing factors for cyfluthrin residues.

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors	PF (Mean, median or best estimate)	RAC-STMR	RAC-STMR×PF
Orange	Pulp dry	5.3	5.3	0.06	0.318
Apple	Pomace, dry	0.11, 16	16	0.02	0.32
Cotton	Hulls	1.9	1.9	0.1	0.19
Cotton	Meal	0.08	0.08		0.008
Cotton	Oil, crude	1.9	1.9		0.19
Cotton	Oil, refined	1.2	1.2		0.12

The Meeting decided to make maximum residue level recommendations for citrus pulp (dry) and cotton seed hulls. Based on an estimated high residue value of 1.06 mg/kg (5.3 × 0.2 mg/kg) for citrus pulp (dry), the meeting recommended a maximum residue level of 2 mg/kg for citrus pulp (dry). The Meeting also recommended a maximum residue level of 1 mg/kg for cotton seed oil, crude based on an estimated high residue of 0.988 mg/kg (1.9 × 0.22 mg/kg).

The Meeting also decided to use the default generic processing factor of 7 to estimate a maximum residue level for chilli pepper (dry) of 1 mg/kg based on an HR-P of 0.84 mg/kg (7 × 0.12) and STMR-P of 0.42 mg/kg (7 × 0.06).

Residues in animal commodities

Farm animal dietary burden

The Meeting estimated the dietary burden of cyfluthrin in farm animals on the basis of the diets listed in Annex 6 of the 2006 JMPR Report (OECD Feedstuffs Derived from Field Crops). Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities. The percentage dry matter is taken as 100% when the highest residue levels and STMRs are already expressed as dry weight.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6. The calculations were made according to the animal diets from US-Canada, EU and Australia in the OECD Table (Annex 6 of the 2006 JMPR Report).

	Animal dietary burden, cyfluthrin, ppm of dry matter diet					
	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	1.87	0.31	3.00	0.49	5.89 [1]	0.68 [3]
Dairy cattle	1.84	0.26	3.00 [2]	0.49 [4]	2.47	0.36
Poultry - broiler	0.009	0.009	0.0152	0.015	0.003	0.003
Poultry - layer	0.009	0.009	1.3 [5]	0.16 [6]	0.003	0.003

[1] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat

[2].Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk

[3]Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[4] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[5]Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs.

[6]Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs.

The cyfluthrin dietary burdens for animal commodity MRL and STMR estimation (residue levels in animal feeds expressed on dry weight) are: beef cattle 5.89 and 0.68 ppm, dairy cattle 3.06 and 0.50 ppm and poultry 1.3 and 0.16 ppm.

Farm animal feeding studies

The Meeting received information on the residue levels arising in animal tissues and milk when dairy cows were dosed with cyfluthrin for 28 days at the equivalent of 4.5, 13 and 40 ppm in the diet. Average residues in milk of the 40 ppm dose group were 0.22 mg/kg at day 14 and 0.14 mg/kg at day 28. Cyfluthrin residues in the fat were higher than in other tissues. Transfer factors (average residue level in tissue ÷ residue level in feed) for each tissue and milk for the three dosing levels (3 animals per dose group) were: fat, 0.056, 0.054, 0.066; muscle, < 0.002, < 0.001, 0.00075; kidney, 0.0042; liver, 0.0032; milk 28 days, 0.0037, 0.0036, 0.0036.

In an additional dosing study conducted at levels equivalent to 11, 36 and 112 ppm in the diet average residues in milk at day 28 were 0.45 mg/kg for the 112 ppm dose group. As for the previous study, residues were highest in fat with only low levels of cyfluthrin detected in other tissues. Transfer factors for each tissue and milk for the three dosing levels (3 animals per dose group) were: fat, 0.11, 0.074, 0.061; muscle, < 0.0009, 0.001, 0.00063; kidney, < 0.0009, < 0.0008, 0.00045; liver, < 0.0036, < 0.00028, 0.00018; milk 28 days, 0.0055, 0.0033, 0.0041.

The Meeting also received information on the residue levels arising in tissues and eggs when laying hens were dosed with cyfluthrin for 28 days at the equivalent of 6 and 20 ppm in the diet. Residues in eggs were below the LOQ for both feed levels. At the 2 ppm feeding level the residues in tissues were below the LOQ of the analytical methods. For the 20 ppm feed level, residues in fat were substantially higher than residues in other tissues 0.05 mg/kg compared to < 0.01-0.01 mg/kg. Transfer factors based on residues for fat were 0.0025 for the 20 ppm feed levels. Transfer factors (mean residue) for muscle and liver were < 0.0005 and < 0.0005 respectively for the 20 ppm feeding level while that for skin was 0.0005.

Farm animal direct treatment

No studies were received on the residues of cyfluthrin arising from direct animal treatment. The Meeting noted that JECFA has evaluated cyfluthrin residues arising from direct animal treatment at its 48th Meeting in 1997 and recommended maximum residue limits for cattle of 20 μg/kg for muscle, liver and kidney, 40 μg/kg for milk and 200 μg/kg for fat. The marker residue that applied to the residue limits was cyfluthrin.

Animal commodity maximum residue levels

The maximum dietary burden for beef and dairy cattle is 5.89 and 3.06 ppm respectively, so the levels of residues in tissues can be obtained by interpolation between the high residues obtained in tissues and at the 4.5 and 13 ppm feeding levels for milk, muscle and fat and from the 40 ppm feed level for kidney and liver as these are the only kidney and liver samples subjected to strong extraction required to release the majority of cyfluthrin residues. Maximum residues expected in tissues are: fat 0.37 mg/kg, muscle < 0.01 mg/kg, liver 0.021 mg/kg, kidney 0.027 mg/kg and the mean residue for milk 0.0136 mg/kg. No data was available on the partitioning of residues in milk between aqueous and fat phases of milk.

The Meeting estimated maximum residue levels for meat (from mammals other than marine mammals) 1 mg/kg (fat); kidney of cattle, goats, pigs and sheep 0.05 mg/kg; liver of cattle, goats, pigs and sheep 0.05 mg/kg and milks 0.04 mg/kg. The recommendation of 0.04 mg/kg milk replaces the previous recommendation of ML 0812 Cattle milk 0.01 F mg/kg, which also incorporated direct animal treatment. The Meeting noted the recommendation for cattle milk arising from exposure to cyfluthrin through the cattle diet is the same as proposed by JECFA for direct animal treatment.

The STMR dietary burdens for beef and dairy cattle are 0.68 and 0.50 ppm respectively. Transfer factors from the average residues from the 4.5 ppm feeding level were used to estimate STMR values as for cyfluthrin. The estimated STMRs are: meat (from mammals other than marine mammals) < 0.01 mg/kg, fat (from mammals other than marine mammals) 0.0378 mg/kg, kidney of cattle, goats, pigs and sheep < 0.01 mg/kg, liver of cattle, goats, pigs and sheep < 0.01 mg/kg and milks 0.0027 mg/kg.

The highest individual tissue residue from the relevant feeding group was used in conjunction with the highest residue dietary burden to calculate the likely highest animal commodity residue level. As only a single animal is available per feeding group, the tissue residues from the animals in the relevant feeding groups were used in conjunction with the STMR dietary burden to estimate the animal commodity STMR values. For milk, the mean milk residue at the plateau level from the relevant feeding group was used to estimate both the maximum residue level and the STMR.

Dietary burden (mg/kg)[1] Feeding level [ppm][2]		Cyfluthrin residues, mg/kg[3]								
		Milk	Fat		Muscle		Liver		Kidney	
		Mean	High	mean	High	mean	high	mean	High	mean
MRL beef	(5.89) [4.5] high		*(0.37)* 0.30		*(< 0.01)* < 0.01		*(0.021)* 0.14[4]		*(0.027)* 0.18[4]	
MRL dairy	(3.06) [4.5] av	*(0.0136)* 0.02								
STMR beef	(0.68) [4.5] av		*(0.0378)* 0.25		*(< 0.01)* < 0.01		*(< 0.01)* < 0.01		*(< 0.01)* < 0.01	
STMR dairy	(0.50) [4.5] av	*(0.0022)* 0.02								

[1] Values in parentheses are the estimated dietary burdens

[2] Values in square brackets are the actual feeding levels in the transfer study

[3] Residue values in parentheses in italics are interpolated from the dietary burden, feeding levels in the transfer study and the residues found in the transfer study. High is the highest individual animal tissue residue in the relevant feeding group. Mean is mean animal tissue (or milk) residue in the relevant feeding group.

[4]Residue values for kidney and liver were obtained from the dosing level equivalent to 40 ppm in the feed as only these samples were subject to reanalysis using a stronger extraction process

The maximum dietary burden for poultry is 1.3 ppm. No residues above the LOQ of the analytical method used were observed in the feeding study for laying hens at the lowest dose level equivalent to 2 ppm in the diet. Maximum residues expected are: muscle, fat, liver, kidney and eggs are all < 0.01 mg/kg.

The Meeting estimated maximum residue levels for poultry meat 0.01(*) mg/kg (fat); poultry offal 0.01(*) and eggs 0.01 (*) mg/kg.

As no residues are observed at the maximum feeding level for poultry, the STMRs for poultry meat, edible offal and eggs are the same as the maximum residue levels.

DIETARY RISK ASSESSMENT

Long-term intake

The evaluation of cyfluthrin has resulted in recommendations for MRLs and STMRs for raw and processed commodities. Consumption data were available for 22 food commodities and were used in the dietary intake calculation. The results are shown in Annex 3.

The International Estimated Daily Intakes for the 13 GEMS/Food regional diets, based on estimated STMRs were in the range 0–2% of the maximum ADI of 0.04 mg/kg bw (Annex 3). The Meeting concluded that the long-term intake of residues of cyfluthrin from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The international estimated short-term intake (IESTI) for cyfluthrin was calculated for the food commodities (and their processing fractions) for which maximum residue levels and HRs were estimated and for which consumption data were available. The results are shown in Annex 4.

For the general population the IESTI varied from 0–120% of the ARfD (0.04 mg/kg bw) while for children the IESTI varied from 0–240% of the ARfD. The IESTI (as a% of the ARfD) for broccoli for children was 120% and 70% for the general population, 240% for head cabbage for children and 100% for the general population.

The Meeting concluded that the short-term intake of residues of cyfluthrin resulting from uses that have been considered by the JMPR, except the uses on broccoli and head cabbage, is unlikely to present a public health concern.

The Meeting noted that no residue data relating to alternative GAP were submitted for broccoli and head cabbage. The information provided to the JMPR precludes an estimate that the dietary intake would be below the ARfD for consumption for broccoli and head cabbage by children.

5.8 LAMBDA-CYHALOTHRIN (146)

TOXICOLOGY

Lambda-cyhalothrin, the ISO approved common name for (*R*)-cyano(3-phenoxyphenyl)methyl (1*S*,3*S*)-rel-3-[(1*Z*)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate is a synthetic cyano-containing type II pyrethroid insecticide (CAS No. 91465-08-6).

Cyhalothrin (CAS No. 68085-85-8) was evaluated by JMPR in 1984, when an ADI of 0–0.02 mg/kg bw was established based on a NOAEL of 20 ppm, equal to 2 mg/kg bw per day, identified on the basis of clinical signs in a 2-year study in mice; a NOAEL of 30 ppm, equal to

1.5 mg/kg bw per day, identified on the basis of decreased body-weight gain in a three-generation study in rats; and a NOAEL of 2.5 mg/kg bw per day, identified on the basis of neurotoxicity in a 6-month study in dogs, and using a safety factor of 100.

At its meeting in 2000, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a temporary ADI of 0–0.002 mg/kg bw based on a LOEL of 1 mg/kg bw per day for induction of liquid faeces in dogs in a 26-week study, and using a safety factor of 500. The high safety factor was used to compensate for the absence of a no-observed-effect level (NOEL) in this study.

At its meeting in 2004, JECFA concluded that the toxicity of cyhalothrin is similar in rats and dogs. The Committee decided that the temporary ADI could be replaced by an ADI of 0–0.005 mg/kg bw, which was determined by dividing the LOEL of 1 mg/kg bw per day in dogs (also the NOEL for rats) by a safety factor of 200. The safety factor incorporated a factor of 2 to compensate for the absence of a NOEL in dogs.

Lambda-cyhalothrin consists of two of the four enantiomers (i.e., the *cis* 1*R*α*S* and *cis* 1*S*α*S* enantiomeric pair) of cyhalothrin. One of the two enantiomers of lambda-cyhalothrin is the insecticidally active gamma-cyhalothrin (CAS No. 76703-62-3). Cyhalothrin comprises about 50% lambda-cyhalothrin.

Lambda-cyhalothrin was evaluated by the present Meeting within the Periodic Re-evaluation Programme of the CCPR. For the present re-evaluation, studies with cyhalothrin and lambda-cyhalothrin were available.

For lambda-cyhalothrin, specifications were established by the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides: lambda-cyhalothrin* [27] (technical material, 2003). For other formulations, specifications also exist.

All pivotal studies with cyhalothrin and lambda-cyhalothrin were certified as being compliant with GLP.

Biochemical aspects

Oral doses of cyhalothrin were readily but incompletely absorbed (30–40% of radiolabel was recovered in urine) in rats and dogs. Peak blood concentrations were reached after 4–7 h. In male rats treated with replacement bile obtained from treatment-naive rats, biliary excretion was about 11%. At a low dose, most (70%) of the administered material was excreted in the faeces and urine within 24 h. After 7 days, 2–3% of the cyhalothrin administered persisted as unchanged residue in fat. Metabolism in rats and dogs was similar, involving initial cleavage of the molecule at the ester bond. In rats dosed with cyhalothrin, major metabolites identified in urine were the sulfate conjugate of 3-(4'-hydroxyphenoxy) benzoic acid (compound XXIII) glucuronide conjugate of (1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanoic acid (i.e., the compound 1a glucuronide). Minor metabolites identified were unconjugated compound XXIII and 3-phenoxybenzoic acid (compound V).

In volunteers given a single dose of lambda-cyhalothrin in capsules, serum and urine contained the metabolites compound XXIII, compound V and compound 1a ((1*RS*)-*cis*-3-(*Z*-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanoic acid, TMFVCA). Their presence suggests that the initial metabolism of this compound in humans is similar to that in rats and dogs.

[27] Available from: http://www.who.int/whopes/quality/en/Lambda-cyhalothrin_eval_specs_WHO_2003.pdf

Toxicological data

The acute oral LD_{50} of lambda-cyhalothrin in rats was 79 mg/kg bw in males and 56 mg/kg bw in females. The observed clinical signs (ataxia, decreased activity, tiptoe gait, splayed gait, loss of stability, dehydration, urinary incontinence, hunched posture, piloerection, salivation, ungroomed appearance and pinched-in sides) were typical of this class of pyrethroids.

In studies with lambda-cyhalothrin in rats, the inhalation LC_{50} value was 60 mg/m^3 (0.06 mg/L), and the dermal LD_{50} was 632 mg/kg bw in males and 696 mg/kg bw in females. Lambda-cyhalothrin was not irritating to the skin and only slightly irritating to the eyes. With respect to dermal sensitization, the results of a maximization test with lambda-cyhalothrin in guinea-pigs were inconclusive. Technical-grade cyhalothrin has been reported to cause skin sensitization in a Buehler test and a maximization test in guinea-pigs.

In a 90-day feeding study in rats given cyhalothrin, the NOAEL was 50 ppm, equal to 2.6 mg/kg bw per day, on the basis of reduced body-weight gain and food consumption. In a 90-day feeding study in rats given lambda-cyhalothrin, the NOAEL was 50 ppm, equivalent to 2.5 mg/kg bw per day, on the basis of reduced body-weight gain and food consumption. In a 26-week study in dogs fed capsules containing cyhalothrin and a 1-year study in dogs fed capsules containing lambda-cyhalothrin, increased incidences of liquid faeces was observed, with an overall NOAEL of 0.1 mg/kg bw per day. The increased incidences of liquid faeces were observed from the first week of treatment. Other pyrethroids produce this effect, which may be the consequence of the local gastrointestinal equivalent of paraesthesia in the skin. In the two studies in dogs, signs of systemic neurotoxicity (ataxia, tremors, and occasionally convulsions) were observed, with an overall NOAEL of 0.5 mg/kg bw per day. Signs of systemic neurotoxicity were observed from the first week and generally occurred within a few hours after treatment.

In a 2-year dietary study with cyhalothrin in mice, the NOAEL was 20 ppm, equal to 1.8 mg/kg bw per day, on the basis of clinical signs (piloerection and hunched posture) in males. An increase in the incidence of mammary adenocarcinomas in the groups receiving the intermediate or highest dose was at the upper limit of the range for historical controls and was not dose-related. The Meeting therefore considered that it was unlikely that these tumours were caused by treatment with cyhalothrin.

In a 2-year dietary study with cyhalothrin in rats, the NOAEL was 50 ppm, equal to 2.3 mg/kg bw per day, on the basis of a reduction in body-weight gain. No treatment-related changes in tumour incidence were observed in this study.

The Meeting concluded that cyhalothrin is not carcinogenic in rodents.

Lambda-cyhalothrin was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test. A number of published studies, largely from the same laboratory, have reported significant increases in DNA damage in vitro (Comet assay) and chromosomal aberrations in vitro and in vivo. The materials tested in these studies were either commercial formulations of unknown composition or were inadequately described. In view of the uniform finding of a lack of genotoxicity in those studies in which lambda-cyhalothrin was adequately characterized, the Meeting concluded that lambda-cyhalothrin is unlikely to be genotoxic.

In view of the lack of genotoxicity of lambda-cyhalothrin and the absence of carcinogenicity shown by cyhalothrin in mice and rats, the Meeting concluded that lambda-cyhalothrin is unlikely to pose a carcinogenic risk to humans.

In a multigeneration dietary study with cyhalothrin in rats, the NOAEL for parental toxicity was 30 ppm, equivalent to 2.0 mg/kg bw per day, on the basis of a reduction in body-weight gain. The NOAEL for offspring toxicity was 30 ppm, equivalent to 2 mg/kg bw per day, on the basis of reduced body-weight gain during lactation. The NOAEL for reproductive toxicity was 100 ppm, equivalent to 6.7 mg/kg bw per day, i.e., the highest dose tested.

The effect of oral exposure to cyhalothrin on prenatal development was investigated in rats and rabbits. In a study of developmental toxicity in rats treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of a reduction in body weight and loss of limb coordination. The NOAEL for foetal toxicity was 15 mg/kg bw per day, i.e., the highest dose tested. In a study of developmental toxicity in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption. The NOAEL for fetotoxicity was 30 mg/kg bw per day, i.e., the highest dose tested.

In a study of acute neurotoxicity in rats given lambda-cyhalothrin by gavage, the NOAEL was 2.5 mg/kg bw per day on the basis of signs of neurotoxicity (increased breathing rate, urinary incontinence, salivation, reduced response to sound).

In a comparative study on the acute effects of pyrethroids in rats treated by oral gavage, in which the data were analysed using a nonlinear exponential threshold model, lambda-cyhalothrin showed decreased motor activity with a benchmark threshold dose (estimate of the highest no-effect level at which the rats would not display any decrease in motor activity) of 0.5 mg/kg bw. In a 90-day dietary study, the NOAEL was 150 ppm (equal to 11 mg/kg bw per day), i.e., the highest dose tested.

In a study of developmental neurotoxicity in rats, the NOAEL for maternal toxicity was 60 ppm, equal to 4.9 mg/kg bw per day, on the basis of reduced body-weight gain during gestation. The NOAEL for offspring toxicity was 60 ppm, equal to 10.7 mg/kg bw per day, based on maternal lambda-cyhalothrin intake, on the basis of reduced body-weight gain during lactation. No evidence for developmental neurotoxicity was observed.

In case reports in humans, no systemic effects were reported. In most cases exposure was by the dermal and inhalation routes. Predominant signs were skin paraesthesia, numbness, irritation of the skin, red eyes, coughing and sneezing.

No toxicological studies on metabolites of cyhalothrin were available. However, the Meeting considered it likely that the metabolites would be less neurotoxic than cyhalothrin, as none contains an intact pyrethroid structure.

The Meeting concluded that the existing database on lambda-cyhalothrin was adequate to characterize the potential hazards to foetuses, infants and children.

Toxicological evaluation

Although increased incidences of liquid faeces were observed in dogs given lambda-cyhalothrin/cyhalothrin, which may represent a consequence of a local gastrointestinal equivalent of paraesthesia in the skin, the Meeting considered that it was not appropriate to base the ADI and ARfD on local effects on the gastrointestinal tract, observed after bolus administration.

The most sensitive systemic effect of lambda-cyhalothrin/cyhalothrin was neurotoxicity (decreased motor activity), which was observed in a study of acute toxicity in rats given lambda-cyhalothrin orally, with a threshold dose of 0.5 mg/kg bw, and in repeat-dose studies with cyhalothrin and lambda-cyhalothrin in dogs treated orally (ataxia, tremors, occasionally convulsions) with a NOAEL of 0.5 mg/kg bw per day. On the basis of these effects, the Meeting established a group ADI for cyhalothrin and lambda cyhalothrin of 0–0.02 mg/kg bw, using a safety factor of 25. Because lambda-cyhalothrin is relatively rapidly absorbed and excreted and the neurotoxic effects are rapidly reversible and dependent on C_{max}, the Meeting considered it appropriate to adjust the safety factor for the reduced variability in C_{max} compared with AUC. The Meeting considered that the ADI of 0.02 mg/kg bw is adequately protective against the other, non-neurotoxic effects of lambda-cyhalothrin/cyhalothrin observed in short- and long-term studies with repeated doses, and in studies of reproductive and developmental toxicity, where the use of a safety factor of 100 would be appropriate.

The Meeting established a group ARfD for cyhalothrin and lambda-cyhalothrin of 0.02 mg/kg bw on the basis of systemic neurotoxicity (decreased motor activity) observed in a study of acute toxicity in rats given lambda-cyhalothrin orally with a threshold dose of 0.5 mg/kg bw per day, and in repeat-dose studies with cyhalothrin and lambda-cyhalothrin in dogs treated orally, in which neurotoxic effects (ataxia, tremors, occasionally convulsions) occurred during the first week, within a few hours after treatment, with an overall NOAEL of 0.5 mg/kg bw per day, and using a safety factor of 25. For the same reasons as described above, the Meeting considered it appropriate to adjust the safety factor for the reduced variability in C_{max} compared with AUC.

A toxicological monograph was prepared.

Levels relevant for risk assessment

(a) Cyhalothrin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity [a]	Toxicity	20 ppm, equal to 1.8 mg/kg bw per day	100 ppm, equal to 9.2 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 51 mg/kg bw per day[c]	—
Rat	Ninety-day study of toxicity[a]	Toxicity	50 ppm, equal to 2.6 mg/kg bw per day	250 ppm, equal to 14 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity [a]	Toxicity	50 ppm, equal to 2.3 mg/kg bw per day	250 ppm, equal to 12 mg/kg bw per day
		Carcinogenicity	250 ppm, equal to 12 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	30 ppm, equivalent to 2.0 mg/kg bw per day	100 ppm, equivalent to 6.7 mg/kg bw per day [d]
		Offspring toxicity	30 ppm, equivalent to 2.0 mg/kg bw per day	100 ppm, equivalent to 6.7 mg/kg bw per day [d]
		Reproductive toxicity	100 ppm, equivalent to 6.7 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	15 mg/kg bw per day
		Fetotoxicity	15 mg/kg bw per day [c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Fetotoxicity	30 mg/kg bw per day [c]	—
Dog	Twenty-six-week study[b]	Toxicity	2.5 mg/kg bw per day	10 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

(b) Lambda-cyhalothrin

Species	Study	Effect	NOAEL	LOAEL
Rat	Ninety-day study of toxicity[a]	Toxicity	50 ppm, equivalent to 2.5 mg/kg bw per day	250 ppm, equivalent to 12.5 mg/kg bw per day
	Acute neurotoxicity[b]	Neurotoxicity	0.5 mg/kg bw [e]	1.3 mg/kg bw [f]

	Ninety-day study of neurotoxicity [a]	Neurotoxicity	150 ppm, equal to 11 mg/kg bw per day [c]	—
	Developmental neurotoxicity [a]	Maternal toxicity	60 ppm, equal to 4.9 mg/kg bw per day	150 ppm, equal to 11.4 mg/kg bw per day
		Offspring toxicity	60 ppm, equivalent to 10.7 mg/kg bw per day [d]	150 ppm, equivalent to 26.3 mg/kg bw per day [d]
		Developmental (neuro)-toxicity	150 ppm, equivalent to 11.4 mg/kg bw per day[c]	—
Dog	One-year study[b]	(Neuro)toxicity	0.5 mg/kg bw per day	3.5 mg/kg bw per day

[a] Dietary administration.

[b] Gavage administration.

[c] Highest dose tested.

[d] Based on maternal intake of lambda-cyhalothrin during lactation.

[e] Threshold dose obtained using a nonlinear exponential threshold model.

[f] ED_{30} (dose associated with a 30% decrease in motor activity) obtained using a nonlinear exponential threshold model.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.02 mg/kg bw

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

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

Critical end-points for setting guidance values for exposure to cyhalothrin/lambda-cyhalothrin

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of absorption	Rapid, incomplete absorption (about 40–50% in rats)
Distribution	Highest concentrations in fat, followed by liver and kidney (rats)
Potential for accumulation	Low
Rate and extent of excretion	Rapid (70% in faeces and urine within 24 h in rats)
Metabolism in animals	Sulfate conjugate of 3-(4'-hydroxyphenoxy) benzoic acid (compound XXIII) and glucuronide conjugate of (1RS)-cis-3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropane carboxylic acid. Unconjugated compound XXIII and 3-phenoxybenzoic acid (compound V) were minor metabolites.
Toxicologically significant compounds in animals, plants and the environment	Cyhalothrin, lambda-cyhalothrin
Acute toxicity	
Rat, LD_{50}, oral	56 mg/kg bw
Rat, LD_{50}, dermal	632 mg/kg bw
Rat, LC_{50}, inhalation	0.060 mg/L
Rabbit, skin irritation	Not an irritant (cyhalothrin)

Rabbit, eye irritation	Slightly irritating (lambda-cyhalothrin)
Guinea-pig, skin sensitization	Sensitizing (cyhalothrin, Buehler test and Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Neurotoxicity, i.e., ataxia, tremors, occasionally convulsions (dogs)
Lowest relevant oral NOAEL	0.5 mg/kg bw per day (lambda-cyhalothrin, dogs)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Decreased body weight gain (rats)
Lowest relevant NOAEL	50 ppm, equal to 2.3 mg/kg bw per day (cyhalothrin, rats)
Carcinogenicity	Not carcinogenic (cyhalothrin, mice, rats)
Genotoxicity	
	Not genotoxic (lambda-cyhalothrin)
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects (rats)
Lowest relevant reproductive NOAEL	100 ppm, equal to 6.7 mg/kg bw per day, i.e., highest dose tested (cyhalothrin, rats)
Developmental target	No developmental effects (rabbits)
Lowest relevant developmental NOAEL	30 mg/kg bw per day (lambda-cyhalothrin, rabbits)
Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	Type II pyrethroid toxicity (choreoathetosis/salivation syndrome)
Lowest relevant oral NOAEL	0.5 mg/kg bw (lambda-cyhalothrin, rats, dogs)
Other toxicological studies	
	No data
Medical data	
	No systemic poisoning reported.
	Skin paraesthesia, numbness, irritation of the skin, red eyes, coughing and sneezing.

Summary for cyhalothrin and lambda-cyhalothrin

	Value	Study	Safety factor
Group ADI	0–0.02 mg/kg bw	Rat, acute neurotoxicity, lambda-cyhalothrin;[a] dog, 1-year, lambda-cyhalothrin	25
Group ARfD	0.02 mg/kg bw	Rat, acute neurotoxicity, lambda-cyhalothrin; dog, 1-year, lambda-cyhalothrin[b]	25

[a] The most sensitive NOAEL for the primary action the chemical and considered protective of other non-neurotoxic effects from studies of repeated doses.

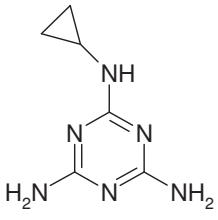
[b] Neurotoxicity occurred a few hours after dosing during the first week of treatment.

5.9 CYROMAZINE (169)

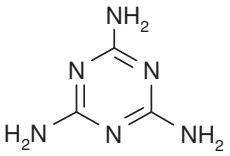
RESIDUE AND ANALYTICAL ASPECTS

Cyromazine was last evaluated by the JMPR in 2006 for toxicology within the Periodic Re-evaluation Programme, where an ADI of 0-0.06 mg/kg bw and an ARfD of 0.1 mg/kg bw were established. The compound was listed at the 38th Session of the CCPR for periodic re-evaluation for residues by the 2007 JMPR. Data submitted by the manufacturer include physical and chemical properties, metabolism in animals and plants, environmental fate in soils, residues in succeeding crops, analytical methods, storage stability, supervised trial on mangos, vegetables and animal commodities and processing studies. Residue and information on good agricultural practices (GAP) was also submitted by the Netherlands.

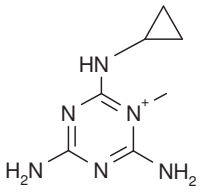
Cyromazine is a selective insecticide that acts by inhibiting the moulting process in insects, particularly in members of the Dipteran family. The figure below shows the compound structure and its main metabolites or degradation products found in animals, plants and/or soils. Metabolism and environmental fate studies submitted to the Meeting were conducted with [triazine-U-^{14}C]cyromazine.



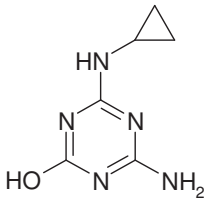
CYROMAZINE



MELAMINE



1-METHYL CYROMAZINE



HYDROXY CYROMAZINE

Animal metabolism

Metabolism studies in rats evaluated by the 2006 JMPR showed that more than 97% of the administered [^{14}C]cyromazine dose was excreted within 24 h, almost exclusively in the urine. Cyromazine was the major compound found in urine (71.5% of the applied radioactivity), with a further 7% attributed to melamine and 8–11% to hydroxy-cyromazine and 1-methyl-cyromazine.

Laying hens that received cyromazine at 5.0 ppm in the feed (equivalent to 0.5 mg/kg body weight/day) for 7 consecutive days had > 99% of the applied radioactivity recovered in the excreta. Egg white and egg yolk had 0.4% and 0.2% of the total applied radioactive dose, respectively; for egg white and egg yolk, an average of 0.15 and 0.12 mg/kg cyromazine equivalents were found in the daily collected eggs of two animals, respectively. Cyromazine represented about 64% TRR in eggs; a metabolite (15.6% TRR) had the same retention volume in an ion exchange column as melamine, but no confirmation of the identity of this compound was performed. Hen tissue residues accounted for 0.1% of the total applied dose, with the highest radioactive levels found in liver, kidney, heart and muscle (0.032, 0.019, 0.10 and 0.09 mg/kg cyromazine equivalents, respectively). The residues in tissues were not characterized. Expired CO_2 and other volatiles accounted for < 0.1% of the applied dose.

Lactating goats dosed with [^{14}C]cyromazine for ten consecutive days at levels of 4.6 ppm (low rate) and 48.4 ppm (high rate) in the feed had most of the administered dose excreted in the urine (86%) and faeces (6.6%). Residues in tissues represented < 2% of the applied dose, mostly found in liver (0.79 and 1.5 mg/kg cyromazine eq. for the low and higher dose, respectively) and kidney (0.043 and 0.44 mg/kg cyromazine eq). About 93% TRR was extracted from the liver, the majority (> 70%) as a non-identified metabolite with cyromazine and melamine representing 2 and 5.6% TRR, respectively. Radioactivity in milk accounted for < 0.4% of the applied dose and plateaued rapidly at both dose levels, with a mean of 0.017 and 0.32 mg/kg cyromazine eq found in the samples collected daily at the low and high dose levels, respectively. The radioactivity in the day 7 milk was mostly associated with the whey fraction, with cyromazine representing about 37% TRR and melamine 9.2 and 4.5% TRR for the low and higher dose, respectively.

In a second study, goats were dosed with cyromazine at 100 ppm in the feed for 4 consecutive days. The TRR was 74% of the applied dose, with 2.7 and 59% eliminated via faeces and urine, respectively; 7.4% of the total dose was found in the gastrointestinal tract. About 6% of the applied dose was recovered in the tissues, mostly in muscle (mean of 2.6%). Highest levels were found in kidney and liver (4.6 and 2.7 mg/kg cyromazine eq, respectively). In liver, the metabolite 1-methylcyromazine was the major compound found (42% TRR), followed by the parent cyromazine (34% TRR). Kidney and muscle had mostly cyromazine (> 70% TRR) and only cyromazine was detected in fat. On average, a total of 0.76% of the applied dose was recovered in the milk, with an average of 0.66 mg/kg cyromazine eq/day. Cyromazine was the major identified compound in milk (68% TRR) followed by melamine (2.9% TRR).

In one study conducted with a mature female sheep fed with [^{14}C]cyromazine for 9 consecutive days at a level of 5 ppm in the diet (equivalent to 0.15 mg/kg bw per day), 94% of the applied radioactivity was recovered, mostly in urine (89%). Tissues represented 0.09% of the applied radioactivity, mostly found in liver (0.17 mg/kg cyromazine eq), kidney (0.048 mg/kg eq) and muscle (0.13 mg/kg eq). While the excreta extracts contained mostly cyromazine, the main compound found in liver extract was melamine (44% total radioactivity), with cyromazine corresponding to 12%.

In summary, animals dosed daily with [^{14}C]cyromazine excreted over 90% of the radioactivity, mainly in urine. Eggs and milk represented < 0.5% and tissues < 2% of the applied or recovered radioactivity. Metabolism of cyromazine involves mainly dealkylation to melamine. Alkylation to 1-methylcyromazine was specific to ruminants; hydroxylation was identified in goats fed at 100 ppm. Cyromazine was the major compound found in hen eggs and milk of goats and sheep. The major compounds found in liver of goat and sheep were the metabolites 1-methylcyromazine and melamine, respectively.

Metabolism in plants

In one greenhouse study conducted at a 0.28 kg ai/ha foliar rate in two phases, celery plants received 2 (1st phase) or 6 applications (2nd phase) and were sampled 7 days after the second application (mature) in the 1st phase or third application (immature) and 14 days after the sixth application (mature) in the 2nd phase. Lettuce received 2 or 4 applications and heads were sampled 7 days after the second application (mature or immature) and 7 days after the fourth application (mature). Radioactivity in the plants was mostly extracted in the aqueous phase (> 74% TRR), with cyromazine representing the major residues (48 to 74% TRR in both plants) and melamine was the only metabolite found (11 to 33% TRR).

In a study conducted with tomatoes in a California field, 6 foliar applications at 0.28 kg ai/ha were made to the crop at 2 weeks intervals. Tomato samples were taken at 0, 7, and 14 days after the 4th or 6th applications and a stalk sample after the last application. TRR in the samples ranged from 64–98% of the applied radioactivity, mostly found in the aqueous extract (56 to 87% TRR) and being characterized as cyromazine (29 to 76% TRR) and melamine (11 to 44% TRR). While the residues ranged from 0.08 to 0.44 mg/kg cyromazine eq. in tomato samples, they reached 37 mg/kg eq in the stalk sample (aerial portion of the plant after removal of the tomato fruits).

Five trials conducted with non-radioactive cyromazine demonstrated that residues did not accumulate significantly in mushrooms under normal use conditions when the crop was grown in amended compost. Cyromazine residues were < 0.05 mg/kg in mushrooms in all trials/flushes with the exception of one trial at a target cyromazine concentration in the compost of 10 mg/kg for which the sample from the third flush contained a residue of 0.08 mg/kg. Residues of melamine were in the range 1.5–6.6 mg/kg at a compost of 5 mg ai/kg, and in the range 3.1–17 mg/kg at a compost of 10 mg/kg.

The results indicated that the major pathway of cyromazine in lettuce, celery and tomato was by dealkylation to form melamine, which can represent about 40% TRR. Melamine was the major compound found in cold treated mushroom.

Environmental fate in soil

Fourteen cyromazine aerobic degradation laboratory studies conducted with a variety of soils were submitted to the Meeting. Soil samples containing from 0.25 to 10.7 mg ai/kg (0.33 to 9.5 kg ai/ha) cyromazine were incubated for up to 12 months in the dark at temperatures ranging from 10 to 25 ˚C. In most studies, TRR was over 90% of the applied radioactivity. Melamine was the only or major degradation product found in the soils. In a 12 months/25 ˚C study conducted with a sandy loam soil (9 mg/kg cyromazine) 15% of the applied dose, at the end of the study, was identified as the carboxylic acid of cyromazine, formed through oxidative opening of the cyclopropane ring. Cyromazine half lives (DT_{50}) varied widely, ranging from 2.9 to 107 days, being lesser at lower temperatures, organic matter content and microbial biomass. In general, the degradation rate of melamine was slower than that of cyromazine.

The photolytic degradation of cyromazine was studied at 20 ˚C under artificial light on soil at a rate of 3.2 µg ai/cm^2 (corresponding to a field rate of 0.32 kg ai/ha). Samples were irradiated for 240 h, equivalent to 30 days natural summer sunlight. In moist irradiated soil, cyromazine degraded rapidly with a calculated DT_{50} of 3.5 days; in dry irradiated soil the level fell rapidly during the first 24 h and the degradation was slow thereafter (DT_{50} > 1 year). In both moist and dry soils, melamine was the major degradation product observed (53 and 15% of applied radioactivity in the moist and dry soil, respectively).

Eleven field soil studies conducted with cyromazine were submitted to the Meeting. In one study cyromazine was applied to bare soil at four different sites in various states in the USA during 1982/83. Plots were treated at an exaggerated rate of 5.6 kg ai/ha as a single application with a second application in Year 2. Residues in the soil at day 0 in both years ranged from 0.39 to 6.6 mg/kg in the 0–15 cm layer, with the highest value occurring from one site in Florida. Cyromazine dissipated at a slow rate at all 4 sites, and could remain unchanged for over 100 days. At the sites in California and Nebraska, essentially no movement of cyromazine was observed below a depth of 15 cm; at the two sites in Florida, cyromazine residues were found to a depth of 30–45 cm in both years. Melamine was detected at all sites down to a depth of 30–45 cm towards the end of each year of the trial.

In four field studies carried out on bare soil plots in potato growing areas of Canada during 1993 and 1994, cyromazine was applied twice at 0.28–0.48 kg ai/ha. Cyromazine residues declined rapidly after the second application and continued to decline over the winter period at a slower rate, with half-lives ranging from 29 to 42 days. The majority of the parent residue was retained in the upper soil layer (0–15 cm) and most of the melamine residues remained in the top 30 cm. Melamine was present from background sources in the three topsoil layers up to 0.011 mg/kg.

In two field studies conducted in Switzerland and France in 2002/2003, soil samples (0–30 cm deep) from plots treated at 0.30 kg ai/ha were taken up to 360 days. In France, no residues were detected in soil below a depth of 20 cm and in Switzerland; no cyromazine residues were found in soil below a depth of below a depth of 10 cm. DT_{50} for cyromazine calculated from the 0–10 cm layer residue data was 17 days and 2.3 days for France and Switzerland, respectively.

In one study conducted in Greece from 2001 to 2003, cyromazine was applied annually 4 times at a rate of 0.30 kg ai/ha at approximately 7 day intervals to bare ground (sandy loam soil type). The plot was cultivated with mustard 1–2 days before the first application in each year. Cyromazine and melamine were found in some occasions at depths of up to 50–70 cm. The degradation of cyromazine was slower during a drought season of the first year (low biological activity) and during the winter.

In a study conducted in Spain, cyromazine was applied yearly to a loamy silt soil in four subsequent early summer applications at 0.30 kg ai/ha (2000–2003). Tomatoes were planted in the second year of the experiment just prior to the cyromazine application. Cyromazine residues were not found in soil layers deeper than 30 cm; melamine could be detected in soil layers up to 100 cm deep in the second and third year of the study, a possible result of higher than average rainfall during this period of the study. DT_{50} for cyromazine, calculated from the first year data, was 51 days.

The proposed metabolic pathway of cyromazine in soil involves the initial cleavage of the cyclopropyl ring moiety to form melamine, with a possible involvement of the carboxylic acid of cyromazine as intermediate. The rate of degradation of cyromazine under laboratory or field conditions vary widely, with estimated half lives ranging from a few days to over 100 days, depending on the soil type and environmental conditions.

Succeeding Crops

Five studies on succeeding crops conducted in USA were submitted to the Meeting. In a greenhouse study conducted in Florida in 1982, celery seedlings were transplanted into a muck soil and [^{14}C]cyromazine mixed with a sample of the soil applied as a top dressing to the crop at 1 kg ai/ha. Celery plants were harvested after 84 days and radishes or sweet corn immediately planted in the same container. About 78% TRR in celery stalks was found the aqueous extract, with cyromazine being the main residue found after 42 and 82 days after treatment (up to 0.45 mg/kg). Radioactivity in radish and mature sweet corn planted 84 days following foliar application of the compound to celery and harvested after 130 or 159 days after planting ranged from 0.01 to 0.02 mg/kg cyromazine eq and were too low for characterization.

In a field study conducted in California, tomatoes were treated in the fall with [^{14}C]cyromazine at 6 × 0.28 kg ai/ha. Tomato plants were harvested 14 days after the last application and winter wheat planted immediately after that. Lettuce, carrot, soy bean and sugar beet were planted the following spring. Two samplings (immature and mature harvest) were taken from each crop for analysis. Radioactive residues in succeeding crops were ≤ 0.05 mg/kg for all crop parts other than half-mature carrot tops (0.19 mg/kg cyromazine eq). Most of the residues in this sample (93%) was partitioned into the aqueous layer and was characterized as 14% cyromazine and 79% melamine.

A study was performed in Georgia to determine the fate of cyromazine in chicken manure amended soil and the uptake and metabolism of cyromazine residues by rotational cops. The soil was prepared by incorporating manure fortified with [^{14}C]cyromazine at 5 mg/kg at a rate of 11.2 t manure/ha and aged for 30 days. Spring wheat, lettuce and sugar beets were planted in buckets containing a 7.6 cm top layer of cyromazine-treated soil and grown to maturity in the greenhouse. TRR ranged from < 0.01 to 0.011 mg/kg cyromazine eq in mature and immature lettuce leaves, sugar beet tops and beets and wheat grain. Immature stalks and mature wheat straw and hulls contained residues between 0.022 to 0.11 (straw) mg/kg eq, greater than the initial soil concentration of 0.064 mg/kg. Cyromazine and melamine accounted for 43% and 28% TRR in wheat straw, respectively.

In a study carried out in Mississippi, tomato crops were treated with 12 applications of cyromazine at 0.14 or 0.28 kg ai/ha, the tomatoes harvested at 14 days after last application and wheat planted 10 weeks after the last application. Cyromazine residues in samples of forage, straw and grain harvested 23 to 43 weeks after planting ranged from < 0.05 to 0.08 mg/kg with the highest value in forage. In California and Florida, 21 field trials were carried with celery treated with 11–15 foliar

applications of cyromazine at rates from 0.14 to 0.28 kg ai/ha. Celery was harvested at 0–14 days after the last application and following a post-harvest interval of 1–6 weeks (Florida) or 8 weeks (California) the field plots were re-planted with sweet corn (eight trials), radishes (eight trials) and lettuce (five trials). Residues of cyromazine were detected in sweet corn forage (0.08 to 0.19 mg/kg), radish roots and tops (0.09 to 0.22 mg/kg) and lettuce leaf (0.05 to 0.08 mg/kg).

In summary, detectable residues of cyromazine and melamine can be found in crops cultivated after cyromazine treated plants have been harvested. Crops planted in cyromazine fortified manure amended aged soil also showed detectable residues.

Analytical methods

Four analytical methods for the analysis of cyromazine and melamine in vegetable crops were provided. In methods developed in the 1980´s, residues can be extracted with water/methanol or pure methanol, cleaned up and partitioned with dichloromethane and hexane, followed by C18 and/or cation exchange columns and additional clean-up with amino column or gel permeation chromatography. The analytes are quantified by HPLC with an amino column and UV detection at 214/215 nm or by GC/NPD. Samples of lettuce, celery, tomato, mushrooms, cucurbits, peppers, grapes, peas and alfalfa, cotton, Sudan grass and/or barley products were fortified at levels that varied from 0.04 to 10 mg/kg level of cyromazine and melamine. In most cases, recoveries were within 70−120% range, however only in few cases replicate samples were analysed.

In a method reported in 2001 (REM 174.02), samples of crops with high water content and fruits with high acid content, are macerated with an acidic solution of potassium dihydrogen phosphate and extracted with methanol. Crops with high fat content, cereals and other dry crops are macerated with water and then extracted with methanol by mechanical shaking. Celite is added, the mixture centrifuged, filtered, and the filtrate acidified. Analysis is by column switching HPLC using two cation exchange columns and detection within the range 215−245 nm depending on crop type and co-extractives. This method was fully validated for sunflower seeds, tomatoes, oranges, beans and potatoes fortified at 0.05 and 0.5 mg/kg (n=5 in each case). Recoveries of cyromazine and melamine ranged from 80 to 110%, with a coefficient of variation (CV) up to 5.2%.

Twelve methods for the analysis of cyromazine and melamine in food of animal origin were provided. The analytes could be extracted with methanol, methanol/water, ethanol or acetone, and the extracts cleaned-up on cation exchange, silica and/or celite column. In some methods, a solvent partitioning step was included before the clean-up, or replaced the clean-up. Quantification was by GC/NPD or MS in most cases, but also by HPLC/UV 214 nm. The methods were validated for eggs, milk and tissues fortified at 0.04 to 1 mg/kg. Recoveries were satisfactory in most cases, and whenever replicate samples were analysed, CV were < 20%. In one method reported in 2003 (RAM 394/01), cyromazine and melamine residues are extracted from liver, kidney, muscle tissue and milk with acetonitrile/water and from eggs with methanol/water. After centrifugation, aliquots are adjusted to pH 4 with glacial acetic acid, subjected to cation exchange clean-up and the compounds quantified by HPLC-MS/MS. This method was fully validated at 0.01 and 0.1 mg/kg levels, with recoveries from 70 to 107% and CV up to 16% (n=5).

Stability of pesticide residues in stored analytical samples

Residues of cyromazine and melamine in crop samples fortified at 0.5 or 1.0 mg/kg levels were stable after being frozen for up to 2 years, with the amount remaining in the range of 80 to 110%. Residues of cyromazine, melamine and 1-methylcyromazine in beef tissues, eggs and milk fortified at 0.5 or 1.0 mg/kg levels were also stable for over 3 years under freezer conditions. Field-incurred residues of cyromazine and melamine in lettuce, celery and tomatoes samples at levels from 0.07 to 21.5 mg/kg increased more than 150% of the initial concentration after stored for up to 23 months under freezer conditions.

Definition of the residue

In 1990, the JMPR defined the residue for cyromazine in food as cyromazine. At the 1992 JMPR, the possibility of including melamine in the definition was discussed, but the Meeting decided to maintain the previous definition in light of melamine was considered to be less toxic than cyromazine, and that melamine may have originated from sources other than from the use of cyromazine. Nevertheless the Meeting recognized that the monitoring of good agricultural practice in growing mushrooms under certain conditions was not possible when melamine was omitted from the residue definition.

Data submitted to the present Meeting have shown that melamine is the main metabolite found in all crops and most animal products. Cyromazine is the major compound found in all crops, with the exception of mushroom, where melamine can be present at levels higher than cyromazine. Most analytical methods analyse cyromazine and melamine and most of the supervised trials submitted to the Meeting contained data for both compounds. It is known that cyromazine is not the only source of melamine in agriculture and in the environment and that melamine can be a component in fertilizers and is used in a variety of manufacturing processes, including plastics. Data provided by the manufacturer have shown that, with the exception of Switzerland, the residue definition in most countries in all foods is cyromazine.

Based on the present knowledge and for practical purposes, the Meeting agreed that the residue definition for cyromazine for enforcement purposes for food of plant and animal origin should continue to be cyromazine.

Toxicological data evaluated by the 2006 JMPR confirmed that melamine is less toxic than the parent compound. The Meeting agreed that the definition for cyromazine in food of plant and animal origin, for dietary intake purposes, is cyromazine.

The octanol-water partition coefficient of cyromazine is < 1 and the compound does not accumulate in fat of animals dosed with cyromazine. The Meeting concluded that cyromazine is not fat soluble.

Definition of residues (for compliance with MRL and for estimation of dietary intake) for plants and animal commodities: *cyromazine.*

Results of supervised trials on crops

Metabolism studies conducted in plants and plant residue data for melamine (not included in this evaluation) indicate that cyromazine is always present at a higher concentration than melamine in the treated crops considered in this evaluation. Cyromazine might be absent or present at lower concentration than melamine in treated mushrooms.

Mango

Mango is registered in Mexico to be used as a foliar application in the field at rates from 0.07 to 0.10 kg ai/ha. The maximum number of application is not specified on the label and the PHI is 0 day. Six trials were conducted with cyromazine in mango in Mexico from 1984 to 1993. The compound was applied 5 times at 0.09 kg ai/ha and samples harvested from day 0 to day 28 after the last application. Residues of cyromazine in the whole fruit at 0 day PHI were: 0.06, 0.10, 0.11, 0.14(2) and 0.25 mg/kg.

The Meeting estimated a maximum residue level of 0.5 mg/kg, an STMR of 0.125 mg/kg and an HR of 0.25 mg/kg for cyromazine in mango.

Onions

The registered use of cyromazine in bulb vegetables in the USA recommends up to 6 foliar applications at 0.14 kg ai/ha rate with a 7 day PHI. A seed treatment at 5 g ai/100 g seed is also recommended, but with no PHI specified. Eight trials were conducted in 1993 with bulb onions using

the seed treatment at the GAP rate and resulted in residues in the onion bulbs at 98 to 207 days PHI of < 0.05 (7) and 0.06 mg/kg. Residues in the whole plant at 60–75 days PHI were 0.09, 0.16, 0.27, 0.34, 0.35, 0.44, 0.83 and 1.7 mg/kg. Dried onion bulb samples (fresh bulbs dried in the field) from all trials gave residues of < 0.05 and 0.06 mg/kg. In nine trials conducted in 1999, using foliar application within US GAP, residues found in onion bulbs were: < 0.05 (7) and 0.07 (2) mg/kg.

The Meeting agreed that trials conducted at GAP using foliar and seed application gave residues in onion bulbs at the same level and could be combined. Residues in ranked order (median underlined) were: < 0.05 (14), 0.06 and 0.07 (2) mg/kg. The Meeting estimated a maximum residue level of 0.1 mg/kg, an STMR of 0.05 mg/kg and an HR of 0.07 mg/kg for cyromazine in bulb onions.

Four trials were conducted with cyromazine in spring (green) onions in USA in 1999 using 6 foliar applications at 0.14 to 0.17 kg ai/ha. Residues in the whole plant within 7 days PHI were 0.26, 0.30, 0.75 and 0.78 mg/kg. Data from immature plant from the bulb onion trials can be considered for the estimation for spring onion and the results from the trials can be combined. Residues in ranked order (median underlined) were: 0.09, 0.16, 0.26, 0.27, 0.30, 0.34, 0.35, 0.44, 0.75, 0.78, 0.83 and 1.7 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg, a STMR of 0.35 mg/kg and a HR of 1.7 mg/kg for cyromazine in spring onion.

Broccoli and cabbage

In the USA, cyromazine is registered for the brassica leafy vegetable group to be applied 6 times as a foliar application in the field at 0.41 kg ai/ha and 7 days PHI. In six trials conducted in broccoli at GAP, residues found in flower head and stem at 7 days PHI were: < 0.05, 0.05, 0.09, 0.21, 0.26 and 0.51 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg, an STMR of 0.15 mg/kg and an HR of 0.51 mg/kg for cyromazine in broccoli.

In six trials conducted at GAP rate in USA, residues in cabbage head (with wrapper leaves) were: 0.06, 0.10, 0.24, 0.28, 0.50 and 6.1 mg/kg.

The Meeting estimated a maximum residue level of 10 mg/kg, an STMR of 0.26 mg/kg and an HR of 6.1 mg/kg for cyromazine in cabbage, head.

The IESTI calculation indicates that the consumption of cabbage at the HR level of 6.1 mg/kg will lead to an exceedance of the ARfD, but no residue data was available from an alternative GAP to estimate a lower maximum residue level.

Fruiting vegetables, cucurbits

Cyromazine is registered for use in cucumber, melon and summer squash in many European countries at similar rates, with the PHI ranging from 3 to 14 days. In France, the maximum GAP rate is 0.3 kg ai/ha (field and protected), with a PHI of 3 days for cucumber and summer squash. Fifteen protected cropping or field trials were conducted in cucumber from 1999 to 2001 in France (6), Greece (2), Italy (5), Spain (1) and Switzerland (1) using 3 to 4 applications at 0.30−0.34 kg ai/ha rate. Residues found at 3 days PHI, in ranked order were: 0.30, 0.32, 0.33, 0.40, 0.43, 0.46, 0.50, 0.52(2), 0.62, 0.71, 0.74, 0.79, 0.88 and 1.3 mg/kg.

In USA, cyromazine is registered for the Cucurbits crop group with a recommendation of a maximum of 6 foliar applications at 0.14 kg ai/ha at 7 day intervals with a 0 day PHI. Due to the rapid rate of growth exhibited by cucurbits, it was considered unlikely that early applications would contribute significantly to the final residues. As a result, trials conducted with a higher number of applications were considered to comply with the US GAP. In five trials conducted in the USA on cucumbers in 1986 and 1990 where 6 or 8 applications, at the GAP rate were made, residues at 0 days

PHI were: 0.16 (2), 0.20, 0.22 and 0.56 mg/kg. In four trials conducted at the double rate, residues found were within the same range.

The Meeting considered that the data from the 20 trials conducted in cucumbers according to the GAPs of Europe and the USA belonged to the same population and could be combined. Residues in ranked order (median underlined) were: 0.16 (2), 0.20, 0.22, 0.30, 0.32, 0.33, 0.40, 0.43, 0.46, 0.50, 0.52(2), 0.56, 0.62, 0.71, 0.74, 0.79, 0.88 and 1.3 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg, an STMR of 0.048 mg/kg and an HR of 1.3 mg/kg for cyromazine in cucumbers.

The Meeting recommended withdrawing the previous recommendation of 0.2 mg/kg for cyromazine in cucumbers.

In eight field trials conducted in summer squash in France, Italy and Spain, between 2000 and 2003, using 3 applications of 0.3 to 0.35 kg ai/ha (0.03 kg ai/hL) with a 3 day PHI, residues found in ranked order were: 0.11, 0.14, 0.15, 0.16, 0.18, 0.21 and 0.27 (2) mg/kg.

In seven trials conducted in the USA from 1986 to 1990 based on the cucurbits GAP rate (6 or 8 applications), residues at 0 days PHI were 0.07 (2), 0.11(2), 0.18, 0.22 and 1.0 mg/kg. In five trials conducted at double rate, residues were within the same range.

The Meeting considered that the residues from 15 trials conducted in summer squash according to European GAP at 3 days or 14 days PHI and in USA according to US GAP belonged to the same population and could be combined. Residues in ranked order (median underlined) were: 0.07 (2), 0.11 (3), 0.14, 0.15, 0.16, 0.18(2), 0.21, 0.22, 0.27 (2) and 1.0 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg, an STMR of 0.16 mg/kg and an HR of 1 mg/kg for cyromazine in summer squash.

GAP for melons in Spain is 0.3 kg ai/ha (14–30 days interval) with a PHI of 3 days. In France, the rate is also 0.3 kg ai/ha (10–15 days interval) and a PHI of 7 days. A total of fourteen trials (field and glasshouse) matching French GAP were conducted in melons in France, Italy and Spain from 1998 to 2001 using 3 applications of 0.28 to 0.33 kg ai/ha (7 days interval). Residues in the whole fruit were: < 0.05, 0.06 (3), 0.07, 0.08, 0.09(2), 0.11, 0.12, 0.13, 0.16, 0.18 and 0.25 mg/kg.

In seven trials conducted in the USA in 1986 in melons and cantaloupe using 8 applications at the GAP rate, residues at 0 days PHI were: < 0.05, 0.08, 0.09, 0.11(2), 0.13 and 0.45 mg/kg. In one trial conducted in watermelon at the same rate, residues found were 0.13 mg/kg.

The Meeting considered that residues from trials conducted in melons according to GAP in Europe and USA appeared to be from similar populations and could be combined. Residues in melons from the 21 trials, in ranked order (median underlined), were: < 0.05 (2), 0.06 (3), 0.07, 0.08 (2), 0.09 (3), 0.11 (3), 0.12, 0.13 (2), 0.16, 0.18, 0.25 and 0.45 mg/kg.

In some of 3 day PHI trials conducted in melons in Europe, residues in fruit were calculated from the levels found in the pulp and in the peel; residues in the pulp were < 0.05(4) mg/kg. The residue ratio fruit/pulp calculated from these and other trials was 1.1, > 1.6, > 2.4, > 2.6, > 3 and > 3.2, with an estimated mean of > 2.3. A fruit/pulp ratio of 2.3 was applied to the median and highest residues for melons (0.09 and 0.45 mg/kg), estimating the median and the highest residue in melon pulp as 0.04 and 0.19 mg/kg.

The Meeting estimated a maximum residue level of 0.5 mg/kg for cyromazine in melons, except watermelons, an STMR of 0.04 mg/kg and an HR of 0.19 mg/kg for cyromazine in melons (pulp).

The Meeting recommends withdrawing of the previous recommendation of 0.2 mg/kg for cyromazine in melons, except watermelons.

Fruiting vegetables, other than cucurbits

Cyromazine is registered in tomato and eggplant in many European countries for either in the field or under protected cropping. In France (F&P) and Italy (F) the maximum application rate is 0.3 mg/kg ai/ha. The recommended PHI is 3 days in France and 14 days in Italy. Eighteen foliar trials complying with French GAP were conducted in France, Greece, Italy and Spain under field or glasshouse conditions with 4 foliar applications at 0.3 to 0.34 kg ai/ha. Residues at 3 days PHI were: 0.05, 0.09, 0.11(3), 0.13 (2), 0.14, 0.15, 0.16, 0.18, 0.21, 0.22, 0.23, 0.29, 0.34, 0.42 and 0.58 mg/kg.

Application through irrigation is also recommended in Italy and Greece, at rates of 0.75 and 0.98 kg ai/ha with a PHI of 14 days. Only one of the four trials conducted in Greece, Italy and Spain using either drip or soil drench irrigation matched the GAP of Italian and Greece, giving residues of cyromazine at 14 days PHI of < 0.05 mg/kg.

In the USA, cyromazine can be applied to tomatoes up to 6 times at a rate of 0.14 kg ai/ha with a 0 day PHI. In 13 trials conducted according to GAP, residues at day 0 were: 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.18, 0.21(2), 0.22, 0.26, 0.28 and 0.30 mg/kg. Five trials conducted at double rate gave residues in the same range.

The Meeting considered that residues in tomatoes from 31 foliar trials conducted in Europe and USA matching GAP belonged to the same population and could be combined. Residues found, in ranked order (median underlined) were: 0.05, 0.09(2), 0.10, 0.11 (4), 0.12, 0.13(3), 0.14 (2), 0.15, 0.16, 0.18 (2), 0.21(3), 0.22(2), 0.23, 0.26, 0.28, 0.29, 0.30, 0.34, 0.42 and 0.58 mg/kg.

In four field trials conducted with eggplant in France and Switzerland 3 applications were made at 0.30 to 0.36 kg ai/ha, residues found at a 3 day PHI were: 0.05, 0.14, 0.23 and 0.26 mg/kg.

The Meeting considered that the residues of cyromazine found in tomatoes and eggplant belonged to the same population and could be combined. Residues from the trials, in ranked order (median underlined) were: 0.05 (2), 0.09 (2), 0.10, 0.11(4), 0.12, 0.13(3), 0.14(3), 0.15, 0.16, 0.18 (2), 0.21(3), 0.22(2), 0.23(2), 0.26(2), 0.28, 0.29, 0.30, 0.34, 0.42 and 0.58 mg/kg.

In the USA cyromazine may be applied to peppers at up to 6 applications at a rate of 0.14 kg ai/ha with a PHI of 0 days. Data from 12 US trials on chilli and bell pepper in 1984/1985 did not comply with GAP as 12 applications were made at 0.14 or 0.28 kg ai/ha, residues found at 0 day ranged from 0.10 to 0.95 mg/kg. Although these trials were conducted with a higher number of applications, residues found at 0 days PHI were within the same range as residues found in tomato and eggplants. The Meeting decided that data from trials on tomato and eggplants, conducted according to GAP, supported the residues found in peppers.

Based on the residues found in tomato and eggplant, the Meeting estimated a maximum residue level of 1 mg/kg, an STMR of 0.16 mg/kg and an HR of 0.58 mg/kg for cyromazine in fruiting vegetables, other than cucurbits, except sweet corn (on-the-cob) and mushrooms.

The Meeting recommends withdrawing the previous recommendations of 0.5 mg/kg for cyromazine in tomato and of 1 mg/kg for cyromazine in peppers.

Mushrooms

Spanish GAP allows cyromazine application to mushrooms at rates up to 0.75 g ai/m^2; Swiss GAP allows treatment at 1 g/m^2 to the casing layer or compost with a PHI of 14/15 days. In France, the application rate is 0.4 g/m^2 with a 14 day PHI. In the USA, cyromazine can be used as a coarse drenching spray at a maximum rate of 0.57 g ai/ m^2, with no specified PHI. Nine mushroom-house trials were conducted in France, Italy and Switzerland from 1986 to 2001 using 1 or 2 applications of cyromazine at 0.4 to 0.8 g ai/m^2. In four trials conducted according to French GAP, residues of cyromazine were 0.37, 1.3, 2.4 and 4.2 mg/kg. Samples collected at a higher PHI in three other trials giving residues of 0.75, 2.2 and 2.8 mg/kg were also considered for MRL estimation. Two trials

conducted at double rate gave residues in the same range. Residues considered for the estimation of an MRL and STMR level were: 0.37, 0.75, 1.3, 2.2, 2.4, 2.8 and 4.2 mg/kg.

The Meeting estimated a maximum residue level of 7 mg/kg, an STMR of 2.2 mg/kg and an HR of 4.2 mg/kg for cyromazine in mushrooms.

The Meeting recommends withdrawing of the previous recommendations of 5 mg/kg for cyromazine in mushroom.

Lettuce

Cyromazine is approved for use on lettuce in a number of European countries. French and Italian GAP for field grown lettuce allows a maximum rate of 0.3 kg ai/ha with a PHI of 21 days and 14 days, respectively. In Spain and Switzerland, GAP comprises a maximum rate of 0.03 kg ai/hL or 0.3 kg ai/ha with a PHI of 7 days in the production of either field grown or protected lettuce. Between 1993 and 2002, 40 trials were conducted in Europe in head and leaf lettuce in both field and greenhouse situations using 3 applications at 0.19 to 0.30 kg ai/ha (0.03 to 0.1 kg ai/hL). These trials were evaluated against either the GAP of Italy (PHI of 14 days) or of Spain/Switzerland (PHI of 7 days).

In three field trials conducted in France in head lettuce complying with the Spanish GAP rate, residues at 14 days PHI were: < 0.03, 0.34 and 1.7 mg/kg. In four protected cropping trials (plastic tunnels), residues were: 2.2, 2.9, 3.0 and 4.9 mg/kg.

In 14 field trials conducted in France, Italy, Spain and Switzerland in Cos lettuce (leaf) complying with the Italian GAP rate, residues at 7 days PHI were: 0.15, 0.18, 0.19, 0.22, 0.24, 0.27, 0.28, 0.34, 0.45, 1.3, 1.5, 1.8(2) and 2.0 mg/kg. Residues in trials according to Spanish or Italian GAP with PHIs of 7 or 14 days in 17 protected trials were: 0.13, 0.55, 1.5, 2.4, 2.8, 3.3, 4.7, 5.2(2), 5.8(2), 6.2, 6.5, 7.9, 11, 14, 15 and 18 mg/kg.

On the basis of the European trials, the Meeting concluded that residues conducted with head and Cos lettuce using the same rate gave residues in the same range. Residues from 17 field trials conducted in lettuce in Europe in ranked order (median underlined), were: < 0.03, 0.15, 0.18, 0.19, 0.22, 0.24, 0.27, 0.28, 0.34(2), 0.45, 1.3, 1.5, 1.7, 1.8(2) and 2.0 mg/kg. Residues from 22 protected trials were: 0.13, 0.55, 1.5, 2.2, 2.4, 2.8, 2.9, 3.0, 3.3, 4.7, 4.9, 5.2(2), 5.8(2), 6.2, 6.5, 7.9, 11, 14, 15 and 18 mg/kg.

In the USA, GAP for leafy vegetables, including brassica leafy vegetables, is a maximum of 5 or 6 applications (6 for head lettuce 5 for all other leafy vegetables) at 0.14 kg ai/ha with a 7 days PHI. In nine trials conducted with head lettuce using 8 applications at 0.14 or 0.28 kg ai/ha rate, residues 7 days after the final application ranged from < 0.05 to 3.8 mg/kg. In nine field trials conducted with leaf lettuce using 5 applications at 0.14 kg ai/ha, residues at 7 days after the final application were: 0.58, 1.5, 1.6, 2.0, 2.8(2), 3.9, 4.4, 5.2 mg/kg.

The Meeting considered the field and protected cropping trials conducted in Europe and the field trials conducted in USA were from different populations and could not be combined. The Meeting considered that the 22 protected trials, conducted in Europe, reflected the most critical use in lettuce.

The Meeting estimated a maximum residue level of 25 mg/kg, an STMR of 2.8 mg/kg and an HR of 18 mg/kg for cyromazine in head lettuce and leaf lettuce.

The IESTI calculation indicates that the consumption of lettuce, at the HR level of 18 mg/kg coming from protected trials, would lead to an exceedance of the ARfD. Consequently, the Meeting used the prospective alternative GAP approach and selected the USA residue data for the maximum residue level estimation.

The Meeting estimated a maximum residue level of 10 mg/kg, an STMR of 2.8 mg/kg and an HR of 5.2 mg/kg for cyromazine in head lettuce and leaf lettuce.

The IESTI calculation indicates that the consumption of lettuce at the HR level of 5.2 mg/kg, coming from the USA field trials, would lead to an exceedance of the ARfD for children. The Meeting once again used the prospective alternative GAP approach and selected the EU residue data population, coming from field trials, for the recommendations.

The Meeting estimated a maximum residue level of 4 mg/kg, an STMR of 0.34 mg/kg and an HR of 2 mg/kg for cyromazine in head lettuce and leaf lettuce.

Mustard greens

Five trials were conducted in the USA according to the GAP for brassicas leafy vegetables (maximum of 5 applications at 0.14 kg ai/ha with a 7 days PHI). Residues in mustard greens (leaves) were: 1.1, 1.6, 2.7, 6.5 and 7.4 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for cyromazine in mustard greens of 10, 2.7 and 7.4 mg/kg.

Spinach

From 16 trials conducted with spinach in the USA, eight were conducted according to GAP for leafy vegetables. Residues at 7 days PHI were: 0.4, 1.1, 1.2, 1.8, 2.3, 4.2, 5.4 and 6.1 mg/kg. The Meeting estimated a maximum residue level of 10 mg/kg, an STMR value of 2.0 mg/kg and an HR value of 6.1 mg/kg for cyromazine in spinach.

The IEST calculation indicates that the consumption of spinach at the HR level of 6.1 mg/kg will lead to an exceedance of the ARfD, however no data was available for an alternative GAP review to estimate a lower maximum residue level.

Beans

US GAP allow 6 applications of cyromazine in lima bean and dry beans at a rate of 0.14 kg ai/ha with a PHI of 7 days. In nine trials conducted in lima beans complying with GAP from the USA, residues found in ranked order (median underlined), were: < 0.05, 0.11, 0.17, 0.19, 0.23(2), 0.32, 0.38 and 0.58 mg/kg. Trials conducted with 8 applications at 0.14 kg ai/ha or 6 applications at 0.28 kg ai/ha gave residues in the range of 0.23 to 1.0 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg, an STMR of 0.23 mg/kg and an HR of 0.58 mg/kg for cyromazine in lima beans.

In nine trials in dry beans from the USA complying with that countries GAP, residues found in ranked order (median underlined), were: 0.23, 0.68, 0.84, 0.97, 1.0, 1.1(2), 1.2 and 1.8 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg and an STMR of 1 mg/kg for cyromazine in beans (dry).

Potato

In Spain cyromazine may be applied to potatoes at a maximum rate of 0.24 kg ai/ha (0.02 kg ai/hL) with a PHI of 21 days. In Italy, field and greenhouse GAP consists of an application rate of 0.3 kg ai/ha with a PHI of 35 days. In eight potato trials conducted in Spain using 3 applications at 0.17 to 0.33 kg ai/ha with samples harvested at 21 or 35 days post application. Four trials could be evaluated against Spanish or Italian GAP, with residues of < 0.05, 0.06, 0.11, and 0.12 mg/kg.

The Meeting decided that there were insufficient trials submitted, complying with GAP, and did not estimate a maximum residue level.

Stalk and stem vegetables

In Spain, cyromazine can be used in artichoke at 0.03 kg ai/hL with a PHI of 7 days. In four Spanish trials conducted complying with that countries GAP, residues were: 0.85, 0.95, 1.1 and 1.3 mg/kg.

The Meeting estimated a maximum residue level of 3 mg/kg, an STMR of 1 mg/kg and an HR of 1.3 mg/kg for cyromazine in artichoke.

In France, cyromazine can be applied to celery in the field or in greenhouses at an application rate of 0.3 kg ai/ha with a PHI of 14 days. In 11 trials conducted in France and Spain from 1998 to 2003 using 3 or 4 applications at 0.3–0.36 kg ai/ha, residues, in ranked order (median underlined), were: 0.27(3), 0.36, 0.57, 0.58, 0.60, 0.68, 1.6, 1.8 and 2.3 mg/kg. The commodity description in the trials specified whole plant or stems and was interpreted by the Meeting as matching the Codex description for celery (whole commodity).

Fourteen trials were conducted in the USA in celery at a greater than GAP application frequency (maximum of 6 applications at 0.14 kg ai/ha with a 7 days PHI). Residues 7 days after the last application ranged from 0.05 to 13 mg/kg.

Based on the European trials, the Meeting estimated a maximum residue level of 4 mg/kg, an STMR value of 0.58 mg/kg and an HR value of 2.3 mg/kg for cyromazine in celery.

Fate of residues in processing

Hydrolysis studies representing food processing procedures of pasteurization, baking, boiling, brewing and sterilization were conducted with [^{14}C]cyromazine in buffer solution at pH 4, 5 and 6, incubated for 20 or 60 minutes at 90–120 ˚C, showed that cyromazine was the only compound found at the end of the incubation period.

In a tomato processing study in the USA, tomatoes were harvested 7 days after the last of 6 applications at 0.140 or 0.250 kg ai/ha. Treated samples were pooled and subject to treatment simulating normal commercial processing. Residues decreased after washing, in canned tomato, tomato juice and in ketchup, with mean processing factors (PF) of 0.71, 0.53, 0.75 and 0.84. Residues in wet pomace remained unchanged, but increased in dry pomace (PF=2.8), puree (PF=1.2) and paste (PF=2.1).

Based on the estimated PFs and STMR for tomato of 0.16 mg/kg, the Meeting estimated an STMR-P of 0.11 mg/kg for washed tomato, 0.09 mg/kg for canned tomato, 0.12 for tomato juice, 0.13 mg/kg for ketchup, 0.19 mg/kg for puree and 0.34 mg/kg for tomato paste.

In one study conducted with potatoes, samples from six trials conducted in USA in 1996 were processed according to commercial practices. Mean cyromazine residues in the raw potato was 0.71 mg/kg, which decreased in peeled/rinsed potatoes with a mean PF of 0.9, but increased in potato chips (PF=1.3) and potato granules (PF=2.8). As no recommendation was made for the raw commodity potato, the Meeting did not make a recommendation for processed potato products.

Residues in animal commodities

Direct treatment of poultry (in-feed use)

In four feeding trials, conducted in Australia, hens were fed at rates of 1.5 mg ai/kg for 35 days, 3 mg ai/kg for 4 days, 5 mg ai/kg for 7 days and 9 mg ai/kg for 4 days, samples were taken of muscle, liver and/or eggs. Muscle and liver samples from hens, treated at the GAP rate of 5 mg ai/kg feed, were taken immediately after treatment and up to 2 days post-treatment. Cyromazine residues in muscle were found to decrease from 0.03 to < 0.02 mg/kg in muscle and from 0.12 to < 0.05 mg/kg in liver.

In two trials conducted in France in 1985/1986, hens were fed cyromazine at 5 mg ai/kg feed for up to 38 days. Residues in eggs (egg white/yolk) sampled from 0 to 22 days, following 15 to 38

days of feeding were: 0.15/0.16, 0.15/0.11, 0.12/0.05, 0.08/0.11, 0.12/0.05, 0.04/0.08, 0.08/0.06, 0.08/0.04, < 0.02/0.04, < 0.02/0.03(3) and < 0.02/0.02(9) mg/kg. As the egg white comprises approximately 66% and the yolk 33% of a typical poultry egg, the levels found in egg white/yolk can be expressed on a whole egg basis, in ranked order, as: < 0.02(12), 0.04, 0.05, 0.07(2), 0.09, 0.10(2), 0.14 and 0.15 mg/kg.

In a trial conducted in Israel in 1987, hens were fed cyromazine at 5 and 50 mg/kg feed for 31 days with the animals sacrificed 4 h after removal of the feed. Residues of cyromazine in muscle and liver ranged from 0.04 to 0.10 mg/kg at the 5 mg/kg treatment level and from 0.36 to 0.76 mg/kg at the higher feeding level. Eggs were not analysed.

In a study conducted in the Philippines in 1982 hens were fed for 60 days, at a rate of 1.6 mg ai/kg of feed, no residues were detected in muscle (< 0.025 mg/kg) and liver (< 0.04 mg/kg) in animals slaughtered 2 to 9 days after cessation of feeding. Residues in eggs collected from animals fed cyromazine from 29 to 60 days, 0 to 9 days after feeding ceased were 0.02, < 0.02 and 0.04 mg/kg.

In ten studies conducted in the USA from 1979 to 1986, hens were fed cyromazine for up to 56 days at levels of 2.5, 5.0, 25 and 50 mg ai/kg of feed. In one trial where hens were fed cyromazine at 5 mg/kg from 14 to 27 days, residues in egg white/yolk at day 0 were: 0.09/0.08, 0.11/0.06 and 0.11/0.07 mg/kg, or 0.09 (2) and 0.10 mg/kg in the whole egg. In three trials conducted at 5.0 mg ai/kg feed, egg samples were collected 0 to 7 days after feeding from animals fed 14 to 56 days. Residues in eggs in ranked order, were: < 0.05(4), 0.11(2), 0.07, 0.09(3), 0.10(7), 0.12(4) and 0.16 mg/kg. In one feeding study conducted at a 2.5 mg/kg feeding level for 14 days, residues at 0 days were < 0.05 mg/kg in muscle, skin and fat and 0.08 mg/kg in liver. The levels found after the animals had been fed for 27 days were 0.05 mg/kg in meat and < 0.05 mg/kg in the other tissues. In all cases, residues in fat were < 0.05 mg/kg.

In one study conducted in Japan in 2000, hens were fed cyromazine for 28 days at the level of 5 mg ai/kg. Residues in egg white/yolk collected from 0 to 2 days after treatment were < 0.02/0.03, < 0.02/0.05 and 0.04/0.07 mg/kg, or < 0.02, 0.04 and 0.05 mg/kg expressed on a whole egg basis. Residues at 0 day were 0.05 mg/kg in muscle, 0.07 mg/kg in liver and 0.09 mg/kg in kidney. Cyromazine levels in all tissues after 1 to 3 days were < 0.02 mg/kg.

In summary, residues of cyromazine in eggs from trials conducted according to GAP were: < 0.02 (13), 0.02, 0.04 (3), < 0.05 (4), 0.05 (2), 0.07(3), 0.09 (4), 0.10(9), 0.11(2), 0.12(4), 0.14, 0.15 and 0.16 mg/kg. Residues at 0 day at 2.5 mg/kg level were < 0.05 and 0.05 mg/kg in muscle, < 0.05 and 0.08 mg/kg in liver and < 0.05 mg/kg in fat.

Lactating dairy cows

In two feeding studies conducted in the USA in 1983 and 1992, Holstein dairy cows were dosed at 5.0, 10, 25, 50 or 100 mg ai/kg diet for up to 28 days. Animals were sacrificed on test days 14, 21 and 28 with tissue samples taken. Milk samples consisted of pooled aliquots from the evening and the following morning's milk. Residues in milk plateaued rapidly on the commencement of dosing with the mean levels, after 28 days treatment, increasing proportionally with the dose, ranging from 0.02 mg/kg at the 5 mg/kg to 0.42 mg/kg at the 100 mg/kg level. In one study, the highest residues in tissues, at the lower dose, were found in the animals sacrificed following 14 days of feeding with 0.13 mg/kg in kidney and 0.12 mg/kg in meat; these levels increased to 1.9 and 0.59 mg/kg, respectively in the animals dosed at 50 mg/kg. In the second study, no residues (< 0.05 mg/kg) were found in tissues from animals dosed at 10 mg/kg level. No residues were found in fat samples from any animal at any dosing level in either study.

Livestock Dietary burden

Dietary burden calculations for beef and dairy cattle and broilers and laying poultry are provided in Annex 6. The calculations were made according to the animal diets from US-Canada, EU and Australia in the OECD Table (Annex 6 of the 2006 JMPR Report). A summary of the results are shown on the Table below.

Animal (feed items)	Livestock dietary burden, cyromazine, ppm of dry matter diet					
	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.31	0.17	8.54	0.57	1.02	0.57
Dairy cattle	0.31	0.17	8.54	0.57	0.31	0.17
Poultry - broiler and layer	0.41	0.06	2.4	0.14	1.4	0.21

Residues in animal commodities

The high and the mean estimated dietary burden for cattle were 8.5 and 0.57 ppm. The estimations were done by interpolation of residues at the 5 ppm feeding level.

Dietary burden (mg/kg)[1] Feeding level [ppm][2]		Cyromazine residues, mg/kg[3]				
		Milk Mean	Muscle High	Liver High	Kidney High	Fat High
MRL cattle beef and dairy	(8.5) [5] high		*(0.204)* 0.12	*(0.153)* 0.09	*(0.221)* 0.13	*(< 0.05)* 0
STMR cattle beef and dairy	(0.57) [5] av		*(0.01)* 0.06	*(0.01)* 0.06	*(0.01)* 0.08	*(< 0.05)* *0*
STMR cattle beef and dairy	0.57) [5] av	*(0.005)* 0.045				

[1] Values in parentheses are the estimated dietary burdens

[2] Values in square brackets are the actual feeding levels in the transfer study

[3] Residue values in parentheses in italics are interpolated from the dietary burden, feeding levels in the transfer study and the residues found in the transfer study. High is the highest individual animal tissue residue in the relevant feeding group. Mean is mean animal tissue (or milk) residue in the relevant feeding group.

The Meeting estimated a maximum residue level of 0.3 mg/kg, an STMR of 0.01 and an HR of 0.20 mg/kg for cyromazine in meat (from mammals other than marine mammals).

The Meeting estimated a maximum residue level of 0.3 mg/kg, an STMR of 0.01 and an HR of 0.187 mg/kg for cyromazine in edible offal (mammalian).

The Meeting estimated a maximum residue level of 0.01 mg/kg and an STMR of 0.005 mg/kg for cyromazine in milks. The Meeting also estimated a median and a highest residue level of 0 mg/kg in mammalian fat.

The high and the mean estimated dietary burden for poultry were 2.4 and 0.14 ppm. The direct treatment for poultry (in-feed use) conducted at 2.5 mg/kg feeding level can be used for estimating residues in poultry tissues.

The Meeting estimated a maximum residue level of 0.1 mg/kg, an STMR of 0.05 mg/kg and an HR of 0.05 mg/kg for cyromazine in poultry meat.

The Meeting estimated a maximum residue level of 0.2 mg/kg, an STMR of 0.065 mg/kg and an HR of 0.08 mg/kg for cyromazine in poultry edible offal. The Meeting also estimated a median and a highest residue level of 0 mg/kg in poultry fat.

The Meeting agreed that for the residue estimation in eggs, the residues coming from the direct use of cyromazine in the feed at 5 mg/kg level, according to GAP (0 day withholding period) represents a better estimation of the likely residues.

The Meeting estimated a maximum residue level of 0.2 mg/kg and an STMR of 0.07 mg/kg and an HR of 0.16 mg/kg for cyromazine in eggs.

The Meeting recommends the withdrawal of the current recommendation of 0.2* mg/kg for cyromazine in eggs, of 0.01* mg/kg in milks, and of 0.05* mg/kg in poultry meat and sheep meat.

DIETARY RISK ASSESSMENT

Long-term intake

The ADI for cyromazine is 0-0.06 mg/kg bw. The International Estimated Daily Intakes (IEDI) for cyromazine was estimated for the 13 GEMS/Food cluster diets using the STMR or STMR-P values estimated by the current Meeting for 35 commodities. The results are shown in Annex 3. The IEDI ranged from 0–2% of the maximum ADI. The Meeting concluded that the long-term intake of residues of cyromazine from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The ARfD for cyromazine is 0.1 mg/kg bw. The International Estimated Short Term Intake (IESTI) for cyromazine was calculated for the plant commodities for which STMRs and HRs were estimated and for which consumption data were available. The results are shown in Annex 4. For the general population, the IESTI was higher than the ARfD for cabbage (120%) and spinach (130% ARfD). For children, the IESTI was higher than the ARfD for cabbage (280%) and spinach (390%). For all the other commodities, the intakes ranged from 0–40%.

The Meeting concluded that the short-term intake of residues of cyromazine from uses other than cabbage and spinach that had been considered by the JMPR is unlikely to present a public concern. The information provided to the JMPR precludes an estimate that the short-term intake of residues of cyromazine from the consumption of cabbage and spinach will be below the ARfD.

The ARfD established by the Meeting in 2006 was based on body-weight loss and decrease in food consumption in dams in developmental toxicity studies. The reason for these effects was unknown and there is a rapid recovery on cessation of administration. The Meeting noted that this ARfD may be conservative. Furthermore, it is possible that the short-term risk assessment conducted by the Meeting may also be conservative.

The Meeting noted that no residue data was available from an alternative GAP to estimate a lower maximum residue level for cyromazine in cabbage and spinach.

5.10 DIFENOCONAZOLE (224)

TOXICOLOGY

Difenoconazole (CAS name, 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1*H*-1,2,4-triazole, CAS No. 119446-68-3) is a systemic triazole fungicide that controls a broad spectrum of foliar, seed and soil-borne diseases caused by *Ascomycetes*, *Basidiomycetes* and

Deuteromycetes in cereals, soya, rice, grapes, pome fruit, stone fruit, potatoes, sugar beet and several vegetable and ornamental crops. It is applied by foliar spray or seed treatment and acts by interference with the synthesis of ergosterol in the target fungi by inhibition of the 14α-demethylation of sterols, which leads to morphological and functional changes in the fungal cell membrane. Difenoconazole is being reviewed for the first time by the present Meeting at the request of the CCPR. All critical studies complied with GLP.

Biochemical aspects

In rats given [^{14}C]difenoconazole labelled in either the triazole or phenyl rings as a single oral dose at 0.5 or 300 mg/kg bw, the radiolabel was rapidly and almost completely absorbed from the gastrointestinal tract, widely distributed, and eliminated from the body with excretion half-lives of about 20 h at the lower dose and about 33–48 h at the higher dose. At doses of 0.5 and 300 mg/kg bw, approximately 13–22% and 8–15%, respectively, of the applied radioactivity was excreted via the urine in rats. Excretion via the faeces accounted for 81–87% (lower dose) to 85–95% (higher dose) of the radiolabel; however, approximately 73% (males) to 76% (females) of the lower dose and approximately 39% (females) to 56% (males) of the higher dose was eliminated from the body via the bile in bile-duct cannulated rats. Thus, bioavailability decreased with increasing dose. When bile from rats given difenoconazole was introduced into the duodenum of other bile-cannulated rats, 80% of the radioactivity was re-eliminated in the bile and 4% in the urine, indicating that there was extensive enterohepatic recirculation. The greater quantities of radioactivity were distributed to the gastrointestinal tract, liver and kidneys. The initial plasma half-life was approximately 4–6 h at the lower dose and 22–24 h at the higher dose and the terminal half-life was approximately 3–4 days at both doses. In spite of these long terminal half-lives, there was no evidence for bioaccumulation of difenoconazole.

After oral administration to male and female rats, the systemically available portion of [^{14}C-phenyl]difenoconazole was rapidly and extensively metabolized to a number of biotransformation products. Three main metabolites isolated from the faeces accounted for approximately 68% of the administered doses. These were: the hydroxylated product of cleavage of the dioxolane ring; its further metabolite resulting from hydroxylation of the chlorophenoxy ring; and the product of the hydroxylation of difenoconazole, occurring directly in the chlorophenoxy ring. A minor metabolic pathway involves cleavage of the alkyl chain between the triazole and the inner phenyl ring, resulting in a hydroxy acetic acid, 2-chloro-4-(4-chlorophenoxy)-benzoic acid and free 1,2,4-triazole. Sulfate conjugates were identified for some hydroxylated metabolites.

Toxicological data

The acute toxicity of difenoconazole was low, with values for the oral LD_{50} being 1453 mg/kg bw in rats and > 2000 mg/kg bw in mice. The dermal LD_{50} in rabbits was > 2010 mg/kg bw and the inhalation LC_{50} in rats was > 3.3 mg/L. Difenoconazole is very slightly and transiently irritating to the skin and moderately and transiently irritating to the eyes of rabbits and is non-sensitizing in a modified Buehler test in guinea-pigs.

Overall, in short-term studies with orally-administered difenoconazole, the signs of toxicity observed in mice, rats and dogs were similar, with reduced body-weight gain and increased liver weights being common features. Histopathology confirmed the liver as a target organ with observation of diffuse or centrilobular hypertrophy of hepatocytes in rats and mice, although this can also be indicative of an adaptive response. Cataracts were found in dogs fed diets containing difenoconazole at a concentration of ≥ 3000 ppm, equal to 96.6 mg/kg bw per day, for 6 months, with an NOAEL of 1000 ppm, equal to 31.3 mg/kg bw per day; however, cataracts were not induced in a second study in dogs given diets containing difenoconazole at up to 1500 ppm, equal to 51.2 mg/kg bw per day, for 1 year. Increased activity of alkaline phosphatase was observed in two studies in rats and in one in dogs. No other blood chemistry changes were consistently observed, although reduced

concentrations of blood protein were observed in dogs given diets containing difenoconazole at 6000 ppm, equal to 157.8 mg/kg bw per day. Also in dogs, a reduction in erythrocyte count of almost 20% was observed in females at this high level of exposure.

For the short-term dietary studies, the NOAELs were: in studies of up to 90 days in rats, 200 ppm (equal to 17 mg/kg bw per day) on the basis of increased hepatocellular hypertrophy and liver weight; in a 90-day dietary study in mice, 200 ppm (equal to 34.2 mg/kg bw per day) on the basis of clinical signs of toxicity and changes in liver weight and increased incidence of centrilobular hepatocellular hypertrophy; in a 28-week study in dogs, 1000 ppm (equal to 31.3 mg/kg bw per day) on the basis of cataracts and liver-weight changes; in a 12-month study in dogs, 100 ppm (equal to 3.6 mg/kg bw per day) on the basis of reduced body-weight gain; in a 4-week study of dermal toxicity with difenoconazole in rats, 100 mg/kg bw per day, on the basis of minimal centrilobular hepatocellular hypertrophy, minimal to moderate thyroid follicular cell hypertrophy and skin lesions at the site of application.

Long-term feeding studies in rats and mice fed with difenoconazole confirmed that the primary target organ was the liver. There was no evidence for any carcinogenic potential in rats, in which hepatic effects were increases in liver weight and hepatocellular hypertrophy in male and female rats. In addition, there were reductions in erythrocyte parameters in female rats at the highest dose, 2500 ppm, equal to 170 mg/kg bw per day.

In mice, there was very high, treatment-related mortality at the beginning of the 18-month study. In groups of 70 mice, there were 52 deaths among females receiving difenoconazole at 4500 ppm and 16 deaths among females at 3000 ppm (reduced to 2500 ppm after 1 week) within the first 2 weeks, while, among the male mice there were 11 deaths in the group at 4500 ppm within the first 3 weeks of the study. There was an increased incidence of hepatocellular adenomas and carcinomas in the group of male and female mice fed diet containing difenoconazole at 2500 ppm, equal to 423 and 513 mg/kg bw per day, respectively, and males at 4500 ppm, equal to 819 mg/kg bw per day. No increase in the incidence of tumours was observed at 300 ppm, equal to 46.3 and 57.8 mg/kg bw per day in males and females, respectively. However, the neoplastic responses occurred at highly toxic doses that also caused the death of substantial proportions of the groups of mice. Among the survivors, biliary stasis and hepatic single-cell necrosis as well as hepatocellular hypertrophy were significantly increased in male and female mice at the tumorigenic doses. On the basis of a study of enzyme activities in male mice, difenoconazole is considered to be a reversible barbiturate-type inducer of metabolizing enzymes in the mouse liver. No peroxisome proliferation was observed. The NOEL was 10 mg/kg, there being no inductive effect on metabolizing enzymes and other parameters in the mouse liver.

The NOAEL in long-term studies in rats was 20 ppm, equal to 1.0 mg/kg bw per day, on the basis of reduced body-weight gains during the first year in males and females, reduced platelet counts in males and hepatic centrilobular hypertrophy in males and females at 500 ppm, equal to 24 mg/kg bw per day. In long-term studies in mice, the NOAEL was 30 ppm, equal to 4.7 mg/kg bw per day, on the basis of decreased body-weight gain in males, increased liver weight in females and hepatocellular hypertrophy in males at 300 ppm, equal to 46.3 mg/kg bw per day.

Difenoconazole was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence for genotoxicity was observed in any test.

The Meeting concluded that difenoconazole is unlikely to be genotoxic.

The Meeting concluded that difenoconazole caused an increase in the incidence of hepatocellular adenomas and carcinomas in mice (but not in rats) by a non-genotoxic mode of action, the nature of which has not been established but which resembles that for phenobarbital in its liver enzyme-inducing characteristics. It is therefore unlikely to pose a carcinogenic risk to humans at exposure levels that do not cause changes in the liver.

The reproductive toxicity of difenoconazole was investigated in a two-generation study of reproduction in rats and in studies of developmental toxicity in rats and rabbits.

Reproductive function was not affected in rats in the two-generation study and the NOAEL for reproductive function was 2500 ppm, equal to 132.1 mg/kg bw per day, the highest dose tested. The NOAEL for systemic toxicity in the parental animals was 250 ppm, equal to 11.5 mg/kg bw per day on the basis of reduced body-weight gain during the pre-mating period in F_0 and F_1 generations at 2500 ppm, equal to 122.7 mg/kg bw per day during these periods. In pups, lower birth weight and subsequent decreased body-weight gain at 2500 ppm, equal to 158.0 mg/kg bw per day, were the effects that defined the NOAEL for offspring toxicity at 250 ppm, equal to 14.1 mg/kg bw per day in the females.

In a study of developmental toxicity in which rats were given difenoconazole by gavage on days 6–15 of gestation, the NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of reduced body-weight gain and excess salivation first observed on day 2 of dosing in 14 out of 23 dams at 100 mg/kg bw per day. There was a statistically significant, but small increased incidence of changes in thoracic vertebral ossification centres in foetuses at 200 mg/kg bw. The NOAEL for foetal toxicity was 100 mg/kg bw per day on the basis of these skeletal anomalies.

In a study of developmental toxicity in which rabbits were given difenoconazole by gavage on days 7–19 of gestation, the NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of reduced body-weight gain in the first few days of dosing at 75 mg/kg bw per day. Examination of the foetuses did not reveal any treatment-related effects in soft or ossified tissues. The NOAEL for developmental toxicity was 75 mg/kg bw per day, the highest dose tested.

In a single-dose study of neurotoxicity in rats treated by gavage, the NOAEL was 25 mg/kg bw based on reduced forelimb-grip strength interpreted as a non-specific response at 200 mg/kg bw. In a 90-day study of neurotoxicity in rats given diets containing difenoconazole, the NOAEL was 40 ppm, equal to 2.8 mg/kg bw per day, on the basis of reduced hind limb-grip strength in males during the final week of the study at 250 ppm, equal to 17.3 mg/kg bw per day. These responses were considered to be non-specific effects of difenoconazole because of the absence of any changes in the multiple end-points of neurotoxicity that were measured and the absence of neuropathological findings.

The Meeting concluded that difenoconazole is unlikely to cause neurotoxicity in humans.

There were no indications of immunotoxicity in general studies of toxicity in dogs, rats and mice.

Some aspects of the toxicology of three plant metabolites of difenoconazole that are also found in rats given difenoconazole were investigated. These metabolites were: 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanone, 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol and 2-chloro-4-(4-chlorophenoxy)-benzoic acid. The LD_{50} value for each of these metabolites was > 2000 mg/kg bw and none of them showed any alerts for mutagenic activity. In addition, there are three metabolites which are common to all parent triazoles: 1, 2, 4-triazole, triazole alanine and triazole acetic acid. 1,2,4-Triazole is a soil metabolite that also appears to a small extent (< 10%) in some studies in plants and is found as at least 10% of the total metabolites in rats administered difenoconazole. Triazole alanine and triazole acetic acid are plant-specific metabolites. The acute toxicity (LD_{50}) values for 1,2,4-triazole, triazole alanine and triazole acetic acid were similar to or higher than the LD_{50} values for difenoconazole. Some recent studies conducted with 1,2,4-triazole have shown certain effects at high doses, i.e., testicular atrophy in mice at $\geq$ 487 mg/kg bw per day; effects on the central nervous system and pathology in rats at $\geq$ 183 mg/kg bw per day; and a reduction in fertility in a two-generation study of reproduction in rats at 218 mg/kg bw per day. The NOAELs for 1,2,4-triazole for these effects were 90 mg/kg bw per day for testicular atrophy in mice, 33 mg/kg bw per day for neurotoxicity in rats and 16 mg/kg bw per day for effects on fertility in rats.

Repeat-dose studies in which triazole alanine was administered for up to 90 days did not show any significant effects other than reduced body-weight gains at 20 000 ppm, equivalent to 2000 mg/kg bw per day in rats and 500 mg/kg bw per day in dogs. The NOAELs in these studies were 5000 ppm,

equivalent to 500 mg/kg bw per day, in rats and 8000 ppm, equivalent to 200 mg/kg bw per day, in dogs. In a two-generation study with triazole alanine in rats, the NOAEL for parental toxicity was 10 000 ppm, equivalent to 500 mg/kg bw per day, the highest dose tested, and the NOAEL for reproductive toxicity was 2000 ppm, equivalent to 100 mg/kg bw per day, on the basis of reduced birth weights at 10 000 ppm. In a study of teratogenicity in rats, the NOAEL for maternal toxicity was 1000 mg/kg bw per day, the highest dose tested, and the NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of retarded ossification at 1000 mg/kg bw per day.

Triazole acetic acid has been tested in a 14-day dietary study in which the NOAEL was 8000 ppm, equal to 704 mg/kg bw per day, respectively, the highest dose tested.

None of these metabolites has been tested for carcinogenicity. The extent to which 1,2,4-triazole, triazole alanine and triazole acetic acid had been tested for mutagenicity varied, but no significant responses were obtained in any of the studies.

Medical surveillance of personnel has been conducted since the early 1990s at sites in several countries where difenoconazole is manufactured. Reports were available up to the end of 2002. There have been no reports of toxicity in workers during manufacture and there was only one case of allergic reaction to a formulated product. There were no reports of poisoning and no reports of sensitization during use of the formulated product.

The Meeting concluded that the existing database was adequate to characterize the potential hazards to foetuses, infants and children.

Toxicological evaluation

An ADI of 0–0.01 mg/kg bw was established for difenoconazole based on the NOAEL of 1.0 mg/kg bw per day in rats, identified on the basis of reduced body-weight gains, reduced platelet counts and hepatic hypertrophy in a 24-month long-term dietary study of toxicity and carcinogenicity. A safety factor of 100 was applied. The increased incidence of hepatocellular adenomas and carcinomas observed in mice at 423 mg/kg bw per day, the NOAEL being 4.7 mg/kg bw per day, in an 18-month long-term dietary study of toxicity and carcinogenicity, was likely to be due to a mode of action without human relevance at exposure levels that do not cause changes in the liver.

An ARfD of 0.3 mg/kg bw was established for difenoconazole. This was based on the NOAEL of 25.0 mg/kg bw in rats, identified on the basis of clinical signs in a single-dose study of neurotoxicity and using a safety factor of 100. This ARfD is supported by the NOAEL of 25 mg/kg bw per day for maternal toxicity in a study of developmental toxicity in rats and rabbits on the basis of excess salivation in rats at 100 mg/kg bw per day and body-weight loss in rabbits during the first few days of treatment at 75 mg/kg bw per day.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity	Toxicity	30 ppm, equal to 4.7 mg/kg bw per day	300 ppm, equal to 46.3 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 46.3 mg/kg bw per day	2500 ppm[a], equal to 423 mg/kg bw per day

Rat	Twenty-four-month studies of toxicity and carcinogenicity	Toxicity	20 ppm, equal to 1.0 mg/kg bw per day	500 ppm, equal to 24 mg/kg bw per day
		Carcinogenicity	2500 ppm[a] equal to 124 mg/kg bw per day	—
	Two-generation study of reproductive toxicity[b]	Reproductive toxicity	2500 ppm[a] equal to 132.1 mg/kg bw per day	—
		Parental toxicity	250 ppm, equal to 11.5 mg/kg bw per day	2500 ppm[a] equal to 122.7 mg/kg bw per day
		Offspring toxicity	250 ppm, equal to 14.1 mg/kg bw per day	2500 ppm[a] equivalent to 158 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	20 mg/kg bw per day	100 mg/kg bw per day
		Embryo and foetal toxicity	100 mg/kg bw per day	200 mg/kg bw per day[a]
	Acute neurotoxicity	Toxicity	25 mg/kg bw	200 mg/kg bw per day[a]
Rabbit	Developmental toxicity[c]	Maternal toxicity	25 mg/kg bw per day	75 mg/kg bw per day[a]
		Embryo and foetal toxicity	75 mg/kg bw per day[a]	—
Dog	One-year study of toxicity	Toxicity	100 ppm, equal to 3.6 mg/kg bw per day	500 ppm, equal to 16.4 mg/kg bw per day

[a] Highest dose tested.

[b] Measurements of intake of the compound are the mean for the pre-mating phases for females.

[c] Gavage administration.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.3 mg/kg bw

Information 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 difenoconazole

Absorption, distribution, excretion and metabolism	
Rate and extent of oral absorption	High, but incomplete (rats)
Dermal absorption	Approximately 8% (rats)
Distribution	Distributed throughout the body; higher concentrations in liver and gastrointestinal tract

Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	High; essentially 100% excretion in bile and urine within 7 days
Metabolism in animals	Extensive; a small number (< 10) of metabolites; little parent compound remaining
Toxicologically significant compounds in animals, plants and environment	Parent, 1,2,4-triazole
Acute toxicity	
Rat, LD_{50}, oral	1453 mg/kg bw
Rat, LC_{50}, inhalation	3.3 mg/L (4 h)
Rabbit, LD_{50}, dermal	2010 mg/kg bw
Rabbit, skin irritation	Very slightly and transiently irritating
Rabbit, eye irritation	Moderately and transiently irritating
Guinea-pig, skin sensitization	Not sensitizing (modified Buehler test)
Short-term studies of toxicity	
Target/critical effect	Liver; body weight
Lowest relevant oral NOAEL	3.6 mg/kg bw per day (12-month study in dogs)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (4-week study in rats)
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver; body weight
Lowest relevant NOAEL	1.0 mg/kg bw per day (24-month study in rats)
Carcinogenicity	Not carcinogenic at levels below those causing changes in the liver
Reproductive toxicity	
Reproductive target/critical effect	None
Lowest relevant reproductive NOAEL	132 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; reduced foetal body weight, delayed ossifications in rats, but not in rabbits
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Single-dose study of neurotoxicity	No signs of neurotoxicity, NOAEL was 25 mg/kg bw (rats)
Ninety-day study of neurotoxicity	No signs of neurotoxicity, NOAEL was 2.3 mg/kg bw (rats)
Other toxicological studies	
	Induction of liver xenobiotic metabolizing enzymes
	Studies of mammalian and plant metabolites
Medical data	
	No reports of toxicity in workers exposed during

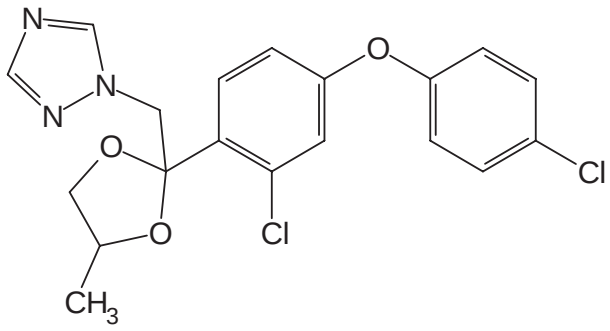
manufacture or use

Summary			
	Value	**Study**	**Safety factor**
ADI	0–0.01 mg/kg bw	Rat, 2-year study of toxicity and carcinogenicity	100
ARfD	0.3 mg/kg bw	Rat, single-dose study of neurotoxicity study, supported by maternal effects in a study of developmental toxicity studying rabbits	100

RESIDUE AND ANALYTICAL ASPECTS

Difenoconazole was considered for the first time by the present meeting. It is a broad-spectrum fungicide used for disease control in many fruits, vegetables, cereals and other field crops. It has preventive and curative action. Difenoconazole acts by inhibition of demethylation during ergosterol synthesis.

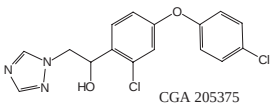
1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl[1,3]dioxolan-2-ylmethyl]-1H-1,2,4-triazole



Animal metabolism

The Meeting received animal metabolism studies with difenoconazole in rats, lactating goats and laying hens. Difenoconazole [^{14}C] labelled in the central phenyl ring or the triazole ring was used in most of the metabolism studies. Difenoconazole [^{14}C] labelled in the chlorophenoxy ring was used in some of the studies.

Difenoconazole is rapidly metabolized, initially to 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol (CGA 205375) and then with cleavage of the triazole moiety from the chlorophenoxyphenyl moiety. Conjugates are formed from hydroxylated metabolites. TRR levels are higher in the liver than in other tissues. Most of the TRR is rapidly excreted.



CGA 205375

Parent difenoconazole has a tendency to fat-solubility, but it is always a minor component of the residue. The major component of the residue in most animal commodities is CGA 205375, which appears to be fat-soluble because residue concentrations in fat are approximately 3 times as high as those in muscle. However, it is not strong fat-solubility because residue concentrations in fat are less than those in kidney and much less than those in liver (typically residues in liver are 6–8 times as high as in the fat).

When rats were orally dosed with labelled difenoconazole it was readily absorbed followed by extensive metabolism and excretion. The following metabolites were identified in excreta: CGA 205375, 1,2,4-triazole, 2-chloro-4-(4-chlorophenoxy)-benzoic acid, 2-chloro-4-(4-

chlorophenoxy)-phenyl-hydroxyacetic acid, hydroxylated difenoconazole and hydroxylated CGA 205375. Sulphate conjugates of the hydroxylated metabolites were identified in urine. (See the toxicology report for more details of laboratory animal metabolism)

When two lactating goats were orally dosed with labelled ([^{14}C]triazole and [^{14}C]phenyl) difenoconazole for 10 consecutive days at 7.5 mg/animal/day, equivalent to 4.7 and 5.6 ppm in the feed, most of the administered [^{14}C] was excreted in the faeces (75% and 67%) and urine (31% and 21%). Residues in milk reached a plateau by day 2 (phenyl) and days 4–7 (triazole). Of the [^{14}C] in milk, 19% and 32% were distributed into the fat portion for the triazole and phenyl labels respectively (metabolite 1,2,4-triazole is water soluble). Residues of [^{14}C] were higher in liver (0.28 and 0.26 mg/kg) than in other tissues. Metabolite CGA 205375 constituted 57–58% of the TRR in liver, with parent difenoconazole at 1% or less. Triazole was the major component identified in milk, constituting 47% TRR.

When four lactating goats were orally dosed with labelled ([^{14}C]triazole and [^{14}C]phenyl) difenoconazole for 4 consecutive days at 150 mg/animal/day, equivalent to 100 ppm in the feed, [^{14}C] recovery was marginal at 40–64%. The TRR in liver (7.5 and 6.0 mg/kg) was much higher than other tissues. CGA 205375 was the major residue in each tissue, accounting for approximately 30–70% of the TRR. Difenoconazole residues in liver (0.62 and 0.40 mg/kg) were higher than in other tissues. Difenoconazole accounted for 1.5–8.3% of the TRR in each of the tissues. In milk, CGA 205375 accounted for 21% and 34% of the TRR (0.38 and 0.14 mg/kg), while difenoconazole (6–9% TRR) and triazole (6% TRR) were minor parts of the residue.

Two lactating goats were dosed orally once daily for 4 consecutive days by gelatin capsule with 150 mg/animal/day of [^{14}C-phenyl]difenoconazole, equivalent to 100 ppm in the feed and were slaughtered approximately 6 h after the final dose for tissue collection. CGA 205375 was the major component of the residue in all tissues and milk. Parent difenoconazole was present in all tissues and milk, but never exceeding 10% of the TRR. A number of metabolites resulted from hydroxylation and conjugation with glucuronic acid, sulphate and glycine. The concentration of the main component, CGA 205375, in fat was 2.3 times its concentration in muscle, but much below its concentration in liver and similar to that in kidney, suggesting borderline fat solubility.

When 4 laying hens were orally dosed with labelled ([^{14}C]triazole and [^{14}C]phenyl) difenoconazole for 14 consecutive days at 0.55 mg/bird/day, equivalent to 5 ppm in the feed, most of the administered [^{14}C] was excreted in the faeces (> 89%). Highest TRR appeared in the kidney (0.43 and 0.49 mg/kg) and liver (0.13 and 0.13 mg/kg). Apparent plateaus for TRR in egg whites and yolks were reached after approximately 4 and 7 days of dosing respectively. The plateau TRR values in egg whites were quite different for the two labels: 0.14 mg/kg for [^{14}C]triazole label and 0.011 mg/kg for [^{14}C]phenyl label, whereas the plateau levels in the yolks were essentially the same (0.28 and 0.29 mg/kg).

When 20 laying hens were orally dosed with labelled ([^{14}C]triazole and [^{14}C]phenyl) difenoconazole for 3 consecutive days at 7.5 mg/bird/day, equivalent to 68 ppm in the feed, most of the administered [^{14}C] was excreted in the faeces (76%). Highest TRR occurred in the liver (4.3 and 4.7 mg/kg) and kidney (1.9 and 2.2 mg/kg). CGA 205375 was the major identified component in each tissue: liver (30% and 34% TRR), kidney (20% and 22%), muscle (8.8% and 35%) and fat (46% and 64%). Parent difenoconazole accounted for less than 5% TRR in each tissue. For eggs from the phenyl label treatment, CGA 205375 was the main component of the residue (73–83% TRR). For the triazole label, triazole accounted for 67% of TRR in egg white and 33% TRR in egg yolk, while CGA 205375 accounted for 7.8% TRR in egg white and 36% TRR in egg yolk. Approximately 4-5% of the TRR in egg yolks was identified as parent difenoconazole.

Five laying hens were dosed orally once daily for 4 consecutive days by gelatin capsule with 12.5 mg/bird/day of [^{14}C-triazole]difenoconazole, equivalent to 121 ppm in the feed and were slaughtered approximately 6 h after the final dose for tissue collection. Significant [^{14}C] levels appeared in all tissues (liver 13 mg/kg, muscle 4.9 mg/kg, fat 10.4 mg/kg) and eggs (whites 4.0 mg/kg, yolks 4.5 mg/kg). CGA 205375 was a major component of the residue in tissues (liver 56%

TRR, muscle 24% TRR, fat 61% TRR) and egg yolk (53% TRR). Triazole was also a significant component of the residue in tissues (liver 18% TRR, muscle 55% TRR, fat 4.6% TRR) and eggs (whites 75% TRR, yolks 31% TRR). Parent difenoconazole was a minor component of the residue in liver, muscle and egg yolk (< 5%TRR) but accounted for 18% of the TRR in fat.

The metabolism of difenoconazole in rats, goats and hens is qualitatively similar.

Plant metabolism

The Meeting received plant metabolism studies with difenoconazole in tomatoes, wheat, potatoes, grapes and oilseed rape. Difenoconazole [^{14}C] labelled in the central phenyl ring, in the triazole ring or in the chlorophenoxy ring was used in the metabolism studies.

Difenoconazole is generally slowly absorbed and metabolized. In most cases, particularly for parts of the plant directly exposed to the treatment, the parent difenoconazole is the dominant part of the residue. Parts of the plant not directly exposed are more likely to contain a residue dominated by a mobile water-soluble metabolite such as triazolylalanine.

The following plant metabolites apparently do not occur as animal metabolites of difenoconazole: triazolylalanine (2-amino-3-(1,2,4]triazol)-1-yl-propionic acid), triazolyl acetic acid (1,2,4-triazol-1-yl-acetic acid) and triazolyl-lactic acid (1,2,4-triazol-1-yl-lactic acid). At least some of these metabolites are common to other fungicides containing the 1,2,4-triazole moiety.

In a tomato metabolism study in USA, tomato plants in pots in a greenhouse were foliar sprayed 6 times at 7-day intervals with [^{14}C]phenyl and [^{14}C]triazole labelled difenoconazole at the equivalent of 0.12 kg ai/ha. Parent difenoconazole was the major part of the residue on foliage. Residue levels on tomato fruits sampled 7 days after the final treatment were insufficient for identification. A field-grown tomato metabolism study produced similar results.

In another tomato metabolism study in USA, tomato plants in pots in a greenhouse were foliar sprayed 6 times at 7-day intervals with [^{14}C]triazole labelled difenoconazole at the equivalent of 0.12 kg ai/ha. In tomato fruits sampled 33 days after the final treatment, parent difenoconazole (12–51% TRR) and metabolite triazolylalanine (19–42% TRR) were major components of the residue (TRR 0.13–0.20 mg/kg). In a parallel study with phenyl labelled difenoconazole, tomato fruits, sampled 33 days after the final treatment, contained parent difenoconazole (66% TRR) as the major part of the residue (TRR 0.17 mg/kg). In both of these studies low concentrations (< 2% TRR) of metabolite CGA 205375 and its ketone (1-(2-chloro-4-(4-chloro-phenoxy)-phenyl)-2-(1,2,4-triazol)-1-yl-ethanone) occurred in the fruits.

In a wheat metabolism study, triazole and triazolylacetic acid were identified in the mature stalks and grain produced from [^{14}C]triazole labelled difenoconazole treated seed. Metabolite CGA 205375 was identified in wheat tops from a parallel wheat metabolism study with [^{14}C]phenyl labelled difenoconazole.

In a greenhouse wheat metabolism study in USA, spring wheat seeds were treated with [^{14}C]phenyl and [^{14}C]triazole labelled difenoconazole at 0.25 and 0.30 g ai/kg seed and grown to maturity. Parent difenoconazole and metabolite CGA 205375 were identified at low levels in wheat tops at 25 % maturity (40 days post sowing).

In a greenhouse wheat metabolism study in USA, spring wheat was foliar sprayed 4 times with [^{14}C]phenyl and [^{14}C]triazole labelled difenoconazole at a rate equivalent to 0.25 kg ai/ha. Mature samples of grain were harvested 29 days after the final application. In grain from the [^{14}C-triazole]difenoconazole treated crop, triazolylacetic acid and triazole accounted for 20% and 10% of the TRR (1.4 mg/kg) respectively. In grain from the [^{14}C-phenyl]difenoconazole treated crop, the TRR (0.064 mg/kg) was much lower, demonstrating that metabolic cleavage of the compound occurred before translocation to the grain. In the mature stalks, difenoconazole accounted for 50% of the TRR (54 and 47 mg/kg) for both labels.

Parent difenoconazole was not identified in mature grain from the wheat metabolism studies.

In a greenhouse potato metabolism study in USA, potato plants were foliar sprayed 6 times with [^{14}C]chlorophenoxy labelled difenoconazole at the equivalent of 0.12 kg ai/ha per application. Very little of the [^{14}C] translocated to the tubers (TRR 0.012 mg/kg) with parent difenoconazole and two primary metabolites identified as low-level components of the residue (< 10% TRR). Parent difenoconazole was the major component (76% TRR) of the foliage residue.

In a parallel study on potatoes with [^{14}C]triazole labelled difenoconazole, triazolylalanine (79% TRR) was the major part of the residue in tubers (TRR 0.087 mg/kg). Parent difenoconazole was again the major component (71% TRR) of the foliage residue.

In a field plot grape metabolism study in USA, grape vines were foliar sprayed 5 times with [^{14}C]phenyl and [^{14}C]triazole labelled difenoconazole. Parent difenoconazole was the major component (51% and 45% TRR) of the residue (TRR 0.13 and 0.12 mg/kg) in grapes harvested 20 days after 3 and 5 sprays. None of the identified metabolites exceeded 10% of the TRR in grapes. Parent difenoconazole was also the major identified component (17% TRR) of the residue (TRR 0.047 mg/kg) in grapes harvested 77 days after the second treatment.

In a field plot oilseed rape metabolism study in Switzerland, spring rape received two foliar sprays with [^{14}C]chlorophenoxy labelled difenoconazole at the equivalent of 0.13 kg ai/ha. Parent difenoconazole was the major identified component of the residue in stalks (17% TRR), seeds (15% TRR) and pods (17% TRR) taken at mature harvest 39 days after the second application and in oil (26% TRR) produced from the seed. Metabolite CGA 205375 exceeded 10% of TRR in the stalks (14%) and pods (11%).

In a parallel oilseed rape study with [^{14}C]triazole labelled difenoconazole, parent difenoconazole was a major identified component of the residue in stalks (17% TRR), pods (14% TRR) from samples taken at mature harvest, 39 days after the second application, and in oil (84% TRR) produced from the seed. Metabolite CGA 205375 exceeded 10% of TRR in the stalks (17%) and pods (13%). Triazolylalanine, the major residue component in the seed (56% TRR) also exceeded 10 % in pods (12%). Triazolylalanine was also the major residue component in the meal (56% TRR). Other identified components of the residue in the meal were triazolylacetic acid, CGA 205375 and difenoconazole.

Parent difenoconazole is the main component of the residues in those parts of the crop directly exposed to treatment. For other parts of the crop, e.g., the grain of cereals and the tubers of potatoes, the main components of the residue are translocatable metabolites, e.g., triazolylalanine, which are common to other fungicides containing the 1,2,4-triazole moiety.

Environmental fate in soil

The Meeting received information on soil aerobic metabolism and soil photolysis properties of difenoconazole as well as studies on the behaviour of difenoconazole residues in crop rotations. Difenoconazole residues are reasonably persistent in soils and are expected to be present in the soil at harvest time for treated root and tuber crops. Difenoconazole residues are also expected to persist in the soil until the sowing of rotational crops. The confined rotational crops studies demonstrate that difenoconazole itself does not appear as a residue in the rotational crop. The water-soluble and mobile metabolites triazolylalanine, triazolylacetic acid and triazolyl-lactic acid have been identified in the rotational crops.

Aerobic soil degradation rates were influenced by the nature of the soil, temperature, moisture status of the soil and dose when [^{14}C]difenoconazole was subjected to laboratory soil incubation. Estimated aerobic soil metabolism half-lives for difenoconazole at 20 °C ranged from 63 to 700 days (n=12) with a median of 181 days. After 220–300 days, mineralization and unextractable residues (20–54% of dose) were major sinks for the [^{14}C] label. The degree of mineralization was different for

the phenyl and triazole label positions, e.g., 0.8–4.6 % of the dose for the triazole label and 3.4–33 % for the phenyl label.

CGA 205375 and 1,2,4-triazole were identified as soil metabolites. Metabolite CGA 205375 consistently reached a maximum (expressed as parent) of 5–10% of the dose and had begun to decline by the end of the observation period. Metabolite 1,2,4-triazole typically reached a maximum (expressed as parent) around 20% of the dose during the observation period. The aerobic soil metabolism of the metabolites, CGA 205375 and 1,2,4-triazole, was studied separately. The major metabolite of CGA 205375 was 1,2,4-Triazole.

Difenoconazole on a soil surface was stable to photolysis during the test period of 30 days.

In rotational crops with the [^{14}C] label in the phenyl moiety, the level of carry-over residues in rotational crops was too low for characterization or identification. With the [^{14}C] label in the triazole moiety and application to bare ground at 0.13 kg ai/ha, metabolites triazolylalanine, triazolylacetic acid and triazolyl-lactic acid were identified in rotational crops: maize grain TRR 0.21 mg/kg (66% triazolylalanine 66%); wheat grain 0.34 mg/kg (44% triazolylalanine, 26% triazolylacetic acid); lettuce heads 0.017 mg/kg (31% triazolylalanine, 43% triazolyl-lactic acid; and sugar beet tops 0.029 mg/kg (25% triazolylalanine, 54% triazolyl-lactic acid).

In outdoor non-confined rotational crop studies in Germany, bare ground was treated directly with difenoconazole at a rate equivalent to 0.75 kg ai/ha and the upper 10 cm soil layer was turned over to mix in the applied material. Carrots or spinach were sown 30–31 days after the difenoconazole application and harvested for analysis 97–136 days (carrots) and 62–77 days (spinach) after the application. Residues of difenoconazole (LOQ 0.02 mg/kg) and triazolylalanine (LOQ 0.05 mg/kg) in the carrots and spinach did not exceed the LOQs. Difenoconazole residue levels in the soil were in the range 0.15-0.23 mg/kg during rotational crop samplings.

Methods of residue analysis

The Meeting received descriptions and validation data for analytical methods for residues of parent difenoconazole in raw agricultural commodities, processed commodities, feed commodities, animal tissues, milk and eggs. Methods were provided also for metabolite CGA 205375 in animal tissues, milk and eggs.

In the methods for plant commodities, macerated samples are typically extracted with methanol or acetonitrile and the extract is cleaned up by solvent partitions and solid phase column chromatography. The final residue may be determined by GLC with ECD or NPD or alternatively by LC-MS-MS. LOQs are typically in the 0.01–0.05 mg/kg range. The analytical methods for animal commodities are similar, but with extraction methods tailored for milk, eggs or animal tissues. The LOQ for milk is 0.005 mg/kg and eggs and tissues 0.01–0.05 mg/kg.

Analytical recovery data were satisfactory for difenoconazole and CGA 205375 (in animal commodities) for numerous commodities.

Residue methods were tested by independent laboratories unfamiliar with the analysis and were found to have satisfactory recoveries and no background interferences.

DFG Method S19 (revision) was demonstrated to be suitable for analysis of difenoconazole residues in a number of crop commodities.

The acetonitrile-water extraction of poultry tissues and eggs, as in the analytical method, was applied to liver, fat, muscle and egg yolk samples from a [^{14}C-triazole]difenoconazole metabolism study and was shown to provide comparable extraction for difenoconazole, CGA 205375 and 1,2,4-triazole with the exhaustive extraction of the metabolism study.

Stability of residues in stored analytical samples

Information was received on the freezer storage stability of parent difenoconazole residues in plant and animal commodities, and of residues of CGA 205375 in animal commodities.

Difenoconazole residues were stable in the following crop commodities for the intervals tested, some for 1 year, but most for 2 years: banana, cotton seed, cotton seed meal, cotton seed oil, lettuce, potatoes, soya beans, tomatoes, wheat forage, wheat grain and wheat straw.

Difenoconazole and metabolite CGA 205375 spiked into animal tissues (0.2 mg/kg) and milk (0.05 mg/kg) were stable when stored at or below -18 °C for approximately 10 months.

Definition of the residue

Parent difenoconazole is the dominant component of the residue in crop commodities and is a suitable analyte for enforcement purposes.

Parent difenoconazole is generally no more than a minor component in animal commodities. The major component of the residue in most animal commodities is metabolite CGA 205375 (1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol).

In the goat metabolism studies, the concentration of CGA 205375 in the fat was approximately 3 times as high as in the muscle, but much lower than in the liver. In the dairy cow feeding studies, the concentration of CGA 205375 in the fat was approximately 3 times as high as in the muscle, but much lower than in the liver. In the laying hen metabolism studies, the concentration of CGA 205375 in the fat was approximately 5–8 times as high as in the muscle, but also much lower than in the liver. The octanol-water partition coefficient of CGA 205375 (log P_{OW}=3.8) suggests fat-solubility.

The Meeting decided the residue would be defined as fat-soluble.

The Meeting recommended a residue definition for difenoconazole.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities: difenoconazole.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: sum of difenoconazole and 1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol), expressed as difenoconazole.

The residue is fat soluble.

Results of supervised residue trial on crops

The Meeting received supervised trials data for difenoconazole uses on oranges, pome fruits (apple, pear), stone fruits (cherries, peach, plum), grapes, olives, tropical fruits (banana, mango, papaya), bulb vegetables (garlic, leek), Brassica vegetables (broccoli, Brussels sprouts, cabbages, cauliflower), watermelon, fruiting vegetables (chilli peppers, tomatoes), lettuce, soya beans, root and tuber vegetables (carrot, potato, sugar beet), stalk and stem vegetables (asparagus, celeriac, celery), cereal grains (rice, wheat) and oilseeds (rape seed, sunflower seed). Residue data were also provided on wheat straw and fodder, rice straw and fodder, sugar beet leaves and tops, oilseed rape fodder and sunflower plant and stubble.

In trials where duplicate field samples from an unreplicated plot were taken at each sampling time and analysed separately, the mean of the two results was taken as the best estimate of the residue from the plot.

Labels (or translations of labels) were available from Australia, Belgium, Brazil, Central America (Belize, Costa Rica, Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua,

Panama), France, Germany, Indonesia, Italy, Poland, Spain, Switzerland and UK describing the registered uses of difenoconazole.

Citrus fruits

In Brazil, difenoconazole may be applied to citrus trees twice at a spray concentration of 0.005 kg ai/hL with a 30 days PHI. In two trials in Brazil matching GAP and two others with a spray concentration of 0.01 kg ai/ha, difenoconazole residue levels were < 0.05 mg/kg.

The number of trials was insufficient for an orange MRL recommendation.

Pome fruit

Spanish GAP allows five applications of difenoconazole to apple or pear trees at 0.075 kg ai/ha with a PHI of 14 days. In three trials from Spain, matching GAP, difenoconazole residues in apples were 0.10, 0.14 and 0.15 mg/kg.

In two apple trials from France with application parameters matching Spanish GAP, difenoconazole residues were 0.11 and 0.28 mg/kg.

In two trials from Greece, also with application parameters matching Spanish GAP, difenoconazole residues were 0.05 and 0.13 mg/kg.

In two trials from Italy also with application conditions matching Spanish GAP, difenoconazole residues were 0.06 and 0.08 mg/kg.

In one pear trial from France and one from Greece, matching Spanish GAP, difenoconazole residues in pears were 0.07 and 0.16 mg/kg, respectively.

The Meeting decided to combine the apple and pear data to support a pome fruit MRL. Residues in the 11 trials in ranked order (median underlined) were: 0.05, 0.06, 0.07, 0.08, 0.10, <u>0.11</u>, 0.13, 0.14, 0.15, 0.16 and 0.28 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in pome fruit of 0.5, 0.11 and 0.28 mg/kg respectively.

Stone fruits

Polish GAP allows 3 applications of difenoconazole to cherry trees at 0.05 kg ai/ha with a PHI of 14 days.

In a cherry trial from France and two from Germany, with application conditions matching Polish GAP, difenoconazole residues in cherries were 0.08, 0.06 and 0.10 mg/kg, respectively.

Italian GAP allows 3 applications of difenoconazole to peach trees with a spray concentration of 0.0075 kg ai/hL with a PHI of 7 days. In five Italian trials matching Italian GAP, difenoconazole residues on peaches were 0.07, 0.11, 0.14, 0.14 and 0.19 mg/kg.

In a peach trial from France and two from Greece with application conditions matching Italian GAP, difenoconazole residues in peaches were 0.18, 0.16 and 0.26 mg/kg, respectively.

In summary, the difenoconazole residues on peaches from eight trials (in ranked order, median underlined) were: 0.07, 0.11, 0.14, <u>0.14</u>, <u>0.16</u>, 0.18, 0.19 and 0.26 mg/kg.

French GAP allows 3 applications of difenoconazole to plum trees with a spray concentration of 0.005 kg ai/hL with a PHI of 14 days. In four French trials matching GAP (accepted variation on spray concentration 0.0035–0.0065 kg ai/hL) difenoconazole residues on plums were 0.02, 0.03, 0.07 and 0.10 mg/kg.

In four German trials on plums with application conditions matching French GAP (accepted variation on spray concentration 0.0035–0.0065 kg ai/hL), difenoconazole residues were < 0.01, 0.01, 0.02 and 0.04 mg/kg.

In two Spanish trials on plums with application parameters matched French GAP, difenoconazole residues were 0.03 and 0.08 mg/kg.

In summary, the difenoconazole residues on plums from 10 trials were: < 0.01, 0.01, 0.02, 0.02, 0.03, 0.03, 0.04, 0.07, 0.08 and 0.10 mg/kg.

The data from the peaches and plums were apparently of different populations and could not be combined.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in peaches of 0.5, 0.15 and 0.26 mg/kg respectively. These values may also be used for nectarines.

The data from plums and cherries were combined for mutual support, residues in 13 trials in ranked order (median underlined) were: < 0.01, 0.01, 0.02, 0.02, 0.03, 0.03, 0.04, 0.06, 0.07, 0.08, 0.08, 0.10 and 0.10 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in plums and cherries of 0.2, 0.04 and 0.10 mg/kg respectively.

Grapes

Italian GAP allows 4 applications of difenoconazole to grape vines with a spray concentration of 0.005 kg ai/hL with a PHI of 21 days. In six Italian trials from 2003–2004 matching GAP, difenoconazole residues on grapes were 0.01, 0.02, 0.02, 0.03, 0.03 and 0.04 mg/kg. In two French trials matching Italian GAP, residues in grapes were 0.04 and 0.07 mg/kg.

In summary, the difenoconazole residues on grapes from eight trials in ranked order (median underlined) were: 0.01, 0.02, 0.02, 0.03, 0.03, 0.04, 0.04 and 0.07 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in grapes of 0.1, 0.03 and 0.07 mg/kg respectively.

Olives

In Spain, difenoconazole may be applied to olive trees three times at a spray concentration of 0.015 kg ai/hL with a 30 days PHI. In seven trials in Spain in 2003–2005 matching GAP, difenoconazole residue levels were 0.22, 0.29, 0.40, 0.42, 0.51, 0.90 and 1.2 mg/kg.

In an olive trial in France with application conditions matching Spanish GAP, difenoconazole residues on olives were 0.76 mg/kg.

In summary, difenoconazole residues in olives from eight trials in ranked order (median underlined) were: 0.22, 0.29, 0.40, 0.42, 0.51, 0.76, 0.90 and 1.2 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in olives of 2, 0.465 and 1.2 mg/kg respectively.

Bananas

In Costa Rica, Guatemala and Honduras difenoconazole may be applied 8 times to bananas at 0.1 kg ai/ha with harvest permitted on the day of application. The use pattern includes aerial application.

In the banana trials in 1997 in Ecuador, Colombia and Honduras, unbagged fruit were chosen for study although these cropping conditions, approved as GAP, rarely occur in commercial banana

production. The trials of 1993 in Costa Rica and Guatemala included both bagged and unbagged fruits. For the purposes of estimating an MRL, only data from unbagged fruit are considered in this case.

In three banana trials in Colombia with conditions matching the GAP of Costa Rica, residues of difenoconazole in whole fruit were < 0.02, 0.02 and 0.04 mg/kg, with residues in pulp all at < 0.02 mg/kg.

In two banana trials in Costa Rica with conditions matching GAP, difenoconazole in whole fruit were 0.03 and 0.04 mg/kg, with residues in pulp both at < 0.02 mg/kg.

In three banana trials in Ecuador with conditions matching the GAP of Costa Rica, difenoconazole in whole fruit were all < 0.02 mg/kg, with residues in pulp also all at < 0.02 mg/kg.

In one banana trial from Guatemala with conditions matching the GAP of Costa Rica, difenoconazole in whole fruit were 0.07 mg/kg, with residues in pulp at < 0.02 mg/kg.

In three banana trials in Honduras with conditions matching the GAP of Costa Rica, difenoconazole in whole fruit were < 0.02, < 0.02 and 0.03 mg/kg, with residues in pulp also all at < 0.02 mg/kg.

In summary, difenoconazole residues in whole bananas from the 12 unbagged trials were: < 0.02 (5), 0.02, 0.02, 0.03, 0.03, 0.04, 0.04 and 0.07 mg/kg. Residues in banana pulp were all < 0.02 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in bananas of 0.1, 0.02 and 0.02 mg/kg respectively.

Mango

In Brazil, difenoconazole may be applied to mango trees three times at a spray concentration of 0.0125 kg ai/hL with a 7 days PHI. In four trials in Brazil in 2003 matching GAP, difenoconazole residues in mango whole fruits were 0.025, 0.025, 0.035 and 0.04 mg/kg. No data were available for residues in edible portion.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in mangos of 0.07, 0.03 and 0.04 mg/kg respectively.

Papaya

In Brazil, difenoconazole may be applied to papayas four times at a spray concentration of 0.0075 kg ai/hL with a 14 days PHI. In four trials in Brazil in 2002 matching GAP, difenoconazole residues in papaya whole fruits were 0.02, 0.03, 0.07 and 0.10 mg/kg and residues in edible portion were all < 0.01 mg/kg. In four trials where the spray concentration was 0.015 kg ai/hL (2 × label) residues in whole papaya fruit were 0.09, 0.09 0.12 and 0.20 mg/kg and residues in edible portion were < 0.01 (3) and 0.02 mg/kg, suggesting residues could occur in the edible portion, i.e., not a nil residue.

The double rate trials provided additional support, particularly in cases such as this for difenoconazole where the residue is generally external and essentially non-systemic.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in papaya of 0.2, 0.01 and 0.02 mg/kg respectively.

Garlic

In Brazil, difenoconazole may be applied to garlic crops six times at a rate of 0.13 kg ai/ha with a 14 days PHI. In four trials in Brazil in 1995 with 6 applications of 0.19 or 0.38 kg ai/ha (1.5 × and 3 ×

label rates), difenoconazole residues in bulbs of garlic were all < 0.02 mg/kg at PHIs of approximately 0, 7, 14 and 21 days.

Data from the exaggerated rates and various sampling intervals suggest that difenoconazole residues do not reach garlic bulbs.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in garlic of 0.02*, 0 and 0 mg/kg respectively.

Leeks

In Germany, difenoconazole may be applied to leek crops 3 times at a rate of 0.1 kg ai/ha with a 21 days PHI. In four trials in Germany with application in line with GAP, difenoconazole residues in whole plants with roots removed were 0.02, 0.07, 0.09 and 0.12 mg/kg.

In four leek trials in France with conditions matching German GAP, difenoconazole residues in whole plants were 0.03, 0.05, 0.13 and 0.21 mg/kg.

In two leek trials from Italy with conditions matching German GAP, difenoconazole residues in whole plants were 0.14 and 0.17 mg/kg.

In two leek trials from Switzerland with conditions matching German GAP, difenoconazole residues in edible portions were 0.02 and 0.04 mg/kg.

The Meeting accepted that the three descriptions of the commodity analysed, i.e., (1) whole plants with roots removed, (2) whole plants and (3) edible parts, were all intended to agree with the Codex description of the commodity for analysis: *Whole vegetable after removal of roots and adhering soil*.

In summary, difenoconazole residue in leeks from the 12 trials, in rank order (median underlined), were: 0.02, 0.02, 0.03, 0.04, 0.05, <u>0.07</u>, <u>0.09</u>, 0.12, 0.13, 0.14, 0.17 and 0.21 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in leeks of 0.3, 0.08 and 0.21 mg/kg respectively.

Broccoli

In Belgium, difenoconazole may be applied twice to broccoli at a rate of 0.13 kg ai/ha with a 14 days PHI. In trials in France, Netherlands and Spain, difenoconazole was applied 3 times rather than twice. Difenoconazole is a reasonably persistent residue as found in the decline trials with residue remaining on the whole plant just prior to the final application. However, carryover on the flower heads is not expected as they were unlikely to be formed at the time of the first application.

In four broccoli trials in France with conditions matching Belgian GAP, except for 3 applications instead of 2, difenoconazole residues in flower heads on days 13–15 after the final application were 0.02, 0.05, 0.08 and 0.10 mg/kg.

In two broccoli trials from The Netherlands, with conditions matching Belgian GAP except for 3 applications instead of 2, difenoconazole residues in flower heads on day 14 after the final application were < 0.02 and 0.03 mg/kg.

In two broccoli trials in Spain, with conditions matching Belgian GAP except for 3 applications instead of 2, difenoconazole residues in flower heads on day 14 and day 21 (higher residues than on day 14) after the final application were 0.41 and 0.15 mg/kg.

In summary, difenoconazole residue in broccoli flower heads from the eight trials, in ranked order (median underlined), were: 0.02, 0.02, 0.03, <u>0.05</u>, <u>0.08</u>, 0.10, 0.15 and 0.41 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in broccoli of 0.5, 0.065 and 0.41 mg/kg respectively.

Brussels sprouts

In France, difenoconazole may be applied to Brussels sprouts 3 times at a rate of 0.13 kg ai/ha with a 21 days PHI.

In four Brussels sprouts trials from Belgium in 1999, with conditions in line with French GAP, difenoconazole residues in buttons on days 20–21 and 28 (higher residues than on day 21) after the final application were 0.02, 0.05, 0.07 and 0.09 mg/kg.

In eight Brussels sprouts trials in the UK, with conditions matching French GAP, difenoconazole residues in buttons on days 21–22 after the final application were 0.04, 0.05, 0.05, 0.06, 0.07, 0.08, 0.10 and 0.14 mg/kg.

In summary, difenoconazole residues in Brussels sprouts buttons from the 12 trials, in ranked order (median underlined), were: 0.02, 0.04, 0.05, 0.05, 0.05, 0.06, 0.07, 0.07, 0.08, 0.09, 0.10 and 0.14 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in Brussels sprouts of 0.2, 0.065 and 0.14 mg/kg respectively.

Cabbage

In France, difenoconazole may be applied to cabbage 3 times at a rate of 0.13 kg ai/ha with a 21 days PHI. In six trials from France, with application parameters in line with GAP, difenoconazole residues in cabbage heads were < 0.01 (2), 0.01, < 0.02 and < 0.05 (2) mg/kg.

In Germany, difenoconazole may be applied to cabbage 3 times at a rate of 0.1 kg ai/ha with a 21 days PHI. In two trials in Germany, with trial parameters in line with GAP, difenoconazole residues in cabbage heads were < 0.02 (2) mg/kg.

In five cabbage trials in Belgium in 1999, with conditions in line with French GAP, difenoconazole residues in cabbage heads on day 21 after the final application were < 0.02 (5) mg/kg.

In two cabbage trials in Germany in 2003, with conditions in line with French GAP, difenoconazole residues in cabbage heads on day 21 after the final application were < 0.02 and 0.19 mg/kg.

In two cabbage trials in The Netherlands in 2002, with conditions in line with French GAP, difenoconazole residues in cabbage heads on day 21 after the final application were < 0.02 (2) mg/kg.

In three cabbage trials in UK in 1990 with conditions in line with French GAP, difenoconazole residues in cabbage hearts on day 21 after the final application were 0.06, 0.10 and 0.13 mg/kg. The Meeting accepted that cabbage “hearts” meant the same as cabbage “heads”.

In summary, difenoconazole residues in cabbages from the 20 trials, in rank order (median underlined), were: < 0.01 (3), 0.01, < 0.02 (10), < 0.05 (2), 0.06, 0.10, 0.13 and 0.19 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in head cabbage of 0.2, 0.035 and 0.19 mg/kg respectively.

Cauliflowers

In France, difenoconazole may be applied to cauliflowers 3 times at a rate of 0.13 kg ai/ha with a 14 days PHI. In 12 trials from France matching GAP, difenoconazole residues in the flower heads were 0.01, < 0.02 (9), 0.03 and 0.10 mg/kg.

In a cauliflower trial in Switzerland in 2005, with conditions in line with French GAP, difenoconazole residues in flower heads on day 14 after the final application were < 0.01 mg/kg.

In two cauliflower trials in the UK in 1999 and 2005, with conditions matching French GAP, difenoconazole residues in flower heads on day 14 after the final application were < 0.02 and 0.02 mg/kg.

In summary, difenoconazole residues in cauliflowers from the 15 trials, in ranked order (median underlined), were: < 0.01, 0.01, < 0.02 (10), 0.02, 0.03 and 0.10 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in cauliflowers of 0.2, 0.02 and 0.10 mg/kg respectively.

Watermelons

Residue data were available only on the edible portion of the watermelons in the four trials provided, so estimation of an MRL was not possible.

Chilli peppers

In Indonesia, difenoconazole may be applied at 7 day intervals to chilli pepper crops at a spray concentration of 0.0063–0.013 kg ai/hL with no required PHI.

One trial from Indonesia matched GAP for maximum spray concentration with harvest on day 6 after treatment. A second Indonesian trial used a spray concentration of 0.025 kg ai/hL (2 × label rate). One trial from Malaysia matched Indonesian GAP for maximum spray concentration and harvest on the day of treatment. A second Malaysian trial used a spray concentration of 0.025 kg ai/hL (2 × label rate).

The Meeting agreed that, for a minor use, a minimum of three trials matching GAP conditions is needed. The Meeting was not able to recommend a maximum residue level for difenoconazole residues in chilli peppers.

Tomatoes

In Italy, difenoconazole may be applied to tomato crops 4 times at a rate of 0.13 kg ai/ha with a 7 days PHI.

In two tomato trials (glasshouse and polytunnel) in France in 2005, with conditions in line with Italian GAP, difenoconazole residues in tomatoes on day 7 after the final application were 0.04 and 0.05 mg/kg.

In five tomato trials (field) in Greece in 2001–2003, with conditions in line with Italian GAP, difenoconazole residues in tomatoes on day 7 and 10 (higher residues than on day 10) after the final application were 0.10, 0.13, 0.18, 0.28 and 0.36 mg/kg.

In a tomato trial (glasshouse) in UK in 2005, with conditions in line with Italian GAP, difenoconazole residues in tomatoes on day 7 after the final application were 0.10 mg/kg.

In two tomato trials (field) in Spain in 2003, with conditions in line with Italian GAP, difenoconazole residues in tomatoes on day 7 after the final application were 0.03 and 0.09 mg/kg.

In a tomato trial (polytunnel) in Spain in 2005, with conditions in line with Italian GAP, difenoconazole residues in tomatoes on day 7 after the final application were 0.12 mg/kg.

In summary, difenoconazole residues in tomatoes from the field trials were: 0.03, 0.09, 0.10, 0.13, 0.18, 0.28 and 0.36 mg/kg; and from protected trials were: 0.04, 0.05, 0.10, and 0.12 mg/kg. The data appear to be from similar populations and can be combined.

In summary, difenoconazole residues in tomatoes from the 11 trials, in ranked order (median underlined), were: 0.03, 0.04, 0.05, 0.09, 0.10, 0.10, 0.12, 0.13, 0.18, 0.28 and 0.36 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in tomatoes of 0.5, 0.10 and 0.36 mg/kg respectively.

Lettuce

In Spain, the registration document states that difenoconazole is registered for use on lettuce at a rate of 0.13–0.20 kg ai/ha with a 14 days PHI. The maximum application rate on the available label was 0.13 kg ai/ha. The Meeting agreed to use the GAP from the registration document.

In eight lettuce trials from Spain in 1991 and 2003 with application rates of 0.17–0.18 kg ai/ha (within 30% of GAP rate) the residues 13–14 days after the final application, in ranked order (median underlined), were: 0.07, 0.08, 0.29, 0.31, 0.51, 0.56, 0.65 and 1.0 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in head lettuce and leaf lettuce of 2, 0.41 and 1.0 mg/kg respectively.

Soya beans

In Brazil, difenoconazole may be applied to soya bean crops once at a rate of 0.075 kg ai/ha with a 30 days PHI. In six soya bean trials in 2000 and 2003 in Brazil with conditions in line with GAP, except that there were 2 applications in place of 1, difenoconazole residues in the dry beans on day 30 and 31 after the final application were < 0.01 (3) and < 0.02 (3) mg/kg.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in soya beans of 0.02* and 0.02 mg/kg respectively.

Carrots

In France, difenoconazole may be applied to carrot crops 3 times at a rate of 0.13 kg ai/ha with a 14 days PHI. In nine carrot trials in 1991–1993, 1996 and 2000 in France, with conditions in line with GAP, difenoconazole residues in the carrots on days 14 or 15 after the final application were 0.02, 0.02, 0.03, 0.03, 0.04, 0.05, 0.07, 0.11 and 0.13 mg/kg.

In two carrot trials in 1987 in Switzerland, with conditions in line with French GAP, difenoconazole residues in carrots on day 14 after the final application were 0.07 and 0.12 mg/kg.

In summary, difenoconazole residues in carrots from the 11 trials, in rank order (median underlined), were: 0.02, 0.02, 0.03, 0.03, 0.04, 0.05, 0.07, 0.07, 0.11, 0.12 and 0.13 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in carrots of 0.2, 0.05 and 0.13 mg/kg respectively.

Potatoes

In Spain, difenoconazole may be applied to potato crops 4 times at a rate of 0.2 kg ai/ha with a 30 days PHI. In seven potato trials in 2003 and 2005 in Spain with conditions in line with GAP except that only 2 applications were made, difenoconazole residues in the potato tubers on days 27–31 after the second and final application were < 0.01 (6) and 0.01 mg/kg.

In a trial in 2005 in Italy with the application rate in line with Spanish GAP, difenoconazole residues in potato tubers on day 29 after the second application were < 0.01 mg/kg.

The potato metabolism studies suggest that parent difenoconazole residues in tubers should be below LOQ. However, residues might be occasionally expected in tubers with surface exposure to spray application.

In summary, difenoconazole residues in potatoes from the eight trials, in rank order (median underlined), were: < 0.01 (7), 0.01 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in potatoes of 0.02, 0.01 and 0.01 mg/kg respectively.

Sugar beet

In Germany, difenoconazole may be applied to sugar beet crops twice at a rate of 0.1 kg ai/ha with a 28 days PHI. In 14 sugar beet trials in 1987–88 and 1995-96 in Germany with conditions in line with GAP except that in some trials 3 applications were made, difenoconazole residues in the sugar beet roots on days 27–30, or later if higher residues, after the second application were < 0.02 (4), 0.02 (4), 0.03, 0.03, 0.06, 0.08, 0.08 and 0.10 mg/kg.

In three sugar beet trials in 1985 and 1991 in France, with conditions in line with German GAP, difenoconazole residues in sugar beet tubers on days 25, 29 and 33 after the second application were all < 0.02 mg/kg.

In a sugar beet trial in Denmark with conditions matching German GAP, difenoconazole residue in sugar beet root 37 days after the second application was 0.08 mg/kg.

In a sugar beet trial in the UK with conditions matching German GAP, difenoconazole residue in sugar beet root 35 days after the second application was 0.08 mg/kg.

In summary, difenoconazole residues in sugar beet from the 19 trials, in ranked order (median underlined), were: 0.01, < 0.02 (7), <u>0.02</u> (4), 0.03, 0.033, 0.06, 0.08, 0.08, 0.08 and 0.10 mg/kg.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in sugar beet of 0.2 and 0.02 mg/kg respectively.

Asparagus

In France, difenoconazole may be applied to asparagus crops 3 times at 0.13 kg ai/ha. In asparagus crops protected by 6 to 8 applications of fungicide per year, the difenoconazole product should be used for the first three treatments and other products that act in a different way should be used to complete the season.

In four asparagus trials in France, two in Italy and two in Switzerland where difenoconazole was applied 4–8 times at 0.13 kg ai/ha and asparagus shoots were harvested for analysis 179–290 days later (approximating French GAP), the resulting difenoconazole residues were < 0.02 (7) and 0.02 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in asparagus of 0.03, 0.02 and 0.02 mg/kg respectively.

Celeriac

In Belgium, difenoconazole may be applied to celeriac 4 times at a rate of 0.13 kg ai/ha with a 14 days PHI. In three Belgian trials matching GAP, difenoconazole residues in celeriac roots 15 days after the final treatment were 0.08, 0.12 and 0.22 mg/kg.

The Meeting acknowledged that celeriac is a minor crop and decided to estimate an MRL based on the three trials. The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in celeriac of 0.5, 0.12 and 0.22 mg/kg respectively.

Celery

In France, difenoconazole may be applied to celery crops 4 times at a rate of 0.13 kg ai/ha with a 14 days PHI.

The Codex description of the sample to be analysed is: "*Whole commodity as marketed after removal of obviously decomposed or withered leaves.*" For celery, the commodity marketed is usually

trimmed celery, i.e., most foliage removed. In a number of the celery trials, leaf and stems had been detached and analysed separately. The Meeting agreed to use the stem data where stems and leaf were analysed separately.

In four celery trials in 2003–04 in France, with conditions in line with GAP, difenoconazole residues in celery stems on day 14 after the final application were 0.03, 0.04, 0.14 and 0.26 mg/kg.

In two celery trials in 1990 in Italy, with conditions in line with French GAP, difenoconazole residues in celery edible parts and celery stems on day 14 after the final application were 1.2 and 2.0 mg/kg respectively.

In two celery trials in 2004 in Spain and one in Switzerland in 1988, with conditions in line with French GAP, difenoconazole residues in celery stems on day 14 after the final application were 0.04, 0.05 and 0.17 mg/kg. Data from a second trial in Switzerland were not used because difenoconazole residues (0.02 mg/kg) in a sample from the control plot were significant with respect to the residue (0.058 mg/kg) in the treated plot.

In summary, difenoconazole residues in celery from the nine trials, in ranked order (median underlined), were: 0.03, 0.04, 0.04, 0.05, <u>0.14</u>, 0.17, 0.26, 1.2 and 2.0 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in celery of 3, 0.14 and 2.0 mg/kg respectively.

Rice

In Indonesia, difenoconazole may be applied to rice at 0.050 to 0.10 kg ai/ha, with one application at the mid booting stage (45 days after sowing) and one at the 75% flowering stage (approximately 60 days after sowing). These growth stages are interpreted as equivalent to BBCH 43–45 and BBCH 63–67 growth stages.

In two rice trials in Indonesia with application rates of 0.063 kg ai/ha (37% below maximum GAP) and with timing to match GAP, residues in rice grain were 1.3 and 0.75 mg/kg.

In three rice trials in Malaysia with application rates of 0.064–0.075 kg ai/ha and timing to match Indonesian GAP, difenoconazole residues in rice grain harvested 28–30 days after the second application were 0.15, 0.16 and 0.37 mg/kg. In another trial in Malaysia at 0.12 kg ai/ha and with similar timing, residues of difenoconazole in rice grain were 0.76 mg/kg.

In summary, difenoconazole residues in rice grain from the six trials were: 0.15, 0.16, 0.37, 0.75, 0.76 and 1.3 mg/kg.

The Meeting decided that six trials (some at application rates not close enough to maximum GAP) were insufficient for a major commodity such as rice and did not estimate a maximum residue level.

Wheat

In Switzerland, difenoconazole may be applied once to wheat crops at a rate of 0.13 kg ai/ha up to growth stage BBCH 61.

In three wheat trials in Denmark, three in France and one in Switzerland where the difenoconazole was applied at 0.13 kg ai/ha up to growth stage BBCH 61, residues of difenoconazole in wheat grain were all < 0.02 mg/kg.

In nine wheat trials in France and seven in the UK, where the difenoconazole was applied at 0.12–0.15 kg ai/ha from growth stages BBCH 61 to 87, residues of difenoconazole in wheat grain were also all < 0.02 mg/kg.

In summary, difenoconazole residues in wheat grain from the 23 trials were all < 0.02 mg/kg.

The metabolism studies suggest that parent difenoconazole residues should not occur in the grain. The Meeting agreed that the evidence supported an STMR of nil residues in wheat.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in wheat of 0.02* and 0 mg/kg respectively.

Rapeseed

In the UK, difenoconazole may be applied twice to oilseed rape crops at a rate of 0.13 kg ai/ha up to the end of flowering (growth stage BBCH 69).

In four oilseed rape trials in 1996 in Germany, with conditions in line with GAP of the UK, difenoconazole residues in rape seed on days 56–80 after the second application were all < 0.02 mg/kg.

In three oilseed rape trials in 1997 in Germany with the second of two applications of difenoconazole of 0.13 kg ai/ha at growth stages BBCH 69–75, i.e., later than approved in UK GAP, difenoconazole residues in rape seed on days 55–56 after the second application were all < 0.02 mg/kg.

In two oilseed rape trials in 1988 in France with two applications of difenoconazole of 0.13 kg ai/ha and harvest 83 days after the second application (probably before end of flowering), i.e., within the conditions of UK GAP, difenoconazole residues in rape seed were both 0.04 mg/kg.

In summary, difenoconazole residues in rape seed from the nine trials, in ranked order (median underlined), were: < 0.<u>02</u> (7), 0.04 and 0.04 mg/kg.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in rape seed of 0.05 and 0.02 mg/kg respectively.

Sunflower seed

In Switzerland, difenoconazole may be applied once to sunflower crops at a rate of 0.13 kg ai/ha up to growth stage BBCH 51. In three trials on sunflower in 2004–2005 in Switzerland according to the conditions of GAP, except that 2 applications were made instead of 1, difenoconazole residues in sunflower seed, harvested 68–73 days after the second application were all < 0.01 mg/kg.

In six sunflower trials in 2004–05 in France, with conditions matching Swiss GAP, except for 2 applications instead of 1, difenoconazole residues in sunflower seed harvested 59–101 days after the second application were < 0.01 (5) and 0.01 mg/kg.

In two sunflower trials in 2005 in Spain with conditions matching Swiss GAP, except for 2 applications instead of 1, difenoconazole residues in sunflower seed harvested 74 and 87 days after the second application were both < 0.01 mg/kg.

In summary, difenoconazole residues in sunflower seed from the 11 trials, in ranked order (median underlined), were: < 0.<u>01</u> (10), 0.01 mg/kg.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in sunflower seed of 0.02 and 0.01 mg/kg respectively.

Wheat straw and fodder

In Switzerland, difenoconazole may be applied once to wheat crops at a rate of 0.13 kg ai/ha up to growth stage BBCH 61. In a Swiss trial on wheat in 1989 with conditions matching GAP, difenoconazole residues in wheat straw harvested 45 days after the single application were 1.2 mg/kg.

In three wheat trials in 1989–1990 in Denmark with conditions in line with Swiss GAP, difenoconazole residues in wheat straw on days 57, 58 and 75 after the single application were 0.26, 0.64 and 0.31 mg/kg.

In two wheat trials in 1989 in France, with conditions in line with Swiss GAP, difenoconazole residues in wheat straw on days 57 and 63 after the single application were 0.73 and 0.82 mg/kg.

In summary, difenoconazole residues in wheat straw from the six trials, in ranked order (median underlined), were: 0.26, 0.31, 0.64, 0.73, 0.82 and 1.2 mg/kg.

The Meeting estimated a maximum residue level, an STMR value and a highest residue value for difenoconazole in wheat straw and fodder of 3, 0.685 and 1.2 mg/kg respectively.

Sugar beet leaves or tops

In Germany, difenoconazole may be applied to sugar beet crops twice at a rate of 0.1 kg ai/ha with a 28 days PHI. In 14 sugar beet trials in 1987–1988 and 1995–1996 in Germany with conditions in line with GAP except that in some trials 3 applications were made, difenoconazole residues in the sugar beet leaves or tops on days 27–30 after the second application were 0.084, 0.087, 0.09, 0.11, 0.20, 0.25, 0.25, 0.26, 0.43, 0.43, 0.47, 0.53, 0.62 and 0.95 mg/kg.

In a sugar beet trial in 1985 in France, with conditions in line with German GAP, difenoconazole residues in sugar beet leaves 24 days after the second application were 0.17 mg/kg.

In a sugar beet trial in Denmark with conditions matching German GAP, difenoconazole residues in sugar beet leaves 37 days after the second application were 0.45 mg/kg.

In a sugar beet trial in the UK, with conditions matching German GAP, difenoconazole residues in sugar beet leaves 27 days after the second application were 0.09 mg/kg.

In summary, difenoconazole residues in sugar beet leaves or tops from the 17 trials in ranked order (median underlined), were: 0.084, 0.087, 0.09, 0.09, 0.11, 0.17, 0.20, 0.25, 0.25, 0.26, 0.43, 0.43, 0.45, 0.47, 0.53, 0.62 and 0.95 mg/kg.

The Meeting estimated an STMR value and a highest residue value for difenoconazole in sugar beet leaves or tops of 0.25 and 0.95 mg/kg (fresh weight), respectively.

Fate of residues during processing

The Meeting received information on the fate of difenoconazole residues during the processing of apples for juice, carrots for juice and canning, grapes for wine and dried grapes, olives for oil, rape seed for oil, sugar beet for sugar and molasses, and tomatoes for juice and puree. Also information was provided on hydrolysis studies of difenoconazole to assist with identification of the nature of the residue during processing.

Processing factors have been calculated for difenoconazole residues in apples, carrots, grapes, olives and tomatoes. The data for rape seed and sugar beet could not be used as residue levels in the raw commodity did not exceed the LOQ.

Difenoconazole was stable under the hydrolysis conditions (pH, temperature, time) representing the food processes pasteurisation, baking, brewing and boiling and sterilisation.

Apples from difenoconazole field trials at exaggerated application rates were washed, sliced and pressed to separate pomace from juice. The juice was pasteurised at 80–82 °C for 30 minutes. Puree was produced by boiling washed apples until the puree passed through a sieve. Sugar, citric acid and ascorbic acid were added until the puree reached a pH of 3.0–4.5 and then was heated at 95 °C for 20 minutes.

In a grape drying trial in Chile, grapes were harvested 63 days after the third of 3 applications of difenoconazole at 1 × and 5 × the label rate. The grapes were washed for about one minute and then placed in wooden trays with mesh bottoms and subjected to sulphur dioxide fumigation for 12 h. The trays of grapes were then dried in ovens at 65 °C for about 36–40 h losing approximately two-thirds of their weight, 30 kg grapes producing 10 kg dried grapes.

Wine was produced from grapes in a series of supervised field trials in France and Spain. Difenoconazole residues appeared in the pomace, but not in the wine. In grape trials in Chile, difenoconazole residues appeared in the pomace, but not in the juice.

Olives from a difenoconazole field trial at an exaggerated rate (2 ×) were processed into virgin oil and refined oil. The virgin oil was separated by centrifuging the mixture of olive pulp (from milling) and added water. The oil was refined by a sodium hydroxide process to produce soap from free acids. Residue levels in virgin and refined oil were essentially the same.

In a tomato processing trial in France, tomatoes were harvested 7 days after the final of 3 applications of difenoconazole at 0.37 kg ai/ha. In processing to juice, unwashed tomatoes were crushed and sieved to produce juice and pomace. Finished juice was produced by pasteurization for 1 minute at 82–85 °C after citric acid and salt were added to raw juice. In the production of puree, unwashed tomatoes were crushed and concentrated in a saucepan and then sieved. Salt and citric acid were added and the puree, in glass jars, was sterilised for 10 minutes at 115 °C. In the simulation of canning, unwashed tomatoes were blanched and then immediately plunged into cold water to split and loosen the peel which was removed with a knife. The peeled tomatoes, in glass jars, were covered with tomato juice and sterilised for 10 minutes at 115–120 °C.

In a carrot processing trial in France, carrots were harvested 7 days after the final of 3 difenoconazole applications at 0.50 kg ai/ha. In the simulation of canning, carrots were sorted and peeled with both ends removed. The peeled carrots were washed thoroughly and blanched in boiling water for 1 minute and placed in jars with brine and added citric acid to produce a pH of 3.5 and then sealed and sterilized for 10 minutes at 115–120 °C. For cooked carrots, the washed carrots were cooked in boiling water for 15 minutes and packaged in plastic bags under vacuum. For juicing, carrots were washed thoroughly after sorting, peeling and end removal and were then processed in a juice extractor which separated juice from pulp in a centrifugal filter. After the pH of the juice was adjusted to 3.5 with citric acid, the juice was pasteurized at approximately 85 °C and packaged in glass jars.

Calculated processing factors and the median or best estimate are summarized in the following table.

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors.	Median or best estimate
Apple	juice	< 0.02, < 1.0. < 1.0	< 0.02
Apple	dry pomace	15.4	15
Apple	puree	0.14	0.14
Carrot	canned	0.02, 0.03, 0.05, 0.12	0.04
Carrot	juice	0.02, 0.05, 0.06, 0.12	0.055
Grapes	juice	< 0.5	< 0.5
Grapes	dry pomace	9.3, 10.3, 14.0, 15.4	12
Grapes	dried grapes	1.01, 1.4	1.2
Grapes	wine	< 0.18, < 0.20, < 0.20, < 0.29, < 0.33, < 0.33, < 0.33, < 0.50, < 0.50, < 0.50, < 0.50	< 0.18
Olives	refined oil	1.19, 1.40, 1.50, 1.51	1.4
Olives	virgin oil	1.47, 1.50, 1.50, 1.63	1.5
Tomatoes	canned tomato	< 0.05, 0.06, 0.07, 0.08	0.065
Tomatoes	juice	0.14, 0.15, 0.28, 0.32	0.22
Tomatoes	puree	0.54, 0.58, 0.74, 1.00	0.66

The processing factors for dry apple pomace (15), apple juice (< 0.02) and apple puree (0.14) were applied to the estimated STMR for pome fruits (0.11 mg/kg) to produce STMR-P values for dry apple pomace (1.65 mg/kg), apple juice (0.0022 mg/kg) and apple puree (0.015 mg/kg).

The processing factors for dry grape pomace (12), grape juice (< 0.5) and wine (< 0.18) were applied to the estimated STMR for grapes (0.03 mg/kg) to produce STMR-P values for dry grape pomace (0.36 mg/kg), grape juice (0.015 mg/kg) and wine (0.0054 mg/kg).

The processing factor for dried grapes (1.2) was applied to the estimated STMR and HR for grapes (0.03 and 0.07 mg/kg) to produce STMR-P and HR-P values for dried grapes (raisins) of 0.036 and 0.084 mg/kg respectively.

The Meeting estimated a maximum residue level for difenoconazole in dried grapes (= currants, raisins, sultanas) of 0.1 mg/kg. The estimated maximum residue level is the same as for grapes, so no separate MRL recommendation is necessary.

The processing factors for canned carrots (0.04) and carrot juice (0.055) were applied to the estimated STMR for carrots (0.05 mg/kg) to produce STMR-P values for canned carrots (0.002 mg/kg) and carrot juice (0.0028 mg/kg).

The processing factors for tomato puree (0.66), tomato juice (0.22) and canned tomato (0.065) were applied to the estimated STMR for tomatoes (0.10 mg/kg) to produce STMR-P values for tomato puree (0.066 mg/kg), tomato juice (0.022 mg/kg) and canned tomato (0.0065 mg/kg).

The processing factors for virgin olive oil (1.5) and refined olive oil (1.4) were applied to the estimated STMR for olives (0.465 mg/kg) to produce STMR-P values for virgin olive oil (0.70 mg/kg) and refined olive oil (0.65 mg/kg)

Residues in animal commodities

Livestock feeding

The meeting received lactating dairy cow feeding studies and a laying hen feeding study, which provided information on likely residues resulting in animal commodities, milk and eggs from difenoconazole residues in the animal diet.

Lactating dairy cows

Groups of 3 lactating Holstein dairy cows were dosed once daily via gelatin capsule with difenoconazole at 1 ppm (1 ×), 3 ppm (3 ×) and 10 ppm (10 ×) in the dry-weight diet for 29–30 consecutive days. Parent difenoconazole residues did not occur above LOQ in muscle, kidney or fat tissues or milk for any of the test doses, but were present in liver from the 10 ppm feeding-level group. Metabolite CGA 205375 was present in each of the tissues from the 3 and 10 ppm feeding-level groups and in the liver and fat from the 1 ppm feeding-level animals. The concentration of metabolite CGA 205375 in fat was approximately 3.3 times its concentration in muscle. The average concentrations of metabolite CGA 205375 in the tissues from the 10 ppm feeding-level animals were: muscle 0.020 mg/kg; liver 0.30 mg/kg; kidney 0.044 mg/kg; fat 0.072 mg/kg. For metabolite CGA 205375 in liver, the transfer factors for the 3 feeding levels were reasonably consistent. For fat, the transfer factors for metabolite CGA 205375 apparently decreased as the feeding level increased. For the 10 ppm feeding-level animals, metabolite CGA 205375 was consistently present in the milk from day 2 onwards at 0.005–0.009 mg/kg.

In a second study, groups of 3 lactating Holstein dairy cows were dosed once daily via gelatin capsule with difenoconazole at 1 ppm (1 ×), 5 ppm (5 ×) and 15 ppm (15 ×) in the dry-weight diet for 29–30 consecutive days. Parent difenoconazole residues did not occur above LOQ in muscle, kidney or fat tissues or milk for any of the test doses. Parent difenoconazole residues were present in liver from the 5 and 15 ppm feeding-level groups. Metabolite CGA 205375, the major part of the residue, was present in each of the tissues from the 5 and 15 ppm feeding-level animals and in the liver, kidney and fat from the 1 ppm feeding-level group. In the 15 ppm feeding-level group, the concentration of metabolite CGA 205375 in fat was approximately 3.1 times its concentration in muscle. The average concentrations of metabolite CGA 205375 in the tissues from the 15 ppm feeding-level animals were: muscle 0.04 mg/kg; liver 0.57 mg/kg; kidney 0.11 mg/kg; fat 0.12 mg/kg. For metabolite CGA 205375 in liver, the transfer factors for the 5 ppm and 15ppm feeding levels were close. For fat, the transfer factors for metabolite CGA 205375 were also consistent for the 5 ppm and

15 ppm feeding levels. Metabolite CGA 205375 reached a plateau level in milk of approximately 0.012 mg/kg within 2 days from the 15 ppm feeding-level animals. Metabolite 1,2,4-triazole (not included in the difenoconazole residue definition) was consistently present in the milk from the 5 and 15 ppm feeding levels groups where plateau concentrations in milk of approximately 0.017 mg/kg and 0.04 mg/kg respectively were quickly reached.

The two feeding studies were generally in good agreement of transfer factors. The Meeting decided to use the study with the 1 and 3 ppm feeding levels as most closely bracketing the dietary burdens.

Laying hens

Laying white leghorn hens were fed rations treated with difenoconazole at 0.3 ppm, 1 ppm, 3 ppm and 10 ppm for 28 consecutive days. Parent difenoconazole residues did not occur above LOQ (0.01 mg/kg) in muscle, fat, liver or eggs for any of the test doses. Metabolite CGA 205375 also was not present in the tissues above LOQ (0.01 mg/kg). Average levels of 1,2,4-triazole in the tissues from the 10 ppm feeding-level birds were: skin plus attached fat 0.012 mg/kg; peritoneal fat < 0.005 mg/kg; liver 0.02 mg/kg; muscle 0.022 mg/kg. Metabolite CGA 205375 occurred in eggs from the 1, 3 and 10 ppm feeding-level groups reaching a plateau after approximately 9 days with levels of 0.037 mg/kg and 0.13 mg/kg in eggs from the 3 and 10 ppm feeding-level groups respectively. At the 1 ppm feeding level, CGA 205375 was present in eggs at close to the LOQ (0.01 mg/kg). Metabolite 1,2,4-triazole occurred in eggs from the 1, 3 and 10 ppm feeding-level birds. It reached a plateau after approximately 6 days with plateau levels of 0.007, 0.020 and 0.060 mg/kg in eggs from the 1, 3 and 10 ppm feeding-level birds respectively.

Livestock dietary burden

The Meeting estimated the dietary burden of difenoconazole in livestock on the basis of the diets listed in Annex 6 of the 2006 JMPR Report (OECD Feedstuffs Derived from Field Crops). Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities. The percentage dry matter is taken as 100% when the highest residue levels and STMRs are already expressed as dry weight.

Estimated maximum and mean dietary burdens of livestock

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6. The calculations were made according to the livestock diets from US-Canada, EU and Australia in the OECD Table (Annex 6 of the 2006 JMPR Report).

	Livestock dietary burden, difenoconazole, ppm of dry matter diet					
	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.62	0.48	1.85	0.81	1.42	0.9 [2]
Dairy cattle	0.44	0.30	2.10 [1]	0.76 [3]	0.59	0.44
Poultry - broiler	0.01	0.01	0.12	0.05	0.01	0.01
Poultry - layer	0.01	0.01	0.54 [4]	0.20 [5]	0.01	0.01

[1] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat and milk.

[2] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[3] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[4] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs.

[5] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs.

Animal commodities, MRL estimation

For MRL estimation, the residues in the animal commodities are the sum of difenoconazole and CGA 205375 (1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1,2,4-triazol)-1-yl-ethanol)) expressed as difenoconazole.

Cattle

For MRL estimation, the high residues in the tissues were calculated by interpolating the maximum dietary burden (2.10 ppm) between the relevant feeding levels (1 and 3 ppm) from the dairy cow feeding study and using the highest tissue concentrations from individual animals within those feeding groups.

The STMR values for the tissues were calculated by taking the STMR dietary burden (0.95 ppm) as a proportion of the lowest feeding level (1 ppm) multiplied by the feeding-level residue (mean of the 3 animals).

Residues in the milk were below LOQ (0.005 mg/kg) for all samples from the 1 ppm and 3 ppm feeding groups, so the dietary burdens (2.10 and 0.95 ppm) were taken as a proportion of the 3 ppm to calculate the residues resulting from the dietary burdens.

In the table below, dietary burdens are shown in round brackets (), feeding levels and residue concentrations from the feeding study are shown in square brackets [] and estimated concentrations related to the dietary burdens are shown without brackets.

Dietary burden (ppm) Feeding level [ppm]	Milk	Muscle	Liver	Kidney	Fat
MRL					
	mean	highest	highest	highest	highest
MRL dairy cattle (2.10) [1, 3]	< 0.004 [< 0.005, < 0.005]	0.019 [< 0.01, 0.026]	0.11 [0.051, 0.15]	0.016 [< 0.01, 0.021]	0.028 [0.015, 0.038]
STMR					
	mean	mean	mean	mean	mean
STMR beef cattle (0.95) [0, 1]		< 0.01 [0, < 0.01]	0.043 [0, 0.045]	< 0.01 [0, < 0.01]	0.012 [0, 0.013]
STMR dairy cattle (0.76) [0, 1, 3]	< 0.001 [0, < 0.005, < 0.005)]				

The data from the cattle feeding studies were used to support mammalian meat and milk MRLs.

The Meeting estimated a maximum residue level and an STMR value for difenoconazole in milks of 0.005* and 0.001 mg/kg, respectively. No information was available on the distribution of residue between the fat and non-fat milk fractions.

For muscle, the residue arising from a dietary burden of 2.10 ppm was 0.019 mg/kg, while the residue resulting from a dietary burden of 0.95 ppm was < 0.01 mg/kg. For fat, the residue arising from a dietary burden of 2.10 ppm was 0.028 mg/kg, while the residue resulting from a dietary burden of 0.95 ppm was 0.012 mg/kg.

The Meeting estimated a maximum residue level for difenoconazole in mammalian meat (fat) of 0.05 mg/kg. The Meeting estimated STMR and HR values for meat (fat) of 0.012 and 0.028 mg/kg respectively. The Meeting estimated STMR and HR values for meat (muscle) of 0.01 and 0.019 mg/kg respectively.

For liver, the residue arising from a dietary burden of 2.10 ppm was 0.11 mg/kg, while the residue resulting from a dietary burden of 0.95 ppm was 0.043 mg/kg. The Meeting estimated a

maximum residue level, an STMR value and an HR value for difenoconazole in liver of 0.2, 0.043 and 0.11 mg/kg, respectively.

For kidney, the residue arising from a dietary burden of 2.10 ppm was 0.016 mg/kg, while the residue resulting from a dietary burden of 0.95 ppm was < 0.01 mg/kg. Although the residue levels in kidney were somewhat below those in liver, the Meeting decided that it was preferable to have an offal MRL which would be supported by the liver data.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in mammalian edible offal of 0.2, 0.043 and 0.11 mg/kg, respectively.

Poultry

In the table, dietary burdens are shown in round brackets (), feeding levels and residue concentrations from the feeding study are shown in square brackets [] and estimated concentrations related to the dietary burdens are shown without brackets.

Dietary burden (ppm) Feeding level [ppm]	Eggs	Muscle	Liver	Fat	Skin + attached fat
MRL					
	highest	highest	highest	highest	highest
MRL laying hens (0.54) [0, 1]	0.0054 [0, 0.01]				
MRL laying hens (0.54) [0, 3, 10]		< 0.00054 [0, < 0.01, < 0.01]	< 0.00054 [0, < 0.01, < 0.01]	< 0.00054 [0, < 0.01, < 0.01]	< 0.00054 [0, < 0.01, < 0.01]
STMR					
	mean	mean	mean	mean	mean
STMR laying hens (0.20) [0, 1]	< 0.0020 [0, < 0.01]				
STMR laying hens (0.20) [0, 3, 10]		< 0.0002 [0, < 0.01, < 0.01]	< 0.0002 [0, < 0.01, < 0.01]	< 0.0002 [0, < 0.01, < 0.01]	< 0.0002 [0, < 0.01, < 0.01]

The data from the laying hen feeding studies were used to support poultry meat and egg MRLs.

The residue levels of difenoconazole + CGA 205375, expressed as difenoconazole, in poultry tissues and eggs arising from the dietary burdens (0.54 and 0.20 ppm difenoconazole in feed, dry weight) were all less than the analytical method LOQ (0.01 mg/kg).

For poultry tissues, residues were below LOQ (0.01 mg/kg) even at the 10 ppm feeding level, so an estimate of the STMRs was made by dividing the dietary burden (0.20 ppm) by 10 ppm and multiplying by the LOQ (0.01 mg/kg) to produce a value of 0.00020 mg/kg. An estimate of the HRs was made by dividing the dietary burden (0.54 ppm) by 10 ppm and multiplying by the LOQ (0.01 mg/kg) to produce a value of 0.00054 mg/kg.

For eggs, residues were below LOQ (0.01 mg/kg) at the 1 ppm feeding level, so an estimate of the STMR was made by dividing the dietary burden (0.20 ppm) by 1 ppm and multiplying by the LOQ (0.01 mg/kg) to produce a value of 0.0020 mg/kg. Similarly, a calculation for the HR for eggs produced a value of 0.0054 mg/kg.

The Meeting estimated maximum residue levels of 0.01* mg/kg for poultry eggs, poultry meat (fat) and poultry edible offal.

The Meeting estimated STMRs of 0.0020 mg/kg for eggs and 0.00020 mg/kg for poultry meat and poultry edible offal.

The Meeting estimated HRs of 0.0054 mg/kg for eggs and 0.00054 mg/kg for poultry meat and poultry edible offal.

DIETARY RISK ASSESSMENT

Also see the General Report on triazoles.

Long-term intake

The evaluation of difenoconazole resulted in recommendations for MRLs and STMR values for raw and processed commodities. Where data on consumption were available for the listed food commodities, dietary intakes were calculated for the 13 GEMS/Food Consumption Cluster Diets. The results are shown in Annex 3.

The IEDIs in the thirteen Cluster Diets, based on estimated STMRs were 1–10% of the maximum ADI (0.01 mg/kg bw). The Meeting concluded that the long-term intake of residues of difenoconazole from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The IESTI of difenoconazole calculated on the basis of the recommendations made by the JMPR represented 0–10% of the ARfD (0.3 mg/kg bw) for children and 0–7% for the general population.

The Meeting concluded that the short-term intake of residues of difenoconazole resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.

5.11 DIMETHOMORPH (225)

TOXICOLOGY

Dimethomorph is a cinnamic acid derivative for which the chemical name is (*E*,*Z*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)acryloyl]morpholine or (*EZ*)-4-[3-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]morpholine, according to IUPAC and CAS nomenclatures respectively (CAS No. 110488-70-5). Dimethomorph is a mixture of *E*- and *Z*-isomers in the ratio of approximately 1 : 1.

Dimethomorph is a fungicide that disrupts fungal cell-wall formation. Fungicidal activity is primarily associated with the *Z*- isomer.

Dimethomorph has not been evaluated previously by the JMPR and was reviewed at the present Meeting at the request of the CCPR. All pivotal studies with dimethomorph were certified as complying with GLP.

Biochemical aspects

In most studies, the batch of dimethomorph used consisted of mixtures of the *E*- and *Z*- isomers in approximately equal amounts. It was reported that the two isomers can interconvert on exposure to light.

In several studies, the absorption, distribution, metabolism and excretion of dimethomorph were investigated in rats treated orally. After single oral doses of 10 or 500 mg/kg bw administered by gavage to male and female rats, more than 90% of the lower dose was absorbed and excreted via bile and 7% via urine. At 500 mg/kg bw, absorption decreased to 65% in males and 40% in females. Pre-treatment of the animals with nonlabelled dimethomorph at the lower dose did not influence the pattern of excretion. At 10 mg/kg bw, t_{max} for total radioactivity was reached after 1.4–2.8 h and excretion was virtually complete after 48 h. After 24 h, up to 10% of the administered dose was found in the gastrointestinal tract (including contents, 0.4–1% in the gastrointestinal tract only). Less than 1% of the administered dose was found in the carcass and in liver, and 0.2% or less of the

administered dose was found in the kidneys, plasma and erythrocytes. In all other organs, concentrations of radioactivity were no longer quantifiable. At 500 mg/kg bw, some delay in depletion from organs was observed; residual radioactivity in tissues as a percentage of administered dose was approximately threefold that at 10 mg/kg bw. After 168 h, the pattern of distribution was very similar. Dimethomorph is extensively metabolized by demethylation of one of the methoxy groups and formation of *O*-conjugate and degradation products of morpholine ring-opening were found.

Toxicological data

Dimethomorph is of low acute toxicity in rats; the oral LD_{50} was 350 mg/kg bw in females and 4300 mg/kg bw in males. The oral LD_{50}s for the *E*- and *Z*- isomers were similar. In studies with dimethomorph administered dermally or by inhalation, the LD_{50} was > 2000 mg/kg bw and the LC_{50} was > 4.24 mg/L, respectively. Dimethomorph was not a skin irritant and was not a skin sensitizer in the Magnusson & Kligman test. In a test for ocular irritation, all animals showed reddened conjunctivae and slight chemosis. These effects had resolved within 4 h after dosing.

In short- and long-term studies, the liver was consistently a target organ; increased organ weights were often accompanied by hepatocyte hypertrophy.

In short-term studies of toxicity in mice fed diets containing dimethomorph at up to 10 000 ppm, equal to 1145 mg/kg bw per day, dimethomorph was generally well tolerated, but increased liver weights were observed at all doses. In 4-week studies in rats given doses of 220 mg/kg bw per day and greater, decreased body-weight gains, liver-weight increases with increased hepatocyte hypertrophy and, at higher doses, histological changes in the intestine were observed. Generally, the same effects were also seen in two 4-week studies in rats given isolated *E*- and Z- isomers; NOAELs were 10 mg/kg bw per day. In a 13-week feeding study in rats allowed a recovery period after dosing, the NOAEL was 14 mg/kg bw per day on the basis of increased liver weights in females at higher doses; in males, an increase in vacuolation in adrenals was found and slightly changed haematology parameters at higher doses. In both sexes, vascular congestion of the ileum mucosa was also observed. The severity of most effects was reduced in the recovery period. In a later re-examination of histology slides, findings in the ileum of mice and rats, dilatation, mucosal hyperplasia and fibre separation in muscle layers were identified with rats being more sensitive than mice. In a 13-week feeding study in dogs, lip-licking and subdued behaviour were seen usually shortly after feeding at the highest dose of 43 mg/kg bw per day. At this dose, alkaline phosphatase activity in both sexes had nearly doubled, and males showed an increase in relative thymus weight and decreased prostate weight with increased fibrosis, while females showed an increase in liver weight. The NOAEL was 450 ppm, equal to 15 mg/kg bw per day. In the 52-week feeding study in dogs, liver weights in both sexes were increased at a dietary concentration of 450 ppm, equal to 15.2 mg/kg bw per day, and greater. At 1350 ppm, equal to 44.8 mg/kg bw per day, the highest dose, alkaline phosphatase activity was increased in males and females and, as in the 13-week study, prostate weights were decreased, with a very slightly increased severity of fibrosis. Testes weights were statistically significantly increased at the intermediate dose of 450 ppm and greater, but without a histological correlate and therefore this was not considered toxicologically relevant. The NOAEL was 450 ppm, equal to 15.2 mg/kg bw per day, on the basis of weight changes in the liver and prostate, and prostate fibrosis at 1350 ppm.

In a 24-month feeding study in mice, dimethomorph was well tolerated. Treatment-related findings were reduced absolute terminal body weight in males at 1000 mg/kg bw per day without decreased feed intake. In an interim sacrifice of animals at the highest dose at week 52, liver-weight increases and dilatations in the ileum were found; both effects were more pronounced in females. At study termination, more animals (males and females) with enlarged spleens were recorded, without a histological correlate. In females at the highest dose, an increase in the incidence of mammary adenocarcinomas was found that was within the range for historical controls. The Meeting concluded that the increased incidence in mammary adenocarcinoma was not due to a tumorigenic potential of

dimethomorph. The NOAEL was 100 mg/kg bw per day on the basis of body-weight gain decreases at the highest dose.

There were two 24 month feeding studies in rats, one of toxicity and another one of carcinogenicity. In the long-term feeding study in rats, feed intake was not affected in any group. At the highest dose (2000 ppm, equal to 99.9 mg/kg bw per day), body weight was decreased in females and an increase in relative kidney weights in females and a statistically non-significant increase in liver weights were observed in both sexes. Incidences of histological changes in the liver included ground-glass foci, periacinar hypertrophy and pigmentation, and were increased statistically significantly at the highest dose of 2000 ppm. The NOAEL was 750 ppm, equal to 36.3 mg/kg bw per day in males and 57.7 mg/kg bw per day in females, on the basis of body-weight decreases and histological changes in the liver of females at 2000 ppm. Effects observed in the study of carcinogenicity in rats were similar to those seen in the study of toxicity. Feed intake was decreased only decreased by 6% in females at the highest dose only. At the lowest dose of 200 ppm, equal to 8.8 mg/kg bw per day, body-weight gain among females was reduced by 9%, with reductions of 23% and 38% at the next higher doses. Although body-weight gain in females at 750 ppm was statistically significantly decreased, the effect was not considered to be treatment-related because there was high variability in body-weight development in the control and the dosed groups between the two 2-year studies. In males, body-weight gain was only impaired in the group at the highest dose of 2000 ppm, equal to 94.6 mg/kg bw per day. In animals at the highest dose, an increase in swollen hind feet/limbs was seen, with unknown etiology. In males, the frequency of findings of lymph node cysts and dilated blood vessels was also increased. At dietary concentrations of 2000 ppm, statistically significantly increased incidences of histological changes in the liver of males and females were seen, increased pancreatitis being observed in males at the highest dose. As in the study of toxicity, males receiving dimethomorph showed more interstitial-cell hyperplasia and adenoma of the testes than did the controls. In both studies, the incidence of adenoma at 2000 ppm, equal to 94.6 mg/kg bw per day, the highest dose, were close to the upper limit of the range for historical controls. However, there was no clear dose–response relationship and therefore adenoma was considered as part of a continuum with interstitial-cell hyperplasia. Additionally, this type of tumour is usually secondary to hormonal perturbation, an effect that is not suggested by the current toxicology database for any species. In males at the highest dose, there was also an increase in the incidence of benign medulla tumours of the adrenals when compared with concurrent controls, but this was within the range for historical controls. The Meeting concluded that dimethomorph was not tumorigenic in rats. When evaluating the two 2-year studies together, the overall NOAEL was 750 ppm, equal to 36.3 mg/kg bw per day, on the basis of reduced body-weight gain in both sexes and histological changes in the liver of females at 2000 ppm.

Dimethomorph was not carcinogenic in mice or rats.

With the exception of three assays for chromosomal aberration in V79 cells and in human lymphocytes, dimethomorph gave negative results in a battery of appropriate tests for genotoxicity. A slight increase in the frequency of aberrant cells was found in V79 cells and in human lymphocytes at high doses, with reduced mitotic indices and slight precipitation of the compound.

The Meeting concluded that dimethomorph was unlikely to be genotoxic in vivo.

On the basis of the absence of carcinogenicity and genotoxicity, the Meeting concluded that dimethomorph is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity studying rats, there was a reduction in body-weight gain in dams at the highest concentration of 1000 ppm, equal to 80 mg/kg bw per day, in the pre-mating period; this was compensated for thereafter and had no impact on reproductive performance. In F_1, F_{2a} and F_{2b} pups, the only finding was a slight delay in incisor eruption, which did not affect feeding capacity. Overall, pups developed normally; no other developmental markers, such as eye-opening, pinna unfolding or hair growth, were affected. Therefore, the NOAEL for maternal and reproductive toxicity was 1000 ppm, equal to 80 mg/kg bw per day, the highest dose tested.

In studies of developmental toxicity in rats, reduced feed consumption and reduced body-weight gain were recorded at the highest dose of 160 mg/kg bw per day on days 6–10 of gestation, but not thereafter. On day 20 of gestation, the difference in absolute body weight compared with that in control animals was marginal. At the highest dose, an increase in total litter losses and an increase in post-implantation losses in females with live foetuses at terminal sacrifice were observed. There were no other effects on foetal development. The NOAEL for developmental toxicity was 160 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity and embryotoxicity was 60 mg/kg bw per day, on the basis of intermittent decreases in body-weight gain in dams and post-implantation losses.

In two studies of developmental toxicity in rabbits, dams lost weight or did not show an increase in body weight during days 6–12 of gestation at 600 and 650 mg/kg bw per day, respectively. At this dose, the incidence of total litter losses was increased, but there were no increases in malformations or variations. The NOAEL for maternal and developmental toxicity was 300 mg/kg bw per day.

The Meeting concluded that dimethomorph is not teratogenic.

The Meeting considered that dimethomorph is not neurotoxic on the basis of the available data.

The Meeting concluded that the existing database on dimethomorph was adequate to characterize the potential hazards to foetuses, infants and children.

No health effects related to exposure to dimethomorph were reported in personnel working in a production plant.

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw based on a NOAEL of 15.2 mg/kg bw per day identified on the basis of the liver weight and clinical chemistry changes and prostate weight changes and prostate fibrosis observed at higher doses in the 13-week and the1-year studies in dogs. A safety factor of 100 was applied.

The Meeting established an ARfD of 0.6 mg/kg bw based on a NOAEL of 60 mg/kg bw per day identified on the basis of post-implantation losses at higher doses in the study of developmental toxicity in rats. A safety factor of 100 was applied.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 mg/kg bw per day	1000 mg/kg bw per day
		Carcinogenicity	1000 mg/kg bw per day[c]	—
Rat	Two-year studies[d]	Toxicity	750 ppm, equal to 36.3 mg/kg bw per day	2000 ppm, equal to 99.9 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 99.9 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	1000 ppm, equal to 80 mg/kg bw per day[c]	—

		Offspring toxicity	1000 ppm, equal to 80 mg/kg bw per day[c]	—
	Developmental toxicity[b]	Maternal toxicity	60 mg/kg bw per day	160 mg/kg bw per day
		Embryo/fetotoxicity	60 mg/kg bw per day	160 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	300 mg/kg bw per day	650 mg/kg bw per day
		Embryo/fetotoxicity	300 mg/kg bw per day	650 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[ad]	Toxicity	450 ppm, equal to 15.2 mg/kg bw per day	1350 ppm, equal to 44.8 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] The results for two studies were combined.

Estimate of acceptable daily intake for humans

0–0.2 mg/kg bw

Estimate of acute reference dose

0.6 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, > 90% within 24 h
Dermal absorption	4.75% after application of single dose of 7.7 mg/kg bw for 8 h
Distribution	Extensive
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Rapid, close to 100% within 48 h, mainly via faeces
Metabolism in animals	Extensive, demethylation and morpholine ring-opening
Toxicologically significant compounds in animals, plants and the environment	Dimethomorph
Acute toxicity	
Rat, LD_{50}, oral	3900 mg/kg bw > 5000 mg/kg bw (*Z*-isomer) 4715 mg/kg bw (*E*-isomer)
Rat, LD_{50}, dermal	> 2000 mg/kg bw; > 5000 mg/kg bw (*Z*-isomer)
Rat, LC_{50}, inhalation	> 4.24 mg/L

Rabbit, skin irritation	Not irritating
Rabbit, eye irritation	Initially slightly irritating
Guinea-pig, skin sensitization (test method used)	Not a sensitizer (Magnusson & Kligman)
Short-term studies of toxicity	
Target/critical effect	Prostate and liver and clinical chemistry (dogs)
Lowest relevant oral NOAEL	15.2 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Unlikely to be genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver histology and body-weight decrease (rats)
Lowest relevant NOAEL	36.3 mg/kg bw per day
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	80 mg/kg bw per day, the highest dose tested
Developmental target/critical effect	Increased incidence of total litter losses (rats and rabbits)
Lowest relevant developmental NOAEL	60 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
	No evidence in conventional studies
Other toxicological studies	
	In pharmacological studies, evidence for an increase in phenobarbital sleeping time
Medical data	
	Medical surveillance of workers in a plant producing dimethomorph did not reveal any adverse health effects.

Summary

	Value	Study	Safety factor
ADI	0–0.2 mg/kg bw	Dog, 13-week and 1-year study	100
ARfD	0.6 mg/kg bw	Rat, study of developmental toxicity	100

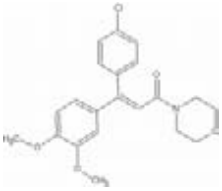
RESIDUE AND ANALYTICAL ASPECTS

Dimethomorph is a morpholine fungicide with protective action against plant pathogenic Phytophthora species and a number of downy mildew diseases of fruit, vegetables and potatoes. It was included on the schedule of new compounds for consideration by the 2007 JMPR. The Meeting received a full data package including animal and plant metabolism studies (goats, hens, grapes, potato, lettuce, tomato), soil metabolism, dissipation and photodegradation, crop rotational studies, information on analytical methods, freezer storage stability, supervised residue trial data from use as a

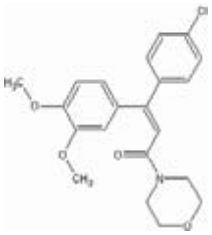
foliar spray on a range of fruit, vegetable, cereal and oil seed crops, processing studies and livestock feeding studies. GAP information was also submitted by Australia.

Chemical name and structure

(E,Z) 4-[3-(4-chlorophenyl)-3-(3,4-dimethoxy-phenyl)-1-oxo-2-propenyl]-morpholine



E- isomer



Z- isomer

The following abbreviations are used for the metabolites discussed below:

*Z*7	4-Chloro-3',4'-dimethoxy-benzophenone
*Z*67	(*E/Z*)-4-(3-(4-Chlorophenyl)-3-(3'-methoxy-4'-hydroxyphenyl)-1-oxo-2-prophenyl)-morpholine
*Z*69	(*E/Z*)-4-(3-(4-Chlorophenyl)-3-(3'-hydroxy-4'-methoxyphenyl)1-oxo-2-prophenyl)-morpholine
*Z*89	N-[3-(4-chlorophenyl)-3-3,4-dimethoxyphenyl)-1-oxo-2-propenyl-glycine
CL 411266	4-[(1Z)-1-(4-chlorophenyl)-3-(4-morpholinyl)-3-oxo-1-propenyl]-2-methoxyphenyl

Animal metabolism

The Meeting received information on the fate of orally dosed dimethomorph in the lactating goat and in laying hens. Experiments were carried out with dimethomorph with the chlorophenyl ring uniformly labelled with [^{14}C]. Metabolism in laboratory animals (rats) was summarized and evaluated by the WHO panel of this JMPR Meeting.

In rats, after oral gavage with a single dose of 10 mg/kg bw per day, dimethomorph is quantitatively absorbed and excreted to more than 90% via bile and to 7% via urine in both sexes. Following a single dose of 500 mg/kg bw per day, absorption was decreased to 65% in males and to 40% in females. At the low dose rate, excretion virtually is complete after 48 h with less than 1% of dose found in carcass and in liver and less or equal to 0.2% in kidneys. Dimethomorph is extensively metabolized by demethylation of one of the methoxy groups and formation of O-conjugate and degradation products of morpholine ring opening were found.

Two lactating goats orally treated twice daily with [^{14}C] labelled dimethomorph at 0.55 mg/kg bw per day (equivalent to 25 ppm in feed for a day). Each animal received 15 doses over 7.5 days, the last being the morning of the 8th day, 4 h before slaughter.

Most of the applied radioactivity was excreted in urine (about 15%) and faeces (about 72%). Total Radioactive Residues (TRR) in edible tissues averaged 7.1 mg/kg in liver, 0.28 mg/kg in kidney, 0.07 mg/kg in fat and 0.03 mg/kg in muscle. In milk, residues reached a plateau of about 0.06 mg/kg after 2 days, with residues generally ranging from 0.03–0.1 mg/kg (average 0.06 mg/kg). About 80% of the TRR in milk was present in whey. The overall recovery of the radioactivity was 88–92%.

The unchanged parent was the primary residue, comprising 72% of the liver TRR, 10% of the kidney TRR, 7.5% of the muscle TRR and 75% of the fat TRR. In milk, the major identified residue

was the polar Z89 metabolite, making up approximately 48% of the milk TRR. Metabolites Z67 and Z69 were also detected in liver at levels of 3–4% of the liver TRR.

The proposed metabolic pathway for dimethomorph in the lactating goat is similar to that suggested for rats, involving the demethylation of one of the phenolic methoxy-groups, with an alternative pathway being the cleavage of the morpholine-ring.

Groups of 6–9 laying hens were orally dosed twice daily for seven consecutive days with 1 mg [^{14}C] labelled dimethomorph/kg bw per day, equivalent to 40 ppm in the feed. The hens were sacrificed 8 h, 7 days and 12 days after the last administration. Most of the administered radioactivity (85%) was found in the excreta, with edible tissues (liver, kidney, muscle and fat) containing about 0.4% and < 0.1% found in eggs. The highest radioactive residues were in liver (1.1 mg/kg dimethomorph equivalents) with lower levels found in kidney (0.3 mg/kg), fat/skin (0.04 mg/kg) and muscle (0.02 mg/kg). In egg whites, TRR reached a maximum of 0.056 mg/kg after 4 days, while in yolks, highest TRR (0.51 mg/kg) was found at day 7. At the end of the depuration period (12 days) the radioactivity levels had decreased to about the background level of 0.01 mg/kg (egg whites) and 0.02 mg/kg (yolks). The overall recovery of the radioactivity (including residues in the cage wash) was around 88%.

Dimethomorph (unchanged parent) was only found in fat and skin, at levels of < 0.02 mg/kg. Metabolites Z67 and Z69 were the major residue components identified in liver (0.13 mg/kg), egg yolks (0.07 mg/kg), kidney (0.03 mg/kg) and muscle (0.003 mg/kg). Low levels (0.02–0.05 mg/kg) of the Z43 and Z95 metabolites were reported in kidney and/or egg yolks.

In general, the metabolism of dimethomorph in farm animals is similar to that in laboratory animals, and is mostly (85–87%) excreted in urine or faeces. Most of the remaining radioactive residues are found in liver and to a much lesser extent in kidney and egg yolk, with other edible tissues and milk containing less than 0.1 mg/kg TRR. Unchanged parent is the predominant residue identified in goat liver (about 5 mg/kg) and is also present in fat, kidney (goats), poultry skin and muscle (goats), but at levels below 0.06 mg/kg. In milk, the major residue is the Z89 metabolite (0.05 mg/kg). The metabolites Z67 and Z69 are the major residue components present in poultry, mostly in liver and kidney but also at low levels in muscle (0.003 mg/kg) and in egg yolks (0.07 mg/kg).

The Meeting concluded that the major residue component in ruminant animal commodities, from the oral administration of dimethomorph, is the parent compound, with the metabolite Z89 being the major residue in milk and that the metabolites Z67 and Z69 are the predominant residues in poultry commodities.

Plant metabolism

The Meeting received information on the fate of dimethomorph in grapes, potato, lettuce and tomato, following treatment with [p-chlorophenyl-U-^{14}C]dimethomorph and also on potato following treatment with [morpholine-U-^{14}C]dimethomorph.

Grape vines grown outdoors under shelter in Germany were treated by syringe with an EC formulation of [^{14}C] labelled dimethomorph at a rate equivalent to 0.09 kg ai/hL or 0.9 kg ai/ha with four applications being made at 9–10 day intervals up to 35 days before harvest. Grapes and leaves were washed with acetone to remove surface residues and the washed samples were then homogenised and remaining residues were further extracted with acetone and methanol. Radioactive residues in the surface washes (leaves and grapes) accounted for 70–72% of the applied radioactivity with about 26% of the TRR in grapes (3.8 mg/kg) being found in the homogenised samples. The majority of the extractable residue was the unchanged parent (83–86% TRR).

Potato plants grown in pots in a glasshouse in Germany were sprayed with [^{14}C]-[chlorophenyl]-dimethomorph (EC) at a rate equivalent to 0.06 kg ai/hL or 0.6 kg ai/ha with four applications at 10 day intervals with the last up to 7 days before harvest. Stems, leaves and tubers

were washed with acetone to remove surface residues and the washed samples were then homogenised and remaining residues extracted with methanol. About 61% of the recovered radioactivity was present as a surface residue. The majority of the extractable residue in foliage was the unchanged parent dimethomorph (68% of the TRR). Only trace amounts of radioactivity (0.01–0.02 mg/kg) were found in tubers.

In a complimentary potato metabolism study, dimethomorph (EC) labelled with [^{14}C] in the morpholine ring was applied to greenhouse potato plants at a rate equivalent to 0.06 kg ai/hL (0.6 kg ai/ha) with four applications at 10 day intervals up to 7 days before harvest. Surface residues were removed in acetone, after which the samples were homogenised and the remaining residues extracted in methanol. In treated foliage the acetone surface wash contained about 72% of the TRR. Small amounts of radioactivity were measured in tubers from treated plants predominantly in peel where radioactive residues of < 0.03 mg/kg were found. The majority of the foliage residue was the unchanged parent (76% of the foliage TRR or 13.8 mg/kg) with the remaining extractable residue consisting of several unknown (mainly polar) metabolites at levels too low to identify.

In an additional study on potato plants grown in a lysimeter, [^{14}C]-[chlorophenyl]-dimethomorph (DC) was applied at a rate equivalent to 0.3 kg ai/ha as a foliar spray three times, 10 days apart, up to 28 days before harvest. About 98% of the recovered radioactivity was found in the foliage, with about 1.5% TRR being found in the tubers, 0.8% (0.12 mg/kg) in the peel and 0.7% (0.025 mg/kg) in the peeled potatoes. Further investigation of the tuber residues identified the unchanged parent compound to be the major residue, predominantly in the peel (about 46% or 0.06 mg/kg), with the metabolites Z67 and Z69 comprising < 10% of the peel TRR.

In field grown lettuce, chlorophenyl ring labelled [^{14}C]dimethomorph (DC) was applied in four successive foliar applications at a rate equivalent to 1.14 kg ai/ha. Applications were made 8 days after transplanting and at intervals of 9, 10 and 11 days, with plants (without roots) being sampled four days after the final application. Close to 99% of the TRR was extracted from macerated samples with acetone or acetone:water with most of this (93%) being the unchanged parent. Trace levels of the metabolites Z7 and Z67 were also reported, each accounting for 0.5% TRR (0.5 mg/kg). The remaining extractable residue (4.5% TRR) consisted of several minor unknown polar components, which were not further characterized.

Tomatoes (young plants) were treated with [^{14}C]-[chlorophenyl]-dimethomorph by the addition of 8 mg ai/L to the hydroponic nutrient solution for 7 days. Plant samples (without roots) were taken 0, 14 and 28 days after termination of the application. Total radioactive residues in leaves and stems, at the end of the 7 day exposure period were 24.5 mg/kg dimethomorph equivalents, reducing to 12.5 mg/kg after 14 days and to 7 mg/kg after 28 days. Dimethomorph was the predominant residue, initially comprising 66% of the TRR and reducing to 28% after 14 days and to 16% after 28 days. The calculated half-life of the parent compound was about 13 days. The demethylated metabolite Z69 (including conjugates) was the major metabolite found (13–34% TRR), with metabolites Z93 (8–17% TRR), Z95 (4–8% TRR) and Z98 (1–7% TRR) also being found.

The Meeting concluded that the metabolic pathways of dimethomorph in plants show a common pattern, with the unchanged parent being the only significant residue in plant commodities. Residues are mostly found on the plant surface, i.e., negligible systemic translocation, with only low residues being found in potato tubers (almost all in the peel). However, when young tomato plants are exposed to dimethomorph in a hydroponic nutrient solution, residues can be taken up by the roots and translocated to leaves and stems. The primary metabolic pathway involves the demethylation of the dimethoxyphenyl ring to produce the metabolites Z67 and Z69, with the probable formation of the associated glucose conjugates. A secondary pathway involves the hydrolysis of dimethomorph to form the Z7 metabolite.

Environmental fate in soil

The Meeting received information on the environmental fate of dimethomorph in soil, including aerobic soil metabolism, soil photodegradation and also confined and field rotational crop studies.

Aerobic soil metabolism

In six laboratory studies the degradation of dimethomorph in soil under aerobic conditions was investigated in sand, loamy sand, sandy loam and silty clay loam, using [^{14}C] labelled dimethomorph (labelled in either the chlorophenyl or the morpholine ring). Under sterile conditions, dimethomorph was stable, with about 90% of the applied radioactivity identified as the parent compound after four months (compared with 36% remaining in a comparable unsterile soil), indicating microbial action was the major source of degradation. As the levels of extractable dimethomorph decreased over time, there was a corresponding increase in unextracted residues, the nature of which was not investigated. The shift in the ratio of *E*- and *Z*-isomers of dimethomorph was investigated in most of these studies, with the initial *E:Z* ratio of about 50:50 shifting to about 40:60 after 60–90 days and 30:70 after 180 days. Half-lives in the laboratory studies ranged from 47 days to 90 days except in one atypical acidic sandy soil (pH 3.5, 99% sand), where 86% of the applied dimethomorph remained after 120 days.

In field studies, where dimethomorph was applied at rates of 0.43–0.6 kg ai/ha to a range of different soil types (sand, loamy sand, sandy loam, clay), dimethomorph residues were only detected in the top 10 cm, with trace amounts of the metabolites Z67 and Z69 being found in the top 20 cm, but only within the first two months after treatment. Half-lives for dimethomorph in these studies ranged from 10–61 days.

Photodegradation in soil

In a soil photolysis study where [^{14}C] labelled dimethomorph was added to sterile sandy-loam soil and exposed to light continuously for 15 days, less than a 10% decrease in dimethomorph residues was observed, with two minor (unidentified) metabolites found at levels up to 4.2% of the applied radioactivity. During the study period, the *E/Z*-isomer ratio shifted from about 40:60 to 34:66.

Residues in rotational crops

In two confined rotational crop studies, [^{14}C] labelled dimethomorph was applied to bare soil at rates equivalent to 4 kg ai/ha and 1.7 kg ai/ha.

In the first study, lettuce, carrots and wheat were planted in soil treated with dimethomorph to simulate the application of 4 kg ai/ha followed by incorporation to a depth of 15 cm and aged for 29, 120 and 361 days. Total radioactive residues of 4.8 mg/kg were found in wheat straw, 1 mg/kg in wheat forage and 0.14–0.2 mg/kg in carrot tops and lettuce planted in the 29 day aged soil. Dimethomorph residues in soil at the time of planting were about 0.8 mg/kg. In the soil aged for 120 days, dimethomorph residues at planting were about 0.05 mg/kg and total radioactive residues in all subsequent crops were < 0.1 mg/kg except wheat foliage (0.24 mg/kg) and wheat straw (0.78 mg/kg).

In the second study, wheat, lettuce, soya beans and radish were planted in soil treated with the equivalent of 1.7 kg ai/ha and aged for 30–394 days. In soil aged for 30 days, total radioactive residues were < 0.1 mg/kg in all crops except wheat straw (0.15 mg/kg). Dimethomorph residues were 0.01 mg/kg or less in all crops and the only metabolite found was CL 411266, at 0.04 mg/kg in wheat straw and 0.01 mg/kg in radish tops and wheat forage. In soil aged for 60 days, total radioactive residues were < 0.05 mg/kg in all crops except wheat straw (0.13 mg/kg). Dimethomorph residues were not found in any crops and the metabolite CL 411266 was measured in wheat straw (0.03 mg/kg) and lettuce (0.02 mg/kg). Radioactive residues did not exceed 0.05 mg/kg in any samples from crops grown in soil aged for 394 days.

Rotational crop field studies were conducted Germany, where carrots, spinach and beans were planted immediately after harvest of a potato crop treated with 3 applications of 0.18 kg ai/ha dimethomorph (PHIs of 2–6 weeks). At the time of planting the rotational crops, dimethomorph residues in soil were 0.08–0.14 mg/kg. Dimethomorph residues in subsequent crops were all 0.02 mg/kg or less, except in spinach sampled 72–76 days after the last soil treatment, where residues of 0.09 mg/kg and 0.21 mg/kg were found. Residues were below the limit of quantification in all three crops at maturity.

The Meeting concluded that dimethomorph is stable to hydrolysis and photolysis and is moderately persistent in soil with field half-lives of 10–61 days. In rotational crops, dimethomorph can be taken up by the roots and dimethomorph residues may occur in early harvest crops (e.g., spinach) planted within 44 days of the last application.

Methods of analysis

The Meeting received data on analytical methods for enforcement and monitoring of dimethomorph and its major metabolites in plant and animal commodities. A number of these methods are capable of determining the individual dimethomorph isomers but in most cases these residues have been combined in the supervised field trial reports, as to minimise isomerization reactions during preparation and analysis, the analytical work needs to be conducted in the absence of light. However these isomerization reactions do not influence the measurement of total residues.

Analytical methods for enforcement and monitoring

The multi-residue analytical method DFG S19, with a modification to use ethyl acetate:cyclohexane instead of dichloromethane in the partition clean-up step, has been validated in a range of commodities as an enforcement-monitoring method for the determination of dimethomorph in plant commodities. With an alternative extraction procedure for fat (DFG Method 5), this method can be used to measure dimethomorph residues in animal matrices. Reported LoQs are 0.01 mg/kg for animal matrices and 0.02 mg/kg for plant matrices.

Analytical methods used in study reports

Analytical methods used in the supervised residue trials and in the animal residue studies generally involve extraction with acetone, acetonitrile or acidified methanol with residues being partitioned into dichloromethane, ethyl acetate or cyclohexane and cleaned-up by gel permeation chromatography prior to analysis. An additional silica gel column clean-up step is included in some methods, and for some matrices, an additional partition step with hexane (to remove fatty constituents) is included. Analysis can be by HPLC-UV, GC-NPD, GC-MS or HPLC-MS/MS. In most of the commonly used methods, LOQs of 0.01 mg/kg or 0.02 mg/kg have been reported. The methods used in the animal studies were capable of measuring the parent compound, the Z89 metabolite and the sum of the Z67 and Z69 metabolite residues.

Validation studies on the more commonly used analytical methods generally reported mean recovery rates of 73–116% when a wide range of plant and animal matrices were fortified with dimethomorph at concentrations of 0.01–5 mg/kg and with 0.1 mg/kg of the metabolites Z89, Z67 and Z69 in the case of cattle matrices.

The Meeting concluded that adequate analytical methods exist for the determination of dimethomorph in crops and livestock commodities both for data collection and MRL enforcement purposes.

Stability of residues in stored analytical samples

The Meeting received information on the frozen storage stability of residues in cattle milk and edible tissues, grapes, rape seed, hops, tomato, broccoli, spinach, potato and processed grape, hops, tomato

and potato matrices. In all cases, residues were stable in the macerated matrices under conditions of frozen storage for an interval at least as great as the storage interval of supervised field trial or livestock feeding samples.

Dimethomorph residues were stable under conditions of frozen storage for the intervals tested: 24 months in broccoli, grapes, spinach, tomato, 21 months in processed tomato matrices, 18 months in soil, rape seed, hops, beer, spent hops and brewer's yeast, 16 months in processed grape commodities, 14 months in raisins and 6 months in potatoes and processed potato commodities. In cattle meat, milk, liver and kidney, residues of dimethomorph and its metabolites Z67 and Z69 were stable for the 16 month frozen storage interval. The predominant residue in milk (the Z89 metabolite) was also stable over the 16 month test interval.

The Meeting concluded that dimethomorph is stable (less than 10% loss of residues) in most crop, processed commodity, and livestock commodity samples under frozen storage conditions.

Definition of the residue

In plants, dimethomorph is stable to hydrolysis and occurs mostly as surface residue with no significant metabolism. The major residue resulting from foliar applications of dimethomorph is the parent compound, present as a mixture of the *E*- and *Z*-isomers, the ratio of which can change over time as a result of isomerisation reactions stimulated by light.

In animals, while metabolism studies indicate that the parent compound is the major residue in cattle liver and fat, residues of the metabolites Z67 and Z69 are also present in significant amounts and metabolite Z89 is the largest single component in milk. In poultry commodities, the parent compound is only found in fat and skin, with metabolites Z67 and Z69 being the major residues. However the Meeting noted that these results were from feeding studies involving exaggerated dosing regimes, and that under practical conditions, residues are not expected in animal commodities.

A validated multi-residue method is available to measure dimethomorph, as the sum of the *E*- and *Z*-isomers in both plant and animal matrices.

Based on the above, the Meeting agreed:

Definition of the residue in plant commodities for estimation of dietary intake and for compliance with MRLs: dimethomorph (sum of isomers).

Definition of the residue in animal commodities for estimation of dietary intake and for compliance with MRLs: dimethomorph (sum of isomers).

The results of the animal metabolism studies indicate that dimethomorph is not fat-soluble.

Results of supervised trials on crops

Supervised trials were available for the use of dimethomorph as a foliar spray on citrus (oranges), strawberries, grapes, pineapples, onions, green onions, brassica vegetables (cabbage, broccoli, kohlrabi), cucumber, courgettes (zucchini), melons, tomatoes, peppers (sweet), lettuce, spinach and hops.

Supervised trials involving dimethomorph seed-piece treatment on pineapples and as a seed treatment on oil seed rape were also made available.

In many countries, dimethomorph is available in formulations with and without other complimentary fungicides such as mancozeb, chlorothalonil, copper and folpet. For the purpose of this evaluation, the PHIs defined as GAP for each crop are those established for dimethomorph when formulated without other active ingredients. Where dimethomorph is available only as combination products with different PHIs, the shortest PHI has been selected when defining GAP.

Oranges, sweet, sour

The results of residue trials in Spain involving foliar applications on oranges were made available to the Meeting.

The only GAP provided to the Meeting was for stem paint treatments in Thailand and Vietnam.

The Meeting agreed the data was not sufficient to estimate a maximum residue limit for oranges.

Strawberries

In Belgium, GAP is for three root drench applications of 0.05 g ai/plant, just after planting, one month later and again at the start of spring growth (about 2 months before harvest). In trials from Belgium, matching this GAP, residues found were 0.01, 0.01, 0.02 and 0.02 mg/kg.

In Netherlands, GAP for protected strawberries is to apply 0.05 g ai/plant with the nutrient solution as a root drench up to 35 days before harvest. In three outdoor trials match Netherlands GAP, residues found were 0.01, 0.01 and 0.02 mg/kg.

GAP for outdoor strawberries in Netherlands is for a single foliar spray (0.15 kg ai/ha) just after planting and in four trials in Netherlands matching this GAP, residues were all < 0.01 mg/kg.

The Meeting agreed to use the data from the root drench trials in Belgium and Netherlands to give a combined data set of: 0.01 (4), 0.02 and 0.02 mg/kg.

The Meeting estimated a maximum residue level of 0.05 mg/kg for dimethomorph in strawberries and estimated an STMR of 0.01 mg/kg and an HR of 0.02 mg/kg.

Grapes

The results of residue trials in grapes from France, Germany, Greece, Italy, Spain, Australia, New Zealand and Brazil were made available to the Meeting.

Residues in trials in Brazil matching the GAP in Columbia (0.3–0.4 kg ai/ha, PHI 19 days) were: 0.24, 0.31, 0.99 and 1.1 mg/kg.

Residues in trials from Germany matching the GAP of Belgium (0.3 kg ai/ha, up to 3 applications per season, with a PHI of 28 days) were: 0.26, 0.36, 0.6, 0.71, 1.2 and 1.3 mg/kg.

Residues in trials from Spain matching the GAP of Spain (0.03 kg ai/hL, with a PHI of 28 days) were: 0.09, 0.11, 0.14, 0.24 and 0.25 mg/kg.

Residues in trials from France, matching the GAP of Spain (0.03 kg ai/hL, PHI 28 days), were: 0.16, 0.20, 0.27, 0.38, 0.38, 0.39, 0.39, 0.46, 0.47, 0.51, 0.51, 0.61, 0.62, and 1.7 mg/kg.

Residues in trials from Italy, matching the GAP of Spain (0.03 kg ai/hL, PHI 28 days), were: 0.1, 0.18, 0.19, 0.21, 0.42, 0.85, 0.94, and 1.2 mg/kg.

Residues from a single trial from Greece, matching the GAP of Spain (0.03 kg ai/hL, PHI 28 days), were: 0.39 mg/kg.

The Meeting agreed to use the trials in Spain, France, Italy and Greece matching the GAP of Spain. Residues in ranked order (median underlined) were: 0.09, 0.1, 0.11, 0.14, 0.16, 0.18, 0.19, 0.20, 0.21, 0.24, 0.25, 0.27, 0.38, 0.38, 0.39, 0.39, 0.39, 0.42, 0.46, 0.47, 0.51, 0.51, 0.61, 0.62, 0.85, 0.94, 1.2 and 1.7 mg/kg (n=27).

The Meeting estimated a maximum residue level of 2 mg/kg for dimethomorph in grapes and estimated an STMR of 0.39 mg/kg and an HR of 1.7 mg/kg.

Pineapple

GAP for pineapples in Philippines is for pre-plant dip treatments of seed-pieces (0.19 kg ai/hL dipping solution) and up to 3 post-planting foliar spray applications (1.8 kg ai/ha), 4, 7 and 10 months after planting. Pineapples are commonly harvested about 16–17 months after planting, about 6 months after the last foliar spray.

In a set of trials in Philippines, residues in pineapples following pre-plant seed-piece dipping treatments at 2 × and 4 × the recommended rate were < 0.01 (2) mg/kg in both flesh and peel. Residues were also < 0.01 (2) mg/kg in flesh and peel of pineapples following the pre-plant seed-piece dip treatments (2 × and 4 ×) combined with three foliar sprays matching the recommended application rate and timing. Similarly, pineapples treated with a combination of pre-plant seed-piece dipping (2 × and 4 ×) and three foliar sprays (2 ×), residues were also < 0.01 (2) mg/kg in both flesh and peel.

Since residues were all < 0.01 mg/kg in all four trials involving exaggerated (2 × and 4 ×) pre-plant dipping treatment combined with foliar treatments (1 × and 2 ×), the Meeting agreed to use the results of these trials to give a combined data set of < 0.01, < 0.01, < 0.01 and < 0.01 mg/kg.

The Meeting estimated a maximum residue level of 0.01* mg/kg for dimethomorph in pineapple and estimated an STMR of 0 mg/kg and an HR of 0 mg/kg.

Onions, bulb

In Australia, GAP for onions is up to 0.18 kg ai/ha (maximum 3–4 applications per season), with a PHI of 7 days and in one trial in Australia matching this GAP (but with 7 applications), residues were < 0.02 mg/kg.

Residues in two trials from Germany, matching the German GAP of 4 × 0.3 kg ai/ha, with a PHI of 14 days for bulb vegetables, were < 0.01 and < 0.01 mg/kg.

In one trial in France, matching the German GAP, residues were 0.02 mg/kg.

The Meeting agreed the data was not sufficient to estimate a maximum residue limit for onions, bulb.

Green onions

The Meeting received results of residue trials in Australia on green onions (spring onions).

GAP for bulb vegetables in USA is 0.22 kg ai/ha, PHI 0 days, in Australia GAP for onions is 0.18 kg ai/ha, PHI 7 days, maximum 4 applications/season and in Germany, GAP for bulb vegetables is 0.3 kg ai/ha, PHI 14 days.

No trials matching these GAPs were available and the Meeting agreed the data was not sufficient to estimate a maximum residue limit for green onions.

Cabbage, head

The Meeting received results of residue trials in USA on cabbage.

In trials in USA matching the GAP of Cuba (0.2–0.23 kg ai/ha, PHI 7 days for vegetables) residues of dimethomorph in cabbages (including wrapper leaves) were < 0.05, 0.14, 0.25, 0.4, 0.69, 1.1 and 1.4 mg/kg.

The Meeting estimated a maximum residue level of 2 mg/kg for dimethomorph in cabbage and estimated an STMR of 0.4 mg/kg and an HR of 1.4 mg/kg.

Broccoli

The Meeting received results of residue trials in the USA on broccoli.

In trials in USA matching the GAP of Cuba (0.2–0.23 kg ai/ha, PHI 7 days for vegetables), residues of dimethomorph in broccoli were: < 0.05, 0.12, 0.17, 0.2, 0.25 and 0.52 mg/kg.

The Meeting estimated a maximum residue level of 1 mg/kg for dimethomorph in broccoli and estimated an STMR of 0.19 mg/kg and an HR of 0.52 mg/kg.

Kohlrabi

GAP in Germany for kohlrabi is 0.3 kg ai/ha (maximum 2 applications per season), PHI 14 days and in two outdoor trials and three indoor trials in Germany matching this GAP, residues in kohlrabi were: < 0.02, < 0.02, < 0.02, < 0.02 and < 0.02 mg/kg.

The Meeting estimated a maximum residue level of 0.02 mg/kg for dimethomorph in kohlrabi and estimated an STMR of 0.02 mg/kg and an HR of 0.02 mg/kg.

Fruiting vegetables, cucurbits

Cucumber

The Meeting received results of residue trials in outdoor cucumbers in Hungary and Germany. No GAP matched these trials.

The Meeting received results of residue trials on protected cucumbers from France, Greece, Italy and Spain.

GAP for cucurbits in the USA is 0.22 kg ai/ha (maximum 5 applications/season), PHI 0 days. In protected cucumber trials, matching the GAP of USA, residues were: 0.02, 0.05 and 0.08 mg/kg in trials in Spain, 0.03 mg/kg in one trial in France, 0.05 mg/kg in one trial in Italy and 0.07 and 0.07 mg/kg in trials in Greece.

The Meeting noted that these trials involved 3–4 applications per season but agreed to use these results because 1–2 additional treatments applied more than 3–4 weeks before harvest would not contribute significantly to the final residue in rapidly growing protected cucumbers. Residues were: 0.02, 0.03, 0.05, 0.05, 0.07, 0.07 and 0.08 mg/kg.

Squash, summer:

The Meeting received results of residue trials on protected summer squash (courgettes) in Greece, Italy and Spain and on outdoor summer squash (zucchini) in Australia.

Residues from five protected summer squash trials in Greece, Italy and Spain, matching the GAP for cucurbits in the USA (0.22 kg ai/ha, maximum of 5 applications per season, PHI 0 days) were: 0.2 and 0.24 mg/kg (Greece), 0.07, 0.13 and 0.17 mg/kg (Italy) and 0.02 mg/kg (Spain).

The Meeting noted that these trials involved 3 applications per season and agreed that the contribution of 2 additional treatments applied more than 3–4 weeks before harvest would not contribute significantly to the final residue.

In one outdoor summer squash trial in Australia matching the Australian GAP (0.18 kg ai/ha, maximum 4 applications per season, PHI 7 days), residues were < 0.02 mg/kg.

The Meeting noted that the residues in protected summer squash trials matching the USA GAP were higher than those from the outdoor summer squash trial in Australia and agreed to use the data on protected summer squash. Residues found were: 0.02, 0.07, 0.13, 0.17, 0.2 and 0.24 mg/kg.

Melons, except watermelons

The Meeting received results of residue trials from Australia, Brazil, France, Italy and Spain.

Residues in trials in France matching the GAP of Israel (0.18 kg ai/ha, PHI 3 days) were: 0.03 and 0.04 mg/kg (whole fruit).

In two trials in Italy matching the GAP of Israel, whole fruit residues were 0.04 and 0.11 mg/kg.

In trials in Spain matching the GAP of Israel, whole fruit residues were: 0.02, 0.02, 0.2 and 0.24 mg/kg and in a further four trials, residues in melon flesh were: < 0.02, < 0.02, < 0.02 and 0.05 mg/kg.

The Meeting agreed to combine the results of the trials in France, Italy and Spain matching the GAP in Israel. Whole fruit residues were: 0.02, 0.02, 0.03, 0.04, 0.04, 0.11, 0.2 and 0.24 mg/kg and residues in melon flesh were: < 0.02, < 0.02, < 0.02 and 0.05 mg/kg.

The Meeting agreed that the data on cucumbers, summer squash and melons were sufficient to support a group MRL and estimated a maximum residue level of 0.5 mg/kg for dimethomorph in fruiting vegetables (cucurbits).

The Meeting estimated an STMR of 0.15 mg/kg and an HR of 0.24 mg/kg for cucurbits with an edible peel (based on the summer squash data) and an STMR of 0.02 mg/kg and an HR of 0.05 mg/kg for cucurbits with an inedible peel (based on the melon data).

Fruiting vegetables, other than cucurbits

Tomato

In protected tomato trials from France, Greece, Italy and Spain, matching the GAP of Japan (0.025 kg ai/hL, maximum 3 applications per season, PHI 1 day), residues were: 0.03, 0.05, 0.06, 0.07, 0.1, 0.1, 0.1, 0.11, 0.11, 0.13, 0.16, 0.16, 0.19 and 0.26 mg/kg.

Residues in outdoor tomato trials in USA matching the USA GAP (0.22 kg ai/ha, maximum 5 applications per season, PHI 4 days) were: 0.06, 0.08, 0.21, 0.26 and 0.41 mg/kg (n=5).

Residues in a further seven trials from the USA matching this GAP but with 6–7 applications per season were: < 0.05, < 0.05, < 0.05, 0.05, 0.14, 0.14, and 0.51 mg/kg (n=7).

The Meeting agreed that the contribution of 1–2 additional treatments applied more than 4 weeks before harvest would not contribute significantly to the final residue and agreed to use these results to give a combined data set of: < 0.05, < 0.05, < 0.05, 0.05, 0.06, 0.08, 0.14, 0.14, 0.21, 0.26, 0.41 and 0.51 mg/kg (n=12) for outdoor tomatoes.

The Meeting noted that the residues from the protected tomato trials matching the GAP of Japan and the outdoor tomato trials matching the GAP of the USA were from similar populations and agreed to combine the results. Residues in ranked order (median underlined) were: 0.03, < 0.05, < 0.05, < 0.05, 0.05, 0.05, 0.06, 0.06, 0.07, 0.08, 0.1, 0.1, 0.1, 0.11, 0.11, 0.13, 0.14, 0.14, 0.16, 0.16, 0.19, 0.21, 0.26, 0.26, 0.41 and 0.51 mg/kg (n=26).

Peppers sweet

In trials on protected sweet peppers in Greece, Italy and Spain matching the GAP of the USA for fruiting vegetables, except tomatoes (0.22 kg ai/ha, maximum 5 applications per season, PHI 0 days), residues in ranked order (median underlined) were: 0.13, 0.13, 0.16, 0.17, 0.18, 0.21, 0.21, 0.26, 0.31, 0.38, 0.48 and 0.56 mg/kg (n=12).

The Meeting noted that these trials involved 3 applications per season but agreed to use this data because the contribution of 2 additional treatments applied more than 3 weeks before harvest would not contribute significantly to the final residue in rapidly growing protected peppers.

Peppers, chilli

The GAP for peppers in the Republic of Korea is 0.3 kg ai/hL with a maximum of 4 applications per season with a PHI of 3 days. In three outdoor chilli pepper trials in Korea, matching this GAP, residues were 0.22, 0.31 and 0.53 mg/kg.

The Meeting noted that the results of the trials on peppers, sweet and peppers, chilli were from similar populations and agreed to combine the results. Residues in ranked order (median underlined) were: 0.13, 0.13, 0.16, 0.17, 0.18, 0.21, 0.21, 0.22, 0.26, 0.31, 0.31, 0.38, 0.48, 0.53 and 0.56 mg/kg.

The Meeting noted that GAP existed in the USA for the fruiting vegetable group and based on the data for peppers and tomatoes, agreed to establish a group MRL for 'fruiting vegetables, other than cucurbits' except mushrooms and sweet corn of 1 mg/kg and estimated an STMR of 0.22 mg/kg and an HR of 0.56 mg/kg.

Lettuce, head

In protected head lettuce trials in Germany, Greece, Italy and Spain matching the GAP in the USA (0.22 kg ai/ha, maximum 5 applications per season, PHI 0 days), residues were: 1.5, 2.2, 2.2, 2.3, 2.7, 2.9, 3.1, 3.6, 3.9, 3.9, 4.2, 4.3, 4.6, 7.1 and 7.2 mg/kg (n=15).

The Meeting noted that these trials involved 2–3 applications per season and agreed to use these results because the contribution of 2–3 additional treatments applied more than 3 weeks before harvest would not contribute significantly to the final residue in rapidly growing protected lettuce.

In outdoor lettuce trials in Spain, matching the GAP of Spain (0.23 kg ai/ha, PHI 7 days), residues found were: 0.05, 0.06, 0.07, 0.1, 0.16, 0.38, 0.39 and 0.43 mg/kg.

The Meeting noted that the residues from the protected lettuce trials and the outdoor lettuce trials were from different populations and agreed to use the data from the protected lettuce trials.

The Meeting estimated a maximum residue level of 10 mg/kg for dimethomorph in lettuce, head and estimated an STMR of 3.6 mg/kg and an HR of 7.2 mg/kg.

Corn salad

The Meeting received results of residue trials in protected corn salad (Lambs lettuce) from Italy and Spain. Residues in trials matching the GAP for lettuce (including Lambs lettuce) in Spain (0.23 kg ai/ha, PHI 7 days) were: 0.79, 0.79, 1.9, 4.8, 5.3 and 7.1 mg/kg.

The Meeting estimated a maximum residue limit of 10 mg/kg for dimethomorph in corn salad and estimated an STMR of 3.4 mg/kg and an HR of 7.1 mg/kg.

Spinach

The Meeting received results of residue trials in spinach in USA.

No GAP matching these USA trials was available and the Meeting agreed the data was not sufficient to estimate a maximum residue limit for spinach.

Potatoes

The Meeting received results of residue trials from Argentina, Australia, Belgium, Brazil, Canada, Denmark, France, Greece, Germany, Italy, New Zealand, Spain, UK and USA on potatoes.

In trials in Brazil matching the GAP of Brazil (0.4 kg ai/ha, maximum 4 applications per season, PHI 14 days), residues were: < 0.03 (9) and 0.03 mg/kg (n=10).

In trials in USA matching USA GAP (0.22 kg ai/ha, maximum 8 applications per season, PHI 4 days), residues were: < 0.01 (6) and 0.02 mg/kg (n=7).

Residues in trials in UK matching the GAP of the UK (0.15 kg ai/ha, maximum 8 applications per season, PHI 7 days), were: < 0.01 (12), < 0.02 (18), 0.02 and 0.04 mg/kg (n=32).

Residues in trials in France matching the GAP of France (0.18 kg ai/ha, maximum 4 applications per season, PHI 7 days) were: < 0.01 (4) and < 0.05 mg/kg (n=5).

The Meeting agreed to combine the results to give a total data set of: < 0.01(22), < 0.02(18), 0.02(2), < 0.03(9), 0.03, 0.04, < 0.05 mg/kg (n=54).

The Meeting estimated a maximum residue level of 0.05 mg/kg for dimethomorph in potatoes and estimated an STMR of 0.02 mg/kg and an HR of 0.05 mg/kg.

Rape seed

GAP in Germany for rape seed is for a pre-plant seed treatment using 5 g ai/kg of seed and in two trials from Germany matching this GAP, residues in rape seed from plants grown from treated seed were both < 0.02 mg/kg. In two related trials, residues of < 0.02 mg/kg were reported in rape seed from treated plots. However the presence of residues of 0.02 mg/kg in control samples suggested that the samples had been mislabelled.

The Meeting agreed that while residues would not be expected in rape seed following a pre-plant seed treatment, there was insufficient data to estimate a maximum residue level for dimethomorph in rape seed.

Hops

In Austria and Germany, GAP on hops is 0.015 kg ai/hL (maximum 6 applications per season in two sets of three applications), PHI 10 days.

One trial in Germany matched the GAP in Germany, with residues of 28 mg/kg in dried hops. A further eight trials in Germany matching GAP but with 4 applications per season reported residues of 8.3, 8.7, 9.3, 24, 26, 26, 29 and 42 mg/kg.

The Meeting agreed that the final 3 applications in these trials would contribute most to the final residue and agreed to use these results to give a combined data set: 8.3, 8.7, 9.3, 24, 26, 28, 26, 29 and 42 mg/kg for dried hops.

The Meeting estimated a maximum residue level of 80 mg/kg for dimethomorph in hops, dry and estimated an STMR of 26 mg/kg.

Fate of residues during processing

Dimethomorph is stable under the standard hydrolysis conditions used to simulate food processing.

The Meeting received information on the fate of incurred residues of dimethomorph during the processing of grapes, tomatoes, potatoes and hops. The processing factors (PF) shown below were calculated from the residues for the commodities for which MRLs, STMRs and HRs were estimated.

Raw commodity (RAC)	Processed commodity	Calculated processing factors.	Median or best estimate	
Grapes	Red wine	0.06, 0.12, 0.16, 0.17, 0.17, 0.17, 0.17, 0.22, 0.24, 0.24, 0.25, 0.25, 0.27, 0.28, 0.29, 0.29, 0.30, 0.31, 0.34, 0.34, 0.35, 0.36, 0.38, 0.38, 0.47, 0.53, 0.58, 0.67, 0.69, 0.70, 0.8	0.29	0.29
	White wine	0.10, 0.12, 0.13, 0.14, 0.17, 0.18, 0.31, 0.43, 0.50, 0.51, 0.61, 1.24	0.24	
	Pomace, wet (red wine)	1.6, 2.4, 2.7, 2.8, 3.1, 3.3, 4.1, 7.3	3.0	2.75
	Pomace, wet (white wine)	1.7, 2.3	2.0	

Raw commodity (RAC)	Processed commodity	Calculated processing factors.	Median or best estimate
	Raisins	1.5, 2.1	1.8
Tomatoes	Juice	0.5	0.5
	Paste	2.4	2.4
Potatoes	Wet peel	6.4	6.4
Hops	Beer	0.0011, < 0.0012, 0.0025, 0.0035	0.002

Grapes were processed into wine and dried grapes (raisins). Processing factors were 0.29 (wine), 1.8 (raisins) and 2.75 (grape pomace). Based on the STMR value of 0.39 mg/kg for grapes and the median processing factors of 0.29 (red and white wine combined) 1.8 for raisins and 2.75 for wet pomace, the STMR-Ps for dimethomorph residues were 0.11 mg/kg in wine, 0.7 mg/kg in dried grapes and 1.07 mg/kg in grape pomace, wet.

Based on the HR of 1.7 mg/kg estimated for grapes and the processing factor of 1.8 for raisins, the Meeting estimated a maximum residue level of 5 mg/kg for dimethomorph in dried grapes.

Tomatoes were processed into juice, puree and paste with processing factors of 0.5, 1.2 and 2.4 respectively. Based on the STMR value of 0.11 mg/kg for tomato, the STMR-Ps for dimethomorph residues were 0.055 mg/kg (tomato juice) and 0.264 mg/kg (tomato paste).

Potatoes, based on a processing factor of 6.4 for wet peel and an STMR of 0.02 mg/kg, the Meeting estimated an STMR-P for dimethomorph residues in potato process waste of 0.128 mg/kg.

Hops were processed into beer with a processing factor of 0.002. Based on the STMR value of 26 mg/kg for hops, dry, the STMR-P for dimethomorph residues in beer was 0.052 mg/kg.

Peppers, chilli dried. Based on the HR value of 0.56 mg/kg and the STMR value of 0.22 mg/kg for fresh peppers (including chilli peppers) and using the new generic processing factor of 7 for dried chilli peppers, the Meeting estimated a maximum residue level of 5 mg/kg and an STMR-P of 1.54 mg/kg for dimethomorph in peppers, chilli dried.

Estimated maximum and mean dietary burdens of farm animals

The Meeting estimated the dietary burden of dimethomorph in farm animals on the basis of the diets listed in Annex 6 of the 2006 JMPR Report (OECD Feedstuffs Derived from Field Crops). Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities. The percentage dry matter is taken as 100% when the highest residue levels and STMRs are already expressed as dry weight.

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex VI. The calculations were made according to the animal diets from US-Canada, EU and Australia in the OECD Table (Annex 6 of the 2006 JMPR Report).

	Animal dietary burden, dimethomorph, ppm of dry matter diet					
	US-Canada		EU		Australia	
	max	mean	max	mean	max	mean
Beef cattle	0.32	0.32	2.3 [1]	0.96	1.48	1.48 [2]
Dairy cattle	0.11	0.11	2.19	0.85	1.43	1.43 [3]
Poultry - broiler	0	0	0.03	0.01	0	0
Poultry - layer	0	0	0.5 [4]	0.14 [5]	0	0

[1] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat and milk.

[2] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat.

[3] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[4] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs.
[5] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs.

Farm animal feeding studies

The Meeting received information on feeding studies with lactating cows.

A residue transfer study in livestock was conducted with 4 groups of 3 Friesian cows that were fed for 28 to 35 days with diets containing dimethomorph, administered (in corn oil) in the diet, corresponding to feeding levels of 0–12.5–37.5–125 ppm. Milk was collected daily at morning and afternoon milking and pooled for analysis. Sub-samples of milk were separated into cream and skim milk on days 14 and 28, with the day 28 milk also being pasteurised, separated into cream (35% butterfat) and skimmed milk (fat content about 0.1%) or treated and centrifuged to obtain acid whey. Liver, kidney muscle, and fat (subcutaneous and peritoneal) were taken within 24 h of the final administration for analysis. Residues in whole milk, pasteurised milk, skimmed milk, acid whey and cream were all below the limits of quantitation (0.01 mg/kg for dimethomorph and metabolite Z89) and 0.02 mg/kg for metabolites Z67/Z69) except in the 125 ppm dose group, where trace residues of dimethomorph (0.01 mg/kg) were found in cream samples.

In animals from the 12.5ppm dose group, residues of dimethomorph and metabolites Z69 and Z67 were not detectable or below the limit of quantification (0.01 mg/kg) in all tissues analysed. Residues were also all below the limit of quantification for all tissues from the 37.5 ppm dose group except for liver, where residues of up to 0.02 mg/kg of the Z69 metabolite were found. Only in the highest dose group (125 ppm) were significant residues found, mostly in liver and kidney, where residues of the Z69 metabolite were measured at levels up to 0.15 mg/kg and 0.14 mg/kg respectively and residues of the parent compound were found in liver (up to 0.05 mg/kg), and in fat (up to 0.03–0.04 mg/kg).

Residues in animal commodities

The maximum calculated animal burden estimated for dairy and beef cattle is 2.3 ppm. In the cattle feeding study, where lactating cows were dosed at 12.5 ppm (more than 5 times higher than the calculated animal burden), no dimethomorph residues were detected in tissues and milk. Therefore, the Meeting concluded that no residues are to be expected at the maximum calculated dietary burden.

The maximum animal burden estimated for poultry (layers) is 0.5 ppm. In the metabolism study where laying hens were fed the equivalent of 40 ppm in feed for seven days, dimethomorph residues in fat and skin were < 0.02 mg/kg and were not detected in eggs or other edible tissues. Metabolites Z67 and Z69 were the major residue components identified in liver (0.13 mg/kg), egg yolks (0.07 mg/kg), kidney (0.032 mg/kg) and muscle (0.003 mg/kg). Low levels (0.02–0.05 mg/kg) of the Z43 and Z95 metabolites were reported in kidney and/or egg yolks.

On the basis that the maximum calculated dietary burden is about 80 times lower than the dose rate in the metabolism study, the Meeting concluded that no residues of dimethomorph, or its primary metabolites, are to be expected at the maximum calculated dietary burden of 0.5 ppm.

The Meeting estimated a maximum residue level of 0.01* mg/kg in meat (from mammals except marine mammals) and estimated HRs and STMRs of 0 mg/kg.

The Meeting also estimated a maximum residue level of 0.01* mg/kg in edible offal (mammalian) and estimated HRs and STMRs of 0 mg/kg.

For milks, the Meeting estimated a maximum residue level of 0.01* mg/kg and estimated an STMR of 0 mg/kg.

The Meeting estimated a maximum residue level of 0.01* mg/kg in poultry meat, poultry offal and eggs and estimated HRs and STMRs of 0 mg/kg.