Developmental neurotoxicity of monocrotophos and lead is linked to thyroid disruption

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Abstract

Aim: A role of thyroid disruption in developmental neurotoxicity of monocrotophos (MCP) and lead is studied.

Materials and Methods: A total of 24 female rats after conception were randomized into four groups of six each and treated as follows: Group I - Sham was administered distilled water orally. Group II - A positive control was administered methyl methimazole at 0.02% orally in drinking water. Group III - MCP orally at 0.3 mg/kg and Group IV - Lead acetate at 0.2% orally in drinking water. The drug was administered from gestation day 3 through post-natal day 21 in all the groups. Acetylcholinesterase (AChE) inhibition, thyroid profile (thyroid stimulating hormone, T3 and T4), neurodevelopment (brain wet weights, DNA, RNA and protein), and neurobehavioral (elevated plus maze, photoactometry, and Morris water maze) parameters were assessed in pups. A histopathology of thyroid of dams and brain of progeny was conducted.

Results: Inhibition of AChE was <20%. Thyroid profile decreased in the treatment groups. Neurodevelopmental and neurobehavioral parameters did not reveal any significant changes. Thyroid architecture was affected significantly with MCP and lead. Cortical layers too were affected. The three layers of cerebellum either had abnormal arrangement or decreased cellularity in all treated groups relating to thyroid disruption.

Conclusion: MCP and lead might have affected the development of cerebrum and cerebellum via thyroid disruption leading to developmental neurotoxicity.

Keywords: behavioral alterations, developmental neurotoxicity, lead, monocrotophos, thyroid disruption.

Introduction

The potential harmful chemicals or substances, such as heavy metals, pesticides, and hydrocarbons, are dumped either or released into the water bodies [1]. When these pollutants flow into water bodies in a higher concentration than permissible limits then these result in the form of heavy mortalities of all life form residing in those aquatic systems such as fish and shellfish, etc., while in a lower concentration these lead to bioaccumulation of these pollutants and ultimately go through the food web to human beings. These pollutants can also alter other hormonal processes of fish like the development of bones and proper thyroid functioning. Dimethoate and lambda-cyhalothrin showed the lethal effect on thyroid hormone of Labeo rohita [2]. Fetal exposure to environmental chemicals could affect the development of nervous system. In this chemical age, certain of the developmental defects do not have a definite etiology and the only pointer could be exposure during development that too at a critical time. With the rise in the use of pesticides, surfacing of behavioral disorders became common. A majority of children suffer from neurodevelopmental disorders and exposure to xenobiotics has been identified as one of the risk factors. About 8 million children suffer from one or other mental disorders, and 1.1 million are exposed to organophosphate (OP) insecticides above the safety levels. One of the facets of OP toxicity is chronic OP-induced neuropsychiatric disorders. While researching on the developmental neurotoxicity of OPs, especially chlorpyrifos (CPS), their cholinesterase-independent actions came into the fore and have surpassed the receptor level and are lingering at the cell signaling mechanisms. One aspect that has been attempted albeit on a lesser scale is the interference of endocrine mechanisms by OPs that could contribute to the existing neurotoxicity on in-utero exposure.

Lead (Pb) has been implicated in a variety of behavioral disorders since its use in 1900 as leaded gasoline and other forms. Although a unified mechanism of action has been elusive, it is believed to be
the outcome of a yet to be identified abnormal process or toxic insult in-utero or during early post-natal life. The subsequent challenge in the adult life of the exposed fetus could cause behavioral abnormalities.

Maternal thyroid hormone availability is crucial for the development of fetal brain [3] and influence the expression of genes in neurogenesis, gliogenesis, maturation, differentiation and migration. All these developmental activities are time-dependent, and any delay could literally compromise the cytoarchitecture of the brain and is manifested as abnormal behavior.

Against this backdrop, the present study was proposed to link the developmental neurotoxicity of monocrotophos (MCP) (an extensively used OP pesticide) and lead (a ubiquitous heavy metal and environmental pollutant) with thyroid disruption.

Materials and Methods

Ethical approval

This study was conducted after approval by the Research Committee and Institutional Animal Ethics Committee.

Experimental design

Rats of Sprague-Dawley strain were procured from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad and maintained under standard conditions. Institutional Animal Ethics Committee, College of Veterinary University, Rajendra Nagar, permission was obtained before the conducting of the experiment and standard humane procedures were adopted. MCP, (purity 77.4%) was supplied by Hyderabad Chemicals Pvt. Ltd., India as a gratis sample. Methyl methimazole (MMI) (METHIMEZ 10 mg, Sun Pharma Pvt. Ltd.), lead acetate (PbAc), and other chemicals used in the experiment were of analytical grade.

Female rats were mated overnight, and the presence of sperm in the vaginal smear was considered as positive for conception (gestational day [GD] zero). 24 females after conception were randomized into four groups of six each and treated as follows: Group I - Sham was administered distilled water orally (5% of body weight). Group II - a positive control was administered MMI at 0.02% orally as sole source of drinking water. Group III - MCP orally at 0.3 mg/kg b.wt and Group IV - PbAc at 0.2% orally as sole source of drinking water. The drug was administered from GD3 through post-natal day (PND) 21 in all the groups.

Sample collection and preparation

On GD 14, whole blood was collected from dams for estimation of erythrocyte acetylcholinesterase (AChE) [4] in control and MCP treatments. Further, serum was used for estimation of thyroid profile (thyroid stimulating hormone [TSH], T4 and T3) in all the groups by radio immunoassay employing Dia Sorin S.P.A. kits, USA. The radio-labeling was detected by automated clinic gamma counter; LKB Wallace, Finland. On PND 7, brains of the pups were rapidly dissected and neurodevelopmental parameters (brain wet weight, DNA, RNA, protein) assessed. Few brains were fixed in 10% formalin for histopathology examination (HPE). DNA was extracted [5] and estimated by UV7500 UV-VIS Spectrophotometer of Techcomp, Ltd., Hong Kong, whereas, RNA was quantified by Trizol method and protein by biuret method [6]. On the day of weaning, i.e., PND 21, mothers were sacrificed and thyroids collected for HPE [7]. Neurobehavioral studies Morris water maze (MWM), elevated plus maze (EPM), photoactometry were conducted in 21-day-old F1 rats.

Statistical analysis

The data were subjected to statistical analysis by applying one-way ANOVA using Statistical Package for Social Sciences version 15.0. Differences between means were tested using Duncan’s multiple comparison test and a significance level was set at 0.05.

Results

Erythrocyte AChE (%) activity in dams was measured in control and MCP-treated groups only. MCP treatment showed 20.38% of AChE inhibition (Table-1).

Neurodevelopment parameters

Wet weight, DNA, RNA and protein were the parameters assessed to evaluate developmental neurotoxicity in rat pups on PND 7 (Table-1).

The mean wet weight of brains (g) of the progeny in Group I, II, and III were 1.339±0.02, 1.309±0.32, and 1.284±0.05, respectively. There appeared to be no statistical significance between and the treatments. However, in Group IV a statistically significant (p<0.05) increase in brain weights 1.450±0.42 g was noted.

The mean concentration of DNA (μg/g) in brain tissue was 0.0313±0.0065, 0.0183±0.0017, 0.0251±0.0029, and 0.0257±0.0031μg/g of in Groups I, II, III, and IV, respectively. There was a non-significant difference in the concentration of DNA between control and the treated groups. MMI group recorded the least concentration of DNA.

The concentration of RNA in the brain (μg/100 mg of brain matrices) in various groups was 62.50±5.18, 72.50±5.18, 57.00±5.23, and 63.83±0.54, respectively, in Groups I through IV. Group II had the highest concentration of RNA while Group V recorded the lowest when compared to control. The remaining Groups (III and IV) registered a decrease in the levels of RNA as compared to control, but the decrease was non-significant.

Protein % in the whole brain showed a significant difference (p<0.05) in MMI and MCP when compared to control. The protein % was highest in MMI (1.42±0.16), whereas in MCP it was lowest (0.73±0.12).

Thyroid assay included the measurement of TSH, T3 and T4 by radio immunoassay on the 14th day of gestation (Table-2).
TSH (μU/ml) concentration increased in treatment groups as compared to control. Such an increase was apparent but statistically non-significant in Groups III and IV, except MMI-treated Group (II) that showed statistically significant (p<0.05) decrease in TSH concentration.

The concentration of the triiodothyronine (T3) (ng/ml) decreased significantly (p<0.05) in Group II (0.862) as compared to control (0.983). MCP and PbAc treated Groups (III and IV) had a slight non-significant increase in T3 concentration when compared to control.

The mean concentration of thyroxine (T4) (μg/dl) T4 was 2.11±0.12 and 2.19±0.14, respectively, in Groups I and III. All the groups had the T3 concentration on par with control, and there was no statistically significant difference. Group II (MMI) had a decreased concentration of T4 while Group IV (PbAc) showed an increase in T4 concentration (2.75±0.18) as compared to control.

The motor activity of the progeny of the treated rats was determined by photoactometer (activity/minute). The activity of the rats in the control group was 103.16±6.33 s, whereas Groups II, III and IV travelled 150.00±28.99 s, 182.77±27.31 s, and 230.00±28.99 s, respectively. The mean entries into the closed arm were more in Group III and Group IV was 4.73±0.55 and 4.00±0.75, respectively. Duration of time spent (seconds) in the center of maze: Animals of control group spent about 20.23±19.87 s in the center of the maze. All other treated groups spent more time compared to control group. The order of increased time spent was 39.00±25.91 s (Group II), 31.22±27.31 s (Group III), and 23.00±28.97 s (Group IV). Thus, MMI treated rats spent more time in the center of the maze. However, there was no significant difference between control and treated groups. Duration of time spent (seconds) in open arm: MMI and MCP groups (92.90±25.91 s and 78.22±27.31 s, respectively) spent more time, whereas, PbAc (46.62±28.97) spent less time when compared to control (63.00±19.87 s), and were without statistical significance. Duration of time spent (seconds) in the closed arm: Sham rats spent 199.41±19.87 s in closed arm. Groups II and III (182.77±27.31 s, respectively) spent lesser time in closed arm as compared to sham. Rats of Groups IV (245.00±28.99 s) spent more time in closed arm than the control group. Although apparent differences existed, they were statistically non-significant. Total distance travelled: Control group traversed 416.87 cm, whereas Groups II, III and IV travelled 500±64.22, 487.5±108.29 and 443.33±54.13 cm, respectively, which is higher than control.

The results of escape latency (EL) and probe trial of MWM are expressed in seconds and presented in Table-4. The data pertaining to the EL revealed that control group took 44.87±6.56 s to navigate the platform, whereas the treated groups took longer to do so, indicating a decrease in spatial learning ability. However, the differences were not statistically significant.
Histopathology

In thyroid gland of dams, the follicles in the control group were large, circular and oval in a shape filled with homogenous, eosinophilic colloid material. The interlobular stroma was poorly defined. The follicular cells were flattened. Among the treatment groups, MMI, MCP and PbAc rats microfollicles were visible, had an irregular shape, absolutely empty with no colloid material and dense stroma appeared between the lobules (Figures-1 and 2).

In the brain of F1 progeny, the cerebral cortical layers showed a decrease in cellularity in all the treated groups as compared to control (Figures-3-6).

Discussion

The developmental neurotoxicity of MCP and lead on maternal thyroid disruption and fetal outcome in Sprague-Dawley rats was studied.

AChE activity

Erythrocyte AChE activity in MCP treated animals was inhibited upto 20.38% as compared to control, and the inhibition was not significant at the dose employed. Greater than 20% inhibition causes developmental neurotoxicity, whereas 70% inhibition is said to produce cholinergic hyperstimulation [8]. The dose of 0.3 mg/kg was selected to have the minimum or no effect on AChE inhibition. This was done with an aim to specifically evaluate the non-cholinergic mechanisms involved in the developmental neurotoxicity. The inhibition of the enzyme by MCP was dose-dependent in day-old chick brain [9], in mice [10] and Wistar rats. The latter two investigators employed the same dose (0.3 mg/kg) as in this experiment and have reported significant inhibition of AChE; such a variance could be due to the difference in species and strain employed.

Wet weight of brains was recorded in pups on PND 7. Syed and Shafiullah [11] employed the...
same dose of MMI as in our study, and no effects were observed on the organ weights of fetuses. CPS at 5 mg/kg/day administered perinatally from GD 10-PND10 decreased neonatal brain weights [12]. However, an increase in brain weights could probably be due to the higher body weights of the pups in MCP group.

DNA→RNA→protein sequence, the vital lead for the function of the cell and gene expression, is said to be affected by environmental factors, especially contaminants like heavy metals, pesticides, polychlorinated biphenyls, hormones, etc., [13]. PbAc in vitro induced single and double DNA strand breaks and

Figure-1: Empty follicles, shrunken in size, stromal abundance in thyroid gland of dams (Group III) (H and E).

Figure-2: Empty follicles. Disrupted cytoarchitecture in thyroid gland of dams (Group IV) (H and E,×200).

Figure-3: Decreased external pyramidal cells and vacoulation in brain of pups (Group III) (H and E,×200).

Figure-4: Vacoulation in cortical layers with reduced external pyramidal layer in brain of pups (Group IV) (H and E,×200).

Figure-5: Disorganized Purkinje cells and granular layer is loosely arranged in brain of pups (Group III) (H and E,×200).

Figure-6: Focal absence of Purkinje cells in brain of pups (Group IV) (H and E,×200).
DNA-protein cross links [14]. Pesticides are known genotoxicants; hence, the DNA content of brain was estimated. DNA is a good indicator of cell number. MCP, orally, caused dose-dependent alkylation of DNA in mice but 72 h post-treatment damaged DNA was repaired as indicated by a decrease in comet tail length [15]. Diazinon exposure in zebra fish decreased DNA, RNA and total protein of liver [16] whereas, CPS damaged DNA in the liver and brain of rats [17] in a dose-dependent manner. Fetal DNA methylation patterns, as evident in a number of animal studies, have established outcomes that are carried to later life [18]. On the same lines of methylation, demethylation in the embryo also helps to remove epigenetic modifications. Thus, loss or gain of methylation is age and cell, tissue or organ dependent. Genotoxicants like cadmium and pesticides cause DNA strand breaks or fragmentation [19,20]. Poly (ADβ-ribose) polymerase-1 senses breaks and promotes repair. The addition of protein too decreases the toxicity as evidenced in CPS and diazinon inhibited DNA synthesis in vitro in neuronotytic PC-12 cells and gliotypic C6 cells [21]. There was an apparent decrease in DNA in all the treated groups in the present work, when compared with control, but such a decrease was not significant statistically. The results are in agreement with an earlier work on day-old chicks where DNA reduction was not statistically significant. This could be due to age- and time-dependent demethylation of DNA. Furthermore, lead intoxicated neonatal rats displayed retardation of new cell formation in the cerebellum but little effect on the concentration of RNA, DNA and protein content in the developing brain. However, Pb at 0.1 or 1 mg/ml for 1 month in mice had decreased body weight and DNA concentration.

RNA is a link between gene and protein expression. A decrease in DNA is followed by a decrease in RNA and protein in zebra fish exposed to diazinon in hypothyroid animals [22] and in mice embryo liver treated with MCP, whereas in the present study, RNA concentration did not show statistically significant difference from the control as was DNA. RNA in MMI Group (II) was apparently the highest but not different from control. As there was no decrease in DNA content, it might be inferred that the RNA content also remained stable.

Protein was quantified to estimate the cell size of the brain of the pups. As discussed above, a fall in DNA would be subsequently reflected in the levels of RNA and protein. OPs cause adduction of proteins specific to the compound [23]. PbAc inhibited protein synthesis in cultures of rat sertoli cells in vitro. In the present experiment, MMI had high protein levels and MCP the lowest. MMI also had a high concentration of RNA and that might have influenced the protein expression. The difference between the MMI and MCP was statistically significant, albeit the difference between these two groups when compared to control was not significant. The present findings are in agreement with Bharani and Reddy [7] with MCP in neonate chick brain and with parathion in rats.

The most common used endpoints to monitor thyroid function currently are serum thyroid hormone levels (T₄ and T₃), TSH and thyroid histology [24]. Maternal T₄ has a greater influence on early neurodevelopment because the brain receives higher levels of T₄ that is converted subsequently to T₃. In the present investigation, the levels of T₃ and T₄ were reduced but TSH was on the rise in the MMI-treated group, as a compensatory mechanism but such an increase in serum TSH is an issue of timing and could be disso-ciable temporarily [25].

Earlier workers have reported a dose-dependent decrease of T₄ with aracolor 1254 in rat [26], dichlorodiphenyl dichloroethylene and dichlorodiphenyltrichloroethane in human infants [27]. Organochlorine compounds decrease or mimic the actions of thyroid hormones by interfering with HPT axis, induce thyroid metabolizing enzymes and competitively bind to transporter protein. But in PbAc-treated group, an increase in T₃ and a normal TSH could be due to a general resistance to thyroid hormone by the receptors or inhibition of Type II deiodinase or inhibition of microsomal glucuronidation and bile elimination of T₄ in rats. Lead is a known microsomal enzyme inhibitor, and this could have added to the increased T₄ concentration. Such an increase in T₄ could produce impaired cognition, lack of concentration and inattentiveness [28]. Environmental toxicants can interfere with thyroid function (thyroid-dependent) or could act on receptors without affecting the thyroid gland (thyroid-independent) and may produce adverse neurodevelopmental effects, which may not fully agree with hypothyroidism or thyroid toxicity [29]. Pb is both negatively correlated with thyroid hormones and positively correlated [30] as a function of the dose. In contrast, [31] reported no relationship between Pb and thyroid hormones. Furthermore, some of the congeners of a compound may bind to thyroid hormone receptors of a particular species of animals and the same affinity may not be displayed in other species. Thus, thyrotoxicity is dependent on dose, compound and species.

The motor response and the exploratory behavior of treated animals were measured by photocellometry. MMI animals had less exploratory behavior but PbAc group exhibited hyperactivity, although the activity did not differ significantly with control. PbAc is known to cause hyperactivity. These results are consistent with Kuriyama et al. [32] for propyl thiouracil (GD7-21), but in adults, a contrast of reduced locomotor activity was observed for 2 days. Locomotor activity was dependent on the dose and PND of exposure of CPS, ranging from no effect to decreased activity in rats [33] and in mice with disopropyl fluorophosphate. EPM determines the animal’s unconditioned response to a potentially dangerous environment. A normal rodent avoids open arm for fear or anxiety.
and spends most of the time in closed arms, called thigmotaxis, i.e. the tendency to remain close to the walls. PbAc is said to affect the behavior of the animal and thus increase its anxiety. Hence, this might have made the animals stick to the walls. MMI-treated animals spent more time in the open arm, whereas MCP-treated animals had spent more time in closed arm inferring loss of fear and anxious behavior in these two groups, respectively. The findings are consistent with Sanchez-Amate et al. [34], who observed anxiogenic-like response with CPS in dams during GD 9-12 and in OP pesticide applicators.

MWM is the gold standard for determining cognitive skills in animals. Changes in the central cholinergic system can be detected, and cued version is capable of revealing deficits in sensory, motor or motivational processes. Repeated acquisition (learning) and performance (memory) were assessed in all the groups. A detailed analysis of swimming patterns revealed thigmotaxis in MMI and PbAc treatments, i.e. a higher percentage of time in outer zone and lesser time in the middle zone, the zone of platform location. Earlier workers found no changes in spatial reference memory and working memory in Morris water tank with methamidophos in adult rats [35]. The acquisition was not affected with CPS in mice [36] and in Pb treatment [37]. Parathion significantly increased learning, opining that adverse effects were seen only at higher doses. Repeated exposure to OPs could decrease behavioral effects due to tolerance resulting from compensatory changes in central nervous system. ACh in small doses is neurotrophic promoting neural cell differentiation, but as the dose of OP is increased, increase in ACh would cause down-regulation of muscarinic receptors [38]. The differences in neurobehavioral measures depended on the pesticide, specific end point and the time of measurement. Literature contrary to the results obtained shows decreased memory both while treatment [39] after treatment [40] and could affect either repeated acquisition.

Lead-induced (PND1-21) deficits in spatial learning were reversed when rats were raised in the enriched environment [41]. Gilbert et al. [42] exposed rats to 0.2% PbAc, as in the present study, from GD16 to PND21 and reported reduced neurogenesis but no impairment of spatial learning in MWM. Long-term Pb exposure was not associated with permanent brain dysfunction at a blood Pb concentration of 2 mM and some of the neuropsychological deficits seen could be due to attention deficit disorder. The results in the present study agree with the above-mentioned investigators that although learning and memory might have been affected, that could have been reversed or alleviated in the adult stage.

Thyroid gland of dams after PND21, i.e., the day of weaning was subjected to histopathology. The interlobular stroma was poorly defined. The follicular cells were flattened. Among the treatment groups, MMI, MCP and PbAc microfollicles were visible, had an irregular shape, absolutely empty with no colloid material and dense stroma appeared between the lobules (Figures-1 and 2). MMI is an anti-thyroid compound and would cause primary hypothyroidism as evidenced by its carbethoxy derivative carbimazole in first generation pups [43].

Rat cortex (neocortex, parietal, and frontal) develops between GD 14 and 20. The cerebral cortical layers showed a decrease in cellularity in all the treated groups as compared to control (Figures-3 and 4). This proves that the treatment affected cortical neurogenesis. Further, when a xenobiotic interferes with neurogenesis, the precursors are killed without replacement. Such a gap would be filled by abnormal cell migration and differentiation.

The same was the case with the molecular, Purkinje, and granular layers of the cerebellum. In MCP-treated group, the Purkinje cell layer was in total disarray. Focal loss of Purkinje cells was seen in the PbAc-treated group (Figures-5 and 6).

Hence, based on the study, it is concluded that MCP might affect neurodevelopment through its thyrotropic action as evinced by the distorted histology of thyroid, cortex and cerebellum. PbAc too could affect the thyroid, and thus caused developmental anomalies in the brain as evinced by increased T₄.

Region-specific and neurotransmitter-based evaluation need to be taken up to further understand the mechanisms of neurodevelopment and neurobehavioral toxicity.

**Conclusion**

MCP and lead might have affected the development of cerebrum and cerebellum via thyroid disruption leading to developmental neurotoxicity.

**Authors’ Contributions**

The present article was part of BKK’s Ph.D. research work and designed the experimental protocol. AGR, AVK and SSYHQ made critical suggestions in conducting the experiment and critically reviewed the manuscript. PSK drafted and revised the manuscript. All authors read and approved the final manuscript.

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**Competing Interests**

The authors declare that they have no competing interests.

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Rotterdam Convention - Operation of the Prior Informed Consent (PIC) procedure for banned or severely restricted chemicals in international trade

Decision Guidance Document

Monocrotophos

Secretariat for the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade
MANDATE

The Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade was adopted at the Conference of Plenipotentiaries held in Rotterdam on 10 and 11 September 1998. The Rotterdam Convention entered into force on 24 February 2004.

At its ninth session, held in Geneva 30 September to 4 October 2002 the Intergovernmental Negotiating Committee (INC) adopted the decision guidance document for monocrotophos (Decision INC-9/1) with the effect that all formulations of this chemical became subject to the interim PIC procedure.

The Committee also decided that with the circulation of this decision guidance document, countries would be invited to submit a single decision regarding future imports that would apply to all forms of monocrotophos, including the severely hazardous formulations listed in Annex III of the Convention (soluble liquid (SL) formulations of the substance which exceed 600 g a.i./l), unless explicitly exempted in the submitted import response.

At its first meeting, held in Geneva 20 to 24 September 2004, the Conference of the Parties agreed to include monocrotophos in Annex III of the Rotterdam Convention, with the effect that this chemical became subject to the PIC procedure.

The present decision guidance document was communicated to the Designated National Authorities on 1 February 2005 in accordance with Article 7 and 10 of the Rotterdam Convention.

Purpose of the Decision Guidance Document

For each chemical included in Annex III of the Rotterdam Convention a decision guidance document has been approved by the Conference of the Parties. Decision guidance documents are sent to all Parties with a request that they provide a decision regarding future import of the chemical.

The decision guidance document is prepared by the Chemical Review Committee (CRC). The CRC is a group of government designated experts established in line with Article 18 of the Convention, that evaluates candidate chemicals for possible inclusion in the Convention. The decision guidance document reflects the information provided by two or more Parties in support of the national regulatory actions to ban or severely restrict the chemical. It is not intended as the only source of information on a chemical nor is it updated or revised following its adoption by the Conference of the Parties.

There may be additional Parties that have taken regulatory actions to ban or severely restrict the chemical as well as others that have not banned or severely restricted it. Such risk evaluations or information on alternative risk mitigation measures submitted by Parties may be found on the Rotterdam Convention web-site.

Under Article 14 of the Convention, Parties can exchange scientific, technical, economic and legal information concerning the chemicals under the scope of the Convention including toxicological, ecotoxicological and safety information. This information may be provided directly to other Parties or through the Secretariat. Information provided to the Secretariat will be posted on the Rotterdam Convention website (www.pic.int).

Information on the chemical may also be available from other sources.

DISCLAIMER

The use of trade names in this document is primarily intended to facilitate the correct identification of the chemical. It is not intended to imply any approval or disapproval of any particular company. As it is not possible to include all trade names presently in use, only a number of commonly used and published trade names have been included in this document.

While the information provided is believed to be accurate according to data available at the time of preparation of this Decision Guidance Document, the Food and Agriculture Organization of the United
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No part of this publication shall be reproduced without a clear reference to its source.
| Abbreviation | Description |
|--------------|-------------|
| <            | less than   |
| ≤            | less than or equal to |
| <<<          | much less than |
| >            | greater than |
| ≥            | greater than or equal to |
| >>>          | much greater than |
| µg           | microgram   |
| AgDrift      | Spray Drift Taskforce Model |
| a.i.         | active ingredient |
| AchE         | acetylcholinesterase |
| ACGIH        | American Conference of Governmental Industrial Hygienists |
| ADI          | acceptable daily intake |
| ADP          | adenosine diphosphate |
| ALT          | alanine amino-transferase |
| AOEL         | acceptable operator exposure level |
| ARfD         | acute reference dose |
| ATP          | adenosine triphosphate |
| BOEL         | biological operator exposure limit |
| b.p.         | boiling point |
| BSI          | British Standards Institution |
| bw           | body weight |
| °C           | degree celsius (centigrade) |
| CA           | Chemicals Association |
| CAS          | Chemical Abstract Service |
| CCPR         | Codex Committee on Pesticide Residues |
| ChE          | cholinesterase |
| CHO          | Chinese hamster ovary |
| d            | day |
| D            | dust |
| DT₅₀         | period required for 50% dissipation |
| EC           | emulsifiable concentrate |
| EC₅₀         | effect concentration, 50% (median effective concentration) |
| ED₅₀         | effect dose, 50% (median effective dose) |
| EHC          | Environmental Health Criteria |
| FAO          | Food and Agriculture Organization of the United Nations |
| g            | gram |
| GAP          | good agricultural practice(s) |
| GL           | guideline level |
| GR           | granules |
| h            | hour |
| ha           | hectare |
| IARC         | International Agency for Research on Cancer |
| IC₅₀         | inhibition concentration, 50% |
| ICSC         | International Chemical Safety Card |
| ABBREVIATIONS WHICH MAY BE USED IN THIS DOCUMENT |
|------------------------------------------------|
| (N.B. Chemical elements and pesticides are not included in this list) |
| i.m. | intramuscular |
| i.p. | intraperitoneal |
| IPCS | International Programme on Chemical Safety |
| IPM | integrated pest management |
| ISO | International Organisation for Standardisation |
| IUPAC | International Union of Pure and Applied Chemistry |
| JMPR | Joint FAO/WHO Meeting on Pesticide Residues (Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues) |
| k | kilo- (x 1000) |
| kg | kilogram |
| K_{oc} | organic carbon/water partition coefficient |
| K_{ow} | octanol/water partition coefficient |
| K_{ow}logP | logarithm of the octanol/water partition coefficient |
| l | litre |
| LC_{50} | lethal concentration, 50% |
| LD_{50} | lethal dose, 50% |
| LD_0 | lethal dose, 0% |
| LD_{100} | lethal dose, 100% |
| LD_{LO} | lowest lethal dose |
| LOAEL | lowest observed adverse effect level |
| LOD | limit of detection |
| LOEL | lowest observed effect level |
| m | metre |
| mg | milligram |
| ml | millilitre |
| m.p. | melting point |
| mPa | millipascal |
| MRL | maximum residue limit |
| MTD | maximum tolerated dose |
| NCI | National Cancer Institute (United States of America) |
| ng | nanogram |
| NOAEL | no-observed-adverse-effect level |
| NOEC | no observed effect concentration |
| NOEL | no observed effect level |
| NOHSC | National Occupational Health and Safety Commission (Australia) |
| NRA | National Registration Authority for Agricultural and Veterinary Chemicals (Australia) |
| OECD | Organisation for Economic Co-operation and Development |
| OHS | Occupational Health and Safety |
| OP | organophosphorus pesticide |
| p | same as K_{ow} |
| Pa | pascal |
| PHI | pre-harvest interval |
| PIC | Prior Informed Consent |
| POEM | predictive operator exposure model |
| POP | Persistent Organic Pollutant |
| Abbreviation | Description |
|--------------|-------------|
| ppm          | parts per million |
| RfD          | reference dose (for chronic oral exposure. Comparable to ADI) |
| SC           | soluble concentrate |
| SG           | soluble granules |
| SL           | soluble liquid |
| SMR          | standardised mortality ratio |
| STEL         | short term exposure limit |
| SUSDP        | Standard for the Uniform Scheduling of Drugs and Poisons (Australia) |
| TER          | toxicity/exposure ratio |
| TLV          | threshold limit value |
| TMDI         | theoretical maximum daily intake |
| TWA          | time weighted average |
| ULV          | ultra low volume |
| UNEP         | United Nations Environment Programme |
| USEPA        | United States Environmental Protection Agency |
| UV           | ultraviolet |
| Vmd          | volume median diameter |
| VOC          | volatile organic compound |
| WHO          | World Health Organisation |
| WP           | wettable powder |
| wt           | weight |
Monocrotophos

1. Identification and uses (see annex I)

| Common name         | Monocrotophos (BSI, E-ISO)                               |
|---------------------|----------------------------------------------------------|
| Chemical name       | Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate (IUPAC) |
| Other names/synonyms|                                                           |
| CAS-No.(s)          | 6923-22-4 (formerly 919-44-8)                             |
| Harmonized System   |                                                           |
| Customs Code        | 2924.10.00 (technical grade active constituent)          |
|                     | 3808.10.90 (formulated product)                           |
| Category            | Pesticide                                                |
| Regulated Category  | Pesticide                                                |
| Use(s) in regulated category | An organophosphorus contact and systemic insecticide and acaricide used to control a broad spectrum of pests, including sucking, chewing and boring insects and spider mites on cotton, citrus, olives, rice, maize, sorghum, soybeans and tobacco. |
| Trade names         | Azodrin, Bilobrin, Crisodrin, Crotos, Glore Phos36, Harcros Nuvacron, More-Phos, Monocil, Monocron, Monocrotophos 60 WSC, Nuvacron 600 SCW, Plantdrin, Red Star Monocrotophos, Susvin, Phoskil 400. |
| Formulation types   | Available in a variety of soluble, liquid and emulsifiable concentrate formulations including 200, 400, and 600 g a.i./l concentrates, 400, 500, and 600 g a.i./l water-soluble concentrates, and 250 g a.i./litre ULV formulations. Monocrotophos is also available in mixtures with other pesticides. |
| Uses in other categories | No reported uses as an industrial chemical. |
| Basic manufacturers | Agrolinz, Inc.; Bharat Pulverizing Mills Ltd. (India); Cia-Shen Co. Ltd. (China); Comlets Chemical Industrial Co. Ltd. (Taiwan); Cyanamid (Brazil); Hindustan CibaGeigy Ltd. (India); Lupin (India); Nantong Pesticides Factory (China); Hui Kwang (China); National Organic Chemical Industries Ltd. (India); Quimica Estrella SACI el (Argentina); Quingdao Pesticides Factory (China); Sudarshan (India); United Phosphorus (India); Sundat (S) Pte Ltd. (Singapore). |

2. Reasons for inclusion in the interim PIC procedure

Monocrotophos is included in the interim PIC procedure as a pesticide. It is listed on the basis of the final regulatory actions to ban all uses of monocrotophos reported by Australia and Hungary.

Initially, only formulations of monocrotophos exceeding 600 g a.i./l were included in the interim PIC procedure as severely hazardous pesticide formulations, based on the recommendation of the fifth meeting of the FAO/UNEP Joint Expert Group (October 1992). The action was taken because of their acute hazard classification and concern as to their impact on human health under conditions of use in developing countries.
2.1 Final regulatory action: see Annex II for details

Australia

Registration of all monocrotophos products was cancelled from 9 December 1999, with all uses phased out over a year to allow existing stocks to be exhausted. This was seen as the lowest-risk option for disposing of existing stocks of monocrotophos in the light of the risks associated with product recall, storage and disposal. It also allowed users time to change over to other pesticides.

Reason: Occupational health* and environmental concerns.

Hungary

The registration for monocrotophos was withdrawn in 1996 as the reduction of application rates and the restriction of its uses did not reduce the level of adverse impact on wildlife to an acceptable level.

Reason: Environmental concerns.

2.2 Risk evaluation

Australia

Monocrotophos was applied in Australia using aerial, ground-rig and directed sprays to sorghum, sunflowers, tomatoes, cotton, potato, lucerne, soybean and tobacco to control Helicoverpa species, locusts, sorghum midge, western flower thrips, aphids, green vegetable bug, mites, stem borer and potato tuber moth.

On the basis of concerns arising from its risk evaluation and in the absence of a commitment by stakeholders to provide the data necessary to allay these concerns, Australia’s National Registration Authority (NRA) for Agricultural and Veterinary Chemicals concluded that there were reasonable grounds to cancel the registration and approvals for monocrotophos. The key aspects of this evaluation are detailed below.

Occupational safety and health

In the absence of measured worker exposure studies for conditions comparable with those for Australian use patterns and conditions for mixer/loader/applicators (M/L/A), the United Kingdom Predictive Operator Exposure Model (POEM) was used, where possible, in the assessment of risk, i.e. exposure and MOE (margins of exposure).

Exposure was predicted to be high and therefore unacceptable in all usual ground application situations.

On this basis, it was concluded that data would be required for all registered uses for ground application in Australia, including information on the functional efficacy of lower dose rates, if continued use of monocrotophos were permitted.

Environmental impact

The concerns from the environmental assessment are that monocrotophos is very toxic to aquatic invertebrates, birds and mammals and is not compatible with integrated pest management (IPM) programmes. There is a high hazard to birds from uses of monocrotophos when avian food items are sprayed. Spray drift from aerial and orchard air-blast spraying is a significant hazard to aquatic invertebrates. Runoff from recently treated areas was identified as hazardous to aquatic invertebrates from both acute and chronic toxic effects.

* In the Australian context, “occupational exposure” would include exposure to workers involved in:
  - Manufacture;
  - Formulation and re-packaging;
  - Mixing/loading;
  - Application;
  - Post-application activities such as cleaning of equipment; and
  - Re-entry following application for trimming/maintenance, bug-checking etc.

“Occupational exposure” may even go so far as to take into account exposure to “bystanders” such as fellow workers not directly involved in using the chemical. However, by definition, occupational exposure would not include members of the public. This would be included under “public health”.
Hungary

Monocrotophos in Hungary was registered for use on sugarbeet, sunflower, *Solanum nigrum*, maize, soybean, and alfalfa to control *Bothynoderes punctiventris*, *Psalidium maxillosum*, *Tanymecus dilaticollis* and *Tanymecus palliatus*.

Monocrotophos was first registered in Hungary in 1971 and the registration was extended in 1975. Registrations for the use of monocrotophos were modified in 1982 because of its observed adverse impacts on wildlife. Further reduction in application rates and restriction of its uses did not reduce the level of adverse impact upon wildlife to an acceptable level, leading to the withdrawal of all registrations in 1996. The key aspects of this evaluation are detailed below.

**Environmental impact**

The wildlife toxicity studies carried out at pilot and large-scale farms clearly confirmed that the use of Azodrin 40 WSC significantly damaged wildlife, first of all birds. Independently of the age and body weight of the animals and the growth stage of the treated crops, the use of the product caused death to some of the animals and prolonged poisoning in others (6–12 days). The poisoned animals did not respond to stimulus and would not flee, therefore it is probable that most were killed by predators. Additional losses were caused by the fact that the recommended use of the product was at the time of reproduction, thus poisoned animals which survived did not feed for several days, did not return to their nests and so on. In Hungary, in addition to pheasants, field hares (*Lepus europeus*) are the most important small game. In the wildlife toxicity studies carried out at large-scale farms, no hare deaths were observed, though slightly poisoned adults could be seen (3–4 kg). It is therefore probable that Azodrin 40 WSC caused death of young hares of low body weight. Azodrin 40 WSC had been used in Hungary since 1971. The annually treated acreage was 50,000 – 150,000 ha. Considering the very low populations of the dead animals and their unborn progeny, the estimated loss in Hungary amounted to 5 to 10 million pheasants since the use of Azodrin 40 WSC begun (25 years). Losses of other songbirds and granivorous birds of low body weight may be much greater than this figure. No other pesticide has caused damage of this extent in Hungary to the natural wild bird population, and the use of Azodrin 40 WSC has played a significant role in the current very low populations of small game birds and animals in Hungary.

### 3. Protective measures that have been applied concerning the chemical

#### 3.1 Regulatory measures to reduce exposure

**Australia**

Under the conditions of use in Australia, protective measures, including prohibition of application by back-mounted knapsack sprayers, the use of closed cabins for ground spraying and closed systems for mixer loaders, were not considered sufficient to reduce exposure to an acceptable level. As a result, registration for all monocrotophos products was cancelled.

**Hungary**

Protective measures were taken to reduce exposure, including a reduction in application rates and restriction of uses. They were not considered sufficient to reduce the adverse impacts of monocrotophos on wildlife and the compound was banned.

#### 3.2 Other measures to reduce exposure

*This section should be completed only where a chemical has been subjected to severe restriction and the notifying country or countries has or have allowed continued use of the chemical and associated products.*

Where it has been made available, additional information on protective measures (regulatory and other measures) taken in other countries concerning monocrotophos may be found on the Rotterdam Convention website [www.pic.int](http://www.pic.int).
3.3 Alternatives

Monocrotophos is a broad-spectrum contact and systemic insecticide and acaracide used in a wide range of crops. There are a number of alternative products available depending on the individual crop-pest complex under consideration. Limited information on alternatives that have been identified by Australia and Hungary may be found in Annex II.

Where it has been made available, additional information on alternatives to monocrotophos may be found on the Rotterdam Convention website www.pic.int.

*It is essential that before a country considers substituting alternatives, it ensures that the use is relevant to its national needs and the anticipated local conditions of use.*

3.4 Socio-economic effects

No detailed assessment of socioeconomic effects was undertaken by the notifying countries.
4. Hazards and risks to human health and/or the environment

4.1 Hazard Classification (WHO 1998)

| WHO | Technical product: 1b (highly hazardous), classification based on oral toxicity (WHO, 1999) |
|-----|------------------------------------------------------------------------------------------|
|     | Classification of formulations                                                            |
|     | **oral toxicity**                                                                          |
|     | LD₅₀: 14 mg/kg bw                                                                          |
|     | **dermal toxicity**                                                                       |
|     | LD₅₀: 112 mg/kg bw                                                                        |
|     | **Formulation**                                                                           |
|     | a.i. (%) | hazard class | a.i. (%) | hazard class |
| Liquid | >70     | 1a           | >25      | 1b           |
|        | >5      | 1b           | >1       | 11           |
|        | >1      | 11           |          |              |
| Solid  | >30     | 1b           | >90      | 1b           |
|        | >3      | 11           | >10      | 11           |
| E.C.   | Classification of the active substance (E.C. 1998) is:                                     |
|        | Mutagenic category 3 ; R 40: possible risks of irreversible effects;                        |
|        | T+; R 26/28: very toxic by inhalation and if swallowed;                                      |
|        | T; R 24: toxic in contact with skin;                                                        |
|        | N; R 50-53: dangerous to the environment, very toxic to aquatic organisms, may cause long-|
|        | term effects in the aquatic environment.                                                    |
| USEPA  | Category 1 (highly toxic) (USEPA, 1985)                                                    |
| IARC   | Not classified                                                                            |

Notifying countries

**Australia** – Monocrotophos is listed in the Australian National Occupational Health and Safety Commission (NOHSC) List of Designated Hazardous Substances. All monocrotophos products that were part of the Australian review are determined to be hazardous substances because they contain monocrotophos at 40% (w/v), exceeding the NOHSC cut-off concentration for hazardous substances.

It is included in Schedule 7 (Dangerous Poisons) of Australia’s Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

**Hungary** - In compliance with Annex II to Ministerial Decree 6/2001, monocrotophos is on the list of banned active ingredients.

4.2 Exposure limits

**Food**

The Codex Alimentarius Commission has published maximum residue limits for a range of fruits and vegetables, animal products, grains and edible oils. Maximum residue limits (MRLs) for these commodities range between the limit of analytical quantitation (0.02 to 0.05 mg/kg) and 1.0 mg/kg. These MRLs were recommended by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in 1991 and 1994.

JMPR established an acceptable daily intake (ADI) of 0.0006 mg/kg bw in 1993 (FAO/WHO 1993). This value was confirmed in 1995. An acute reference dose of 0.002 mg/kg bw/d was established in 1995 (FAO/WHO 1995).

**Drinking water**

WHO has not established a drinking-water guideline for monocrotophos.
4.3 Packaging and labelling

The United Nations Committee of Experts on the Transportation of Dangerous Goods classifies the chemical in:

| Hazard Class | 6.1, poisonous substance. |
| Packing | UN Pack Group II: substances and preparations presenting a serious risk of poisoning, formulations containing 25–100% monocrotophos. Unbreakable packaging; put breakable packaging into closed unbreakable container. Do not transport with food and feedstuff. |

| International Maritime Dangerous Goods (IMDG) Code | Monocrotophos is classified as a marine pollutant. |

For specific guidance on appropriate symbols and label statements regarding formulations of monocrotophos, countries should consult the FAO Revised guidelines on good labelling practice for pesticides (FAO 1995).

4.4 First aid

NOTE: The following advice is based on information available from the World Health Organization and the notifying countries and was correct at the time of publication. This advice is provided for information only and is not intended to supersede any national first aid protocols.

The signs and symptoms of acute organophosphate poisoning may occur in various combinations and may become manifest at different times. According to the degree of severity of poisoning, the following signs and symptoms may occur: anorexia, headache, dizziness, weakness, anxiety, miosis, blurred vision, slurred speech, nausea, hypersalivation, stomach pains, diarrhoea, vomiting and excessive sweating. In severe cases, respiratory depression and convulsions may also occur. In the case of monocrotophos, “intermediate syndrome” has been reported: this occurs after initial improvement, approximately one to eight days after poisoning. Muscle weakness leading to paralysis and sudden respiratory arrest occur (WHO 1999).

First aid personnel should wear rubber or plastic gloves to avoid contamination. Contaminated clothing and contact lenses should be removed as quickly as possible to prevent further absorption. If skin contact occurs, the area should be washed with soap and water; wash eyes for 15–20 minutes with running water. In the case of ingestion, the stomach should be emptied as soon as possible by careful gastric lavage, preferably within one hour of ingestion. Do not induce vomiting if the formulation contained hydrocarbon solvents. Activated charcoal may be effective. In massive overdoses, acute respiratory failure may occur. It is important to keep the airway open and to prevent aspiration if nausea and vomiting occur (WHO 1999).

Persons who have been poisoned, accidentally or otherwise, must be transported immediately to a hospital and placed under the surveillance of properly trained medical staff. Where possible, show the label of the monocrotophos container when the patient/affected person is presented for medical attention. Antidotes are atropine sulphate and pralidoxime chloride.

Depending on the degree of exposure, periodic medical examination is indicated, particularly since monocrotophos has been known to cause “intermediate syndrome”, which may become manifest some time after acute poisoning effects have worn off. Specific treatment is necessary in the event of poisoning with this substance; the appropriate means, with instructions, must be available.

If the substance is formulated with solvent(s), also consult the International Chemical Safety Cards (ICSC) cards for the solvent(s). Carrier solvents used in commercial formulations may affect the toxicity of the active ingredient by altering the extent of absorption from the gastrointestinal tract or through the skin.
4.5 Waste management

Regulatory actions to ban a chemical should not result in creation of a stockpile requiring waste disposal. For guidance on how to avoid creating stockpiles of obsolete pesticide stocks, the following FAO publications are available: Provisional guidelines on the prevention of accumulation of obsolete pesticide stocks (FAO 1995); Pesticide storage and stock control manual (FAO 1996); and Guidelines for the management of small quantities of unwanted and obsolete pesticides (FAO 1999).

In all cases, wastes should be disposed of in accordance with the provisions of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal, any technical guidelines thereunder and any other relevant regional agreements.

It should be noted that the disposal/destruction methods recommended in the literature, such as high-temperature incineration, are often not available in, or suitable for, all countries. Consideration should be given to the use of alternative destruction technologies. Further information on possible approaches may be found in the FAO/WHO/UNEP provisional technical guidelines for the disposal of bulk quantities of obsolete pesticides in developing countries (FAO 1996).

Australia and Hungary avoided creating a stockpile of monocrotophos by taking a step-by-step approach to the phase-out of permitted uses (see Annex II). It was considered that the risk was manageable for this phase-out period.

Annexes

Annex I  Further information on the substance
Annex II  Details on final regulatory action
Annex III  Addresses of designated national authorities
Annex IV  References
INTRODUCTION TO ANNEX I

The information presented in this Annex reflects the conclusions of the two notifying countries, Australia and Hungary. This information is contained in the documents referenced in the notification of regulatory action as supporting their national regulatory actions banning monocrotophos. These notifications of regulatory action were first reported in the PIC Circular of December 2000.

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) reviewed monocrotophos in 1972, 1975, 1991, 1993 and 1994. The conclusions of JMPR were not substantially different from those reported here. Section 2.2.7 includes a brief comparative summary of the conclusions of the two toxicological evaluations.
Annex I – Further information on the substance

1. Physico-chemical properties (Tomlin, 2000)

1.1 Identity Monocrotophos

1.2 Formula C₇H₁₄NO₅P

1.3 Chemical name (IUPAC) Dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate

1.4 Chemical type Organophosphate

Form Pure monocrotophos: colourless hygroscopic crystals. Technical monocrotophos, a reddish-brown semi-solid, is at least 75% pure

1.5 Solubility At 20°C - in water 100%, methanol 100%, acetone 70%, n-octanol 25%, toluene 6%

K_W logP -0.22 (calculated), K_ow 0.60 (calculated)

1.6 Vapour pressure 2.9 x 10⁻⁴ Pa at 20°C

1.7 Melting point 54–55°C

1.8 Reactivity Hydrolysis – half-life at 20°C calculated from Arrhenius parameters: 96 days at pH 5, 66 days at pH 7 and 17 days at pH 9.

Corrosive to black iron, drum steel and stainless steel.

1.9 Stability Decomposes above 38°C, thermal runaway reaction can occur above 55°C. Unstable in short-chain alcohols, decomposes on some inert materials.

Decomposes on heating or burning, producing toxic and irritating fumes including nitrogen oxides, phosphorus oxides. Attacks iron, steel, brass.

Storage – monocrotophos technical grade active constituent should be stored out of direct sunlight and under cool and dry conditions to minimize any degradation.

1.10 Molecular Weight 223.2

2. Toxicological properties

2.1 General Monocrotophos affects the nervous system by inhibiting acetylcholinesterase, an enzyme essential for normal nerve impulse transmission. The toxicological profile of monocrotophos is typical of organophosphorus compounds, with cholinergic signs (including tremors, convulsions, salivation and trismus) being similar in experimental mammals and humans.

2.1.2 Symptoms of poisoning Symptoms of monocrotophos poisoning are typical of cholinergic signs seen after exposure to other organophosphorus insecticides and include excess salivation and lachrymation, tremors, convulsions, and miosis (see also Section 3.5).
2.1.3 Absorption, distribution, excretion and metabolism in mammals

Monocrotophos is systemically absorbed if it is swallowed, inhaled or comes in contact with the skin. Dermal absorption of $^{14}$C-labelled monocrotophos in humans was about 22% of a single dose applied (in acetone) to the forearm for 24 h. Oral absorption in experimental animals was effectively 100% of the administered dose.

Monocrotophos was rapidly absorbed and excreted, mainly in the urine, within 24 hours after oral dosing in rodents. Very little residual tissue accumulation of monocrotophos or its metabolites occurred. Unchanged monocrotophos was found in the urine of rats at greater than 30% of the administered dose. After oral administration of monocrotophos to rats and goats, parent compound, N-methyl acetoacetamide and 3-hydroxy-N-methyl butyramide were detected in the urine.

There were variations in the rates of absorption, metabolism and elimination but overall the metabolic path for monocrotophos appeared to be similar between species. The metabolic pathway in mammals was determined to be mainly a detoxification route involving ester cleavage of monocrotophos.

2.2 Toxicology studies

2.2.1 Acute toxicity

Oral

Monocrotophos was extremely toxic by oral route for rats and mice, with LD$_{50}$ values of approximately 8 and 10 mg/kg bw respectively.

Dermal

The acute dermal toxicity of monocrotophos was solvent-dependent: it was of low to high toxicity in rats (LD$_{50}$ values ranging from 119 to $>2,000$ mg/kg) and of moderate to high toxicity in rabbits (LD$_{50}$ values ranging from 130 to 709 mg/kg).

Inhalation

Monocrotophos had high inhalation toxicity in rats, with an LC$_{50}$ (4 h) of 80 mg/m$^3$.

Irritation

In rabbits, monocrotophos was slightly irritating to the eyes and skin but it was not a skin sensitizer in guinea pigs.

ARfD

No inhibition of erythrocyte cholinesterase activity or other signs of toxicity were seen in volunteers exposed to single oral doses of monocrotophos at up to 0.0059 mg/kg bw in a 28-day study. Based on this no observed effect level (NOEL), and using a 10-fold safety factor, the acute reference dose (ARfD) for monocrotophos in Australia was established at 0.0006 mg/kg bw.

2.2.2 Short-term toxicity

In short-term studies, the inhibition of cholinesterase activity was the main toxicological effect in experimental animals. When rats were given monocrotophos (technical) in the diet for up to 13 weeks, cholinesterase activity was significantly inhibited, but a 5-week recovery phase following feeding allowed some recovery of cholinesterase activity. In repeat-dose dermal studies, the inhibition of cholinesterase activity was also the main toxicological finding. Even at doses that resulted in clinical signs of intoxication, no significant treatment-related gross or histopathological findings were generally observed.

There did not appear to be any clear difference between monocrotophos binding affinity with plasma (or pseudo- or butyryl-) cholinesterase and with erythrocyte or brain cholinesterase (acetyl- or true cholinesterase). There
was considerable variability in responses to monocrotophos between studies, with brain cholinesterase on occasions being the most sensitive to effects of monocrotophos, while in other studies plasma and/or erythrocyte cholinesterase activities were most sensitive to inhibition by monocrotophos.

The anticipated clinical signs associated with organophosphorus compounds and attributable to an excessive interaction of acetylcholinesterase with muscarinic and nicotinic cholinergic receptors were common to all animal studies using monocrotophos. Measurements of plasma, erythrocyte and brain cholinesterase activity in a variety of studies did not reveal a clear hierarchy of inhibition.

It is Australia’s policy to use human data in preference to animal data where human studies are considered to be adequately conducted and reported according to ethical principles of human experimentation. In two different human studies, volunteers received daily oral doses of monocrotophos at up to 0.0059 mg/kg bw for 28 days. No adverse clinical signs were observed. Erythrocyte acetylcholinesterase activity was not affected at any dose level. Plasma cholinesterase activity was significantly decreased at higher doses but not at the low dose of 0.0036 mg/kg bw/d. The acceptable daily intake (ADI) for monocrotophos in Australia was established as 0.0003 mg/kg bw/d, based on the NOEL of 0.0036 mg/kg bw/d for plasma cholinesterase inhibition and using a 10-fold safety factor.

2.2.3 Genotoxicity (including mutagenicity)

Extensive genotoxicity testing has been conducted with monocrotophos ranging in purity from 36% to 99%. Some in vitro mutagenicity tests in bacteria and in yeast, fungi and mammalian cell cultures showed that monocrotophos and its formulations had weak mutagenic potential, both with and without metabolic activation. Similarly, monocrotophos showed potential to damage chromosomes of human lymphocytes, Chinese hamster ovary cells, and rat tracheal epithelial cells, and to induce unscheduled DNA synthesis in human fibroblasts.

In vivo genotoxicity tests showed predominantly negative results, although a weakly positive result was obtained in a mouse micronucleus assay. Monocrotophos did not induce dominant lethal mutations in mice. The doses at which genotoxic effects were observed in in vivo studies were several orders of magnitude greater than the doses at which cholinesterase inhibition was seen in previous studies.

2.2.4 Long-term toxicity and carcinogenicity

The inhibition of cholinesterase activity was the main toxicological effect in long-term animal studies. A two-year rat study investigated histopathological changes in peripheral and central nerves, and found no evidence for a dose-related increase in abnormalities. Progressive examinations through the two-year period did not provide evidence for any acceleration of normal age-related changes. No other significant pathological findings were observed in long-term studies, even when treatment resulted in clinical signs of intoxication.

There were no carcinogenic effects seen over two years of dosing with monocrotophos at the highest dose tested in CD mice (approximately 1.5 mg/kg bw/d), Charles River rats (approximately 5 mg/kg bw/d), Wistar rats (approximately 0.5 mg/kg bw/d) and Beagle dogs (approximately 0.4 mg/kg bw/d).

2.2.5 Effects on reproduction

Overall, development signs were seen only at doses at or near maternotoxic doses, and there were no significant treatment-related teratogenic findings. A development study using Sprague Dawley rats showed a dose-related decrease in the percentage of male foetuses. However, this effect was not seen in a developmental study using Charles River rats, or in a number of
multi-generation reproduction studies in Wistar or Long-Evans rats. In New Zealand rabbits, there was an increase in the incidence of premature deliveries in one study, but this effect was not seen in a second study using another strain of rabbits. Delayed foetal development, including effects on ossification, were attributed to the maternal toxicity of monocrotophos.

2.2.6 Neurotoxicity/ delayed neurotoxicity

There was no evidence for delayed neurotoxicity effects in a range of studies using hens, varying from single oral administration to a 78-day study.

2.2.7 Summary and overall evaluation

Studies in experimental animals indicate that cholinesterase (ChE) inhibition is the major toxic effect of monocrotophos.

In experimental animals, monocrotophos is of high acute toxicity. The lowest oral LD₅₀ is 8.4 mg/kg bw in rats (10 mg/kg bw in mice) and lowest inhalation LC₅₀ is 80 mg/m³ (4 h) in rats. The acute dermal toxicity of monocrotophos is variable and dependent on the solvent; the lowest dermal LD₅₀ is 123 mg/kg (rats). Monocrotophos is a slight skin and eye irritant in rabbits. It is not a skin sensitizer in guinea pigs.

In animal studies, monocrotophos is rapidly excreted mainly in the urine, without evidence of significant accumulation in the body. The metabolic pathway is a detoxification route ultimately involving the ester cleavage of monocrotophos with the formation of N-methyl acetacetamide and 3-hydroxy-N-methyl butyramide as well as dimethyl phosphate and/or monomethyl phosphate.

Single or repeat dose studies (up to 78 days) in hens did not demonstrate delayed neurotoxicity.

It did not have an adverse effect in reproductive parameters in rodent studies. Developmental toxicity was noted only at or near maternotoxic doses in rats and rabbits; however, no teratogenic findings were observed.

Monocrotophos appears to be a weak mutagen at high doses. Metabolic activation was not required for mutagenic or other genotoxic effects of monocrotophos.

Monocrotophos was not found to be carcinogenic. Two-year dietary administration of the chemical in rats did not indicate nerve damage or acceleration of normal age-related changes. The most conservative no observed effect level (NOEL) for monocrotophos established for animal studies was 0.004 mg/kg/d (LOEL 0.04 mg/kg/d) in one- and two-year dog dietary studies for brain ChE depression.

In a number of trials (monocrotophos given in capsule form for 28 days) in human volunteers, a NOEL of 0.0036 mg/kg/d was established based on plasma ChE depression at the next high dose. Red blood cell cholinesterase was not affected. The NOELs established in short-term human studies are similar to the NOEL for long-term animal studies (0.004 mg/kg bw/d).

Acceptable daily intake (ADI) was established at 0.0003 mg/kg bw/d.

The ADI is based on human studies in which volunteers received daily oral doses of monocrotophos at up to 0.0059 mg/kg bw for 28 days. No adverse clinical signs were observed. Erythrocyte acetylcholinesterase activity was not affected at any dose level. Plasma ChE activity was significantly decreased at higher doses but not at the low dose of 0.0036 mg/kg bw/d. The ADI was established as 0.0003 mg/kg bw/d, based on the NOEL of 0.0036 mg/kg bw/d for plasma cholinesterase inhibition (LOEL 0.0057 mg/kg/d) and using a 10-fold safety factor.

The acute reference dose (ARfD) was established at 0.0006 mg/kg bw. The ARfD is based on human studies in which volunteers were exposed to single
oral doses of monocrotophos at up to 0.0059 mg/kg bw in a 28-day study and no inhibition of erythrocyte cholinesterase activity or other signs of toxicity were seen. The ARfD was established based on this no observed-effect level (NOEL) of 0.0059 mg/kg bw and using a 10-fold safety factor.

FAO/WHO JMPR (1995)
The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluated monocrotophos in 1972, 1975, 1991, 1993 and 1995.

Monocrotophos was not found to be carcinogenic or teratogenic and caused no toxicity other than the cholinergic syndrome.

An acceptable daily intake (ADI) of 0.0006 mg/kg bw was allocated in 1993 and confirmed in 1995.

This ADI was established on the basis of a 28-day human volunteer study with an NOAEL for erythrocyte acetylcholinesterase of 0.006 mg/kg bw/d and using a 10-fold safety factor.

An acute reference dose (ARfD) of 0.002 mg/kg bw was established by JMPR in 1995.

It was concluded that the available toxicological data in humans allowed the establishment of an acute reference dose on the basis of erythrocyte acetylcholinesterase inhibition and using a 10-fold safety factor.

3. Human exposure/risk evaluation

3.1 Food Australia

An estimate of monocrotophos intake was derived from the Australian Market Basket Survey. This procedure is based on measured monocrotophos residues found in food surveys rather than assuming that the pesticide is present at the MRL. In 1994, the estimated intake in the group with the highest consumption of monocrotophos residues (toddlers aged two) was 7.2 nanograms/kg bw/d. This intake accounts for less than 3% of the ADI.

3.2 Air

Not relevant.

3.3 Water

Not relevant.

3.4 Occupational Australia

In accordance with internationally accepted practice, the occupational risk assessment was based on hazard characterization and worker exposure. The latter took into consideration the mixing, loading and application activities involved in the use of the pesticide.

End-use applications

There were no measured worker exposure studies for mixing, loading or application of monocrotophos. Therefore, the UKPOEM was used to estimate exposure from which margins of exposure (MOE) for the Australian use pattern were determined wherever possible.

As a result of the occupational risk assessment, the following conclusions were reached.

Acceptable and supported uses of monocrotophos

Broadacre crops, potatoes and bananas

Broadacre crops including tobacco, cereals, wheat, oilseeds and cotton are treated with monocrotophos mainly by aerial spraying, which was the only application method used to treat bananas with this pesticide in Australia. Aerial spraying of monocrotophos may also be used for potatoes. Based on the qualitative risk assessment, continued use of aerial spraying for these crops would be acceptable as long as it remained available only to licensed and authorized personnel.
As the risk could not be quantified, the following control measures are needed for aerial spraying on these crops:

- Essential uses only;
- Development of enclosed mixing/loading systems;
- Farm chemical user training for workers handling monocrotophos;
- Health surveillance to be conducted, when appropriate, for workers handling monocrotophos;
- Human flagging in aerial operations is not acceptable, unless flaggers are protected by engineering controls such as cabs.

**Unacceptable and not-supported uses of monocrotophos**

**Fruit trees and vegetables**

The risk for workers applying monocrotophos by high-volume airblast spraying based on predicted exposure was high and unacceptable, even if mixer/loader exposure was eliminated. Other uses for pome fruit (apples and pears) are not supported as the risk is unacceptable. Measured worker exposure data is needed to quantify risk for these uses.

Monocrotophos use by high-volume or low-volume boom-spraying on tomatoes, French beans and maize is not supported as the risk is unacceptable. Measured worker exposure data is needed to quantify risk for these uses.

Ground-spraying on broadacre crops is not supported as the risk is also unacceptable. Measured worker exposure data is needed to quantify risk for this use.

**Flowers – control of budworms**

The risk for workers applying monocrotophos by high-volume or low-volume boom-spraying based on predicted exposure was high and unacceptable, even if mixer/loader exposure was eliminated in each case, and thus its use was not supported.

**Re-entry**

Overseas studies on dislodgeable foliar residues indicated low levels of residues at 96 hours post-application. The degradation of monocrotophos under aerobic conditions in soil was rapid, with a half-life of between one and seven days, and thus it is unlikely to persist in soil beyond one week following application. It is not expected to bioaccumulate. Based on currently available data, a re-entry period of five days is acceptable.

**Regulatory advice**

It is recommended that appropriate training courses be identified for all workers involved in the use of monocrotophos.

Aerial spraying is the only application method which is supported due to the comparatively minimal exposure likely to users. In general, the use of monocrotophos products should be restricted to emergency-permit use only.

In Australia, organophosphorus pesticides are placed on the National Occupational Health and Safety Commission’s Schedule for Health Surveillance.

**3.5 Medical data**

Several published clinical case studies involving accidental exposure or
suicide attempts with monocrotophos have reported the development of “intermediate syndrome”. This condition owes its name to the onset of reversible paralysis of cranial nerves, weakness of thorax muscles and respiratory difficulties occurring after exposure, generally after cholinesterase activity has begun to return to normal. Thus, its onset may be delayed after apparent recovery from the acute effects characteristic of muscarinic, nicotinic and CNS nerve overstimulation.

4. Environmental fate and effects

4.1 Fate

4.1.1 Soil

The degradation of monocrotophos under aerobic conditions in soil is fast, with a half-life of between <1 and 7 days, based on five different soils. The major products were carbon dioxide and non-extractable residues. Some minor metabolites were identified in some soils, with the highest at 3.5% of the applied dose. The major degradation pathway appears to be direct metabolism to carbon dioxide or incorporation into the organic fraction of the soil followed by mineralization.

No studies were presented that determined a half-life or examined whether monocrotophos degrades under anaerobic conditions. The photolysis half-life of monocrotophos on soil was less than seven days.

It is concluded that monocrotophos is mobile in soil and that leaching is possible. However, the rapid degradation will limit the extent of leaching that is likely to occur under field conditions.

4.1.2 Water

No studies were presented that determined a half-life. However, monocrotophos was shown to degrade rapidly under aquatic aerobic conditions (a rice paddy in the tropics) but, by contrast, there was no degradation in natural river water at room temperature, consistent with the hydrolysis experiments. It is concluded that the limited studies show that in aquatic systems with high microbial activity, i.e. with soil/sediment, degradation could be rapid. The lack of a suitable aerobic aquatic metabolism study is a significant data gap.

Hydrolysis is unlikely to be a significant contributor to the overall degradation of monocrotophos within the normal environmental pH range. Direct photolysis in water is not expected but indirect photolysis is possible.

4.1.3 Air

Volatilization from soil, or water, is not expected to be a significant route for dissipation, but volatilization from other non-adsorbing surfaces cannot be ruled out. Significant concentrations in air are not expected.

4.1.4 Bioconcentration

Based on water solubility, low K_{ow} and ready soil degradation, significant bioaccumulation in the aquatic environment is not expected.

4.1.5 Persistence

Does not accumulate in soil because it is biodegradable and photolabile. Its half-life is less than 7 days in soil exposed to natural sunlight. Monocrotophos has a half-life of 1.3 to 3.4 days on plant foliage.

4.2 Ecotoxicity – Effects on non-target organisms

4.2.1 Terrestrial vertebrates

Mammals

Monocrotophos is extremely toxic to laboratory rodents by the oral route of exposure, with LD_{50} around 10 mg/kg (see Section 2.2.1). The acute dermal toxicity is somewhat less (Section 2.2.1).

In Australia, tests on the native marsupial *Sminthopsis macroura* showed that a single dietary dose at 80–100 mg/kg bw caused death. A lower dose at 2 mg/kg bw at intervals over 18 days did not cause any deaths. The Australian native rodents *Notomys alexis* and *Notomys mitchelli*
when fed monocrotophos at 668 mg/kg for 5 consecutive days showed reduced body weight and all animals were off their feed by the end of the testing period.

In the Hungarian wildlife toxicity studies carried out at large-scale farms using Azodrin 40 WSC at 1.5 l/ha (maximum label rate), no hare deaths were observed, though slightly poisoned adults could be seen. Therefore it is probable that Azodrin 40 WSC causes death of young hares of low body weight.

Birds

Monocrotophos is rated (by USEPA) as very highly toxic to birds by both the acute oral (reports for 13 species, LD$_{50}$ of 0.19 to 6.49 mg/kg) and dietary routes of exposure (3 species, LC$_{50}$ range 2.4 – 32 ppm). Multi-generation tests (approximately 20 weeks’ exposure) on Japanese quail and Mallard duck showed that effects occurred at low levels, 0.1 and 3.0 mg/kg in feed respectively. [Source: database compiled by the USEPA (Ecological Fate and Effects Division, Office of Pesticide Programs) of studies reviewed by them and judged to meet USEPA guidelines.]

Results in the literature for toxicity also indicate very high toxicity to birds – acute toxicity: 1.0 – 4.21 mg/kg; chronic toxicity: NOEC 0.5 mg/kg/d (Japanese quail, 21 d).

Field reports indicate that monocrotophos has been associated with several incidents of bird kill in the United States of America. These old field studies suggest that where there was either food, i.e. wild seeds, or standing water which attracted birds to either drink or feed in the treated fields, significant mortalities occurred at rates of 1 kg a.i./ha and above, except for one study that showed mortalities at 0.32 kg a.i./ha. Birds entering recently sprayed fields were not affected provided they did not feed or drink in the field. Feeding on sprayed locusts or rodents also led to high mortalities.

There are anecdotal Australian reports of bird kills from label use of Monocrotophos EC, but no reliable reports. There are well-documented reports of monocrotophos causing significant mortalities of Swainson’s hawks in Argentina following use to control grasshoppers.

In Hungary, wildlife toxicity studies at pilot and at large-scale farms clearly confirmed that the use of Azodrin 40 WSC significantly damaged wildlife, mainly birds. Independently of the age and body weight of the animals and the growth stage of the treated crops, the use of the product caused death to some birds and prolonged poisoning to others (6 – 12 days). The poisoned birds did not respond to stimulus and were unable to flee, therefore it is probable that most were killed by predators. Additional losses were caused by the fact that the recommended use of the product in Hungary was at the time of bird reproduction, thus poisoned birds which survived did not feed for several days or return to their nests, and so on.

4.2.2 Aquatic species

Fish

Fish are the least sensitive aquatic species, with LC$_{50}$s ranging from 1.9 to 180 mg a.i./l based on 9 species. Monocrotophos is rated as moderately to slightly toxic to fish, again according to USEPA criteria. Several of these values are old, nominal and not considered reliable, but they have been used by NRA in the absence of other data. The USEPA Office of Pesticide Programs database entries show similar sensitivities for fish, with LC$_{50}$s between 5.2 and 50 mg/l.

Aquatic invertebrates

Monocrotophos is rated according to USEPA classifications as very highly to slightly toxic, with invertebrates being the most sensitive class of organisms. The reported acute toxicity to daphnia is given as
between 0.24–20 µg/l but no study meets current requirements.

**Algae**

Monocrotophos is rated as moderately toxic to one species of green alga, *Chlorella vulgaris*, with EC\textsubscript{50} of 6.8 mg/l (nominal), but non-toxic to *Scenedesmus subspicatus*, another green alga, where the EC\textsubscript{50} was >100 mg/l and NOEC = 100 mg/l. USEPA considers both as insensitive species.

**4.2.3 Honey bees and other arthropods**

Based on the results of 15 reports, monocrotophos is very toxic to all the non-target invertebrates tested, in particular bees, lacewing and a range of other predatory insects. Residues on foliage were very highly toxic to bees 24 hours after application (100% mortality). Some reports show that monocrotophos is more toxic to beneficial insects than to pests.

**4.2.4 Earthworms**

The toxicity to earthworms was 196 mg/kg of soil for one test and 35 mg/kg for another. Tests were stated to be based on OECD Guideline 207. These tests rate monocrotophos as either slightly or moderately toxic to earthworms.

**4.2.5 Soil microorganisms**

No toxicity data were available for these organisms.

**4.2.6 Terrestrial plants**

Direct application to desirable terrestrial plants and vegetation is not expected and monocrotophos is non-phytotoxic when used as directed, although some apple, pear, peach, cherry and sorghum varieties may suffer slight injury. Significant effects on desirable plants are therefore considered unlikely.
5. Environmental exposure/risk evaluation

5.1 Terrestrial vertebrates

Birds

Australia’s environmental assessment calculations using standard methodology show that the overall risk to birds appears high and unacceptable, especially to birds that consume insects, seeds and so on or are directly oversprayed by the chemical. Use of monocrotophos to control locusts at the higher rate is likely to represent a very high risk to avian predators of locusts and is unacceptable. This risk has occurred in Argentina, where large numbers of Swainson’s hawks died following application of monocrotophos to control grasshoppers, and led to use of the chemical being restricted/banned. At the lowest label rate for small locusts, 350 ml/ha, calculations for acute dietary exposure for quail ($LC_{50} = 2.4$ ppm, 50% of feed contaminated) for small insects indicate a high risk and for large insects a moderate risk.

5.2 Aquatic species

Fish/aquatic invertebrates

For aerial application, apart from direct overspray the risk to fish is considered to be acceptable. No risk is expected to algae. However, the risk to sensitive aquatic invertebrates was determined to be unacceptable to beyond 300 metres from spray drift at all aerial application rates, based on AgDRIFT (from the USEPA) and literature reports, when used according to current label directions. At the lowest rate examined, 140 g a.i./ha, the risk to less sensitive aquatic invertebrates was acceptable at 300 metres but only with placement spraying (coarse droplets, vmd 350 $\mu$m). It should be noted that a high risk exists at high rates from runoff as well.

For orchard applications, AgDRIFT showed that for apple and stone-fruit orchards the risk to aquatic invertebrates from orchard air-blast sprayers was moderate at 50 metres and may be acceptable with additional label restrictions. For larger trees and dormant spraying, the risk was high and extended to beyond 100 metres from the orchard. Information from the agricultural assessment and other sources show that use on pome fruit orchards is declining with the introduction of IPM. Considering the lack of data on degradation, the level of risk and also that use of monocrotophos is declining in favour of chemicals more suitable for IPM, Australia’s assessment favoured the removal of pome fruit use from the label.

The spray-drift risk from boom sprayers (given by AgDRIFT) to aquatic invertebrates was high at 30 metres, especially at the application rate tested, 800g a.i./ha (2 l/ha), and just acceptable at 100 metres. At the lowest rate, 140 g a.i./ha (350 ml/ha), the risk at 30 metres was just acceptable. Runoff remained a potential problem for rates $\geq 280$ g a.i./ha. Australia nor could support the use of monocrotophos by boom spray unless the application rate was significantly reduced.

In the aquatic environment, monocrotophos is not expected to persist for an extended period, but based on very limited data, the degradation rate is considered dependent on the level of microbial activity. The field studies showed that degradation was fast in rice paddies but slow in natural water. There were no data for more typical agricultural sediment/water systems in temperate conditions. Assuming a half-life of two days, calculations showed that chronic and subchronic effects on aquatic invertebrates were possible from aerial spray drift but less likely from other application technologies. Although there are no chronic effect data, it was assumed that chronic effects are approximately one
tenth of the acute effect, a common “rule of thumb”. Chronic effects on aquatic organisms could not be ruled out.

5.3 Honey bees and other arthropods

At the application rate of 720 g a.i./ha (1.5 l/ha, the rate for sunflowers, sorghum, and orchards), the risk to bees was determined to be high. The risk from aerial spray drift to bees is high at the higher rates and likewise for other non-target insects, but is acceptable at rates used for locust control, 280 g a.i./ha at 100 metres. However, spray drift from the lowest rate, 140 g a.i./ha is expected to be toxic to Apanteles spp., the most sensitive insects to topical applications of monocrotophos.

5.4 Earthworms

The risk to earthworms from the use of monocrotophos is expected to be low.

5.5 Soil microorganisms

For other soil invertebrates there may be expected to be a high risk but there are no toxicity data for these organisms.

5.6 Summary

Using standard methodology it was concluded that there was a high risk to birds from the current use of monocrotophos when avian food items were sprayed. There was also a high aquatic risk to sensitive invertebrates from spray drift at all application rates, except for boom-spray applications at 140 g a.i./ha, where, provided suitable measures to reduce spray drift are in place, the risk is moderate. The risk to bees and other non-target insects was high. There is also a potentially high risk to aquatic organisms from runoff if rain occurs within days of application.
Annex II – Details on final regulatory actions reported

Country Name: Australia

1. Effective date(s) of entry into force of actions
From 9 December 1999: registration of monocrotophos cancelled, further imports prohibited. Use phased out according to the following schedule:

- **Wholesale supply**: to cease by 30 June 2000;
- **Retail sale**: to cease by 31 December 2000; and
- **MRLs withdrawn**: from 30 June 2002.

2. Succinct details of the final regulatory action(s)
The decision cancels the registrations and all relevant approvals for monocrotophos, halts further imports and phases out its use over a one-year period. The Australian MRL for monocrotophos to be withdrawn on 30 June 2002.

3. Reasons for action
Unacceptable occupational health and safety risks.

4. Basis for inclusion in Annex III
Decision follows a review of monocrotophos under the Australian National Registration Authority’s Existing Chemical Review Programme, which failed to satisfy the National Registration Authority that continued use of monocrotophos products, in accordance with the recommendations for its use, would not harm people or the environment. Importantly, there was no commitment by stakeholders to generate the required data to allay concerns about environmental, occupational health and residue impacts.

The review identified several areas of concern about the use of monocrotophos relating to environmental and worker exposure, residues, and to its particular toxicity to birds.

4.1 Risk evaluation
The review concluded that continued use of monocrotophos would pose an unacceptably high risk to workers, wildlife and trade.

4.2 Criteria used
Risks to the environment, occupational health and safety (OHS), public health and trade.

Relevance to other States and regions
Of special concern to developing countries because of the high risk associated with ground spraying of monocrotophos, even when rigorous OHS practices are employed.

5. Alternatives
The following alternatives are considered to pose lower risks to workers and the environment. WHO hazard classifications are provided as an aid to consideration of relative risks. These classifications are for active constituents. Actual hazard depends on formulations. This list is not exhaustive and other alternatives are available.

**Moderately hazardous:**
- Chlorpyrifos, diazinon; dimethoate; fenitrothion

**Slightly hazardous:**
- Azamethiphos; malathion.

It is recommended that if any of the above chemicals are to be considered as alternatives, advice should be sought from product manufacturers concerning
suitability for the proposed use and for local conditions.

|   | Waste management                                                                 |
|---|----------------------------------------------------------------------------------|
| 6.| Halting imports followed by phase-out of existing stocks                         |

|   | Other                                                                 |
|---|----------------------------------------------------------------------|
| 7.| Australia has established a Health Value of 0.001 mg/l for monocrotophos in drinking water. (The “Health Value” is the concentration of contaminant that is not expected to result in any significant health risk to consumers, assuming a lifetime intake of 2 litres of water/day. The derivation of this value assumes a bodyweight of 70 kg and that intake from drinking water will constitute 10% of the ADI (which is 0.0003 mg/kg bw/d). |
Country Name: Hungary

1. **Effective date(s) of entry into force of actions**
   - Registration of monocrotophos-containing insecticides withdrawn in 1996.

Reference to the regulatory document
- The registration of products with monocrotophos as their active ingredient was reviewed in compliance with Ministerial communiqué 1994/20, by the Plant Protection and Agro-environmental Department of the Ministry of Agriculture and Food, published in the Official Journal of the Ministry. In compliance with Annex II to Ministerial Decree 6/2001 FVM, monocrotophos is on the list of banned active ingredients.

9032/1992; 21175/1996.

2. **Succinct details of the final regulatory action(s)**
   - Banned for all agricultural uses.

3. **Reasons for action**
   - Unacceptably high adverse impact on wildlife.

4. **Basis for inclusion in Annex III**
   - A review based on field observations and studies which showed that monocrotophos has an unacceptably high adverse impact on the environment.

4.1 **Risk evaluation**
   - Scientific studies carried out at small-scale and large farms indicated extremely high risk to birds and bees during and following the application of monocrotophos-containing products.

   The review identified concern about environmental impacts resulting from the extreme adverse impacts on wildlife observed under conditions of commercial use, confirmed by toxicity tests at pilot farms and large-scale farms at the Nature and Wildlife Conservation Station (Fácánkert, Hungary) between 1976 and 1980, and reported by users, hunters, and environmentalists.

   Restrictions on uses and times of application, and of the quantity to be applied per unit area (limited to 0.75-1.0 l/ha to control seedling pests of sugar beet and maize grown in blocks, and crops with poorer wildlife populations) did not reduce the impact on wildlife to an acceptable level.

4.2 **Criteria used**
   - Assessment of impact upon wildlife.
   - Because of the similar ecological parameters (climate, crops and pests), the action by Hungary is highly relevant to neighbouring States.

5. **Alternatives**
   - The product can be replaced with other organophosphorus compounds and other types of products with lower acute toxicities and lower risk to humans and environment.

6. **Waste management**
   - As monocrotophos has not been used in Hungary since 1996, there are no waste management problems.
Monocrotophos was registered for use in Hungary in the form of Azodrin 40 WSC (Shell, UK; Agrokémia Szövetkezet, Hungary) at a rate of 0.75 – 1.0 l/ha to control Bothynoderes punctiventris, Psalidium maxillosum, Tanymecus dilaticollis and Tanymecus palliatus in emerging sugar beet and maize grown in blocks if applied within 30 days of the sowing date. Nuvacron 40 WSC (Ciba-Geigy AG, Switzerland; Nitrokémia Ipartelepek, Hungary), with the same active ingredient, was registered for use on sugar beet against *Aphis fabae, Bothynoderes punctiventris, Chaetocnema tibialis, Pegomya betae* and *Lixus scabricollis* (rate: 0.75 – 1.25 l/ha); *Psalidium maxillosum* (rate: 1.0 – 1.25 l/ha); *Scrobipalpa ocellatella* (rate: 1.5 l/ha); *Mamestra brassicae* (rate: 1.5 – 2.5 l/ha); and spider mites (*Tetranychus urticae*) (rate: 1.5 – 2.0 l/ha).

For maize it was registered at rates of 0.75 - 1.25 l/ha and 1.5 l/ha against *Tanymecus dilaticollis* and *Oscinella frit* respectively. In maize and soya, the following rates were registered to control various pests: noctuid larvae 1.5 – 2.0 l/ha and spider mites 1.5 - 2.0 l/ha. In sunflower and soya, 1.75 – 1.25 l/ha was the registered rate against *Tanymecus spp.*, *Psalidium maxillosum* and *Sitona spp.*. For the control of *Leptinotarsa decemlineata*, 2.4 – 2.8 l/ha was registered in *Solanum nigrum*. Both products were authorized for large-scale farm use only. Biological efficacy of the products was good against the above pests.

Monocrotophos-containing insecticides were registered for use in Hungary from 1971 until 1996. With their withdrawal, no gaps in the pest management programmes for the concerned crops (sugar beet, maize, sunflower, soya and *Solanum nigrum*) appeared. For their major uses (to control *Bothynoderes punctiventris, Chaetocnema tibialis* and *Tanymecus dilaticollis*), several registered organophosphate insecticides such as Danatox 50 EC, Dimecron 50, Nurelle D 50/500 EC, Pyrinex 48 EC and Ultracid 40 WP, organochlorine insecticides such as Thiodan 35 EC and Thionex 35 EC, and insecticides containing other active ingredients, such as Bancol 50 WP and Padan 50, are available. Regent 80 WG will soon have its registration document, including a very efficient solution for pest management programmes. For sugar beet, maize and sunflower, seed-dressing agents containing chloronicotinyl have recently been registered which can be successfully applied against pests of young plants *Bothynoderes punctiventris, Psalidium maxillosum, Tanymecus dilaticollis, Tanymecus palliatus* and *Chaetocnema tibialis*. Other pests such as *Aphis fabae, Pegomya betae* and *Scrobipalpa ocellatella* can be well controlled using several registered organophosphates and synthetic pyrethroids with less mammalian toxicity. The replacement of Azodrin 40 WSC has therefore caused no problems in this area either.
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- C Industrial chemicals
- CP Pesticides and industrial chemicals
- P Pesticides
Annex IV – References

Regulatory actions

Australia

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Rotterdam Convention - Operation of the Prior Informed Consent (PIC) procedure for banned or severely restricted chemicals in international trade

Decision Guidance Document

Monocrotophos

Secretariat for the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade
MANDATE

The Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade was adopted at the Conference of Plenipotentiaries held in Rotterdam on 10 and 11 September 1998. The Rotterdam Convention entered into force on 24 February 2004.

At its ninth session, held in Geneva 30 September to 4 October 2002 the Intergovernmental Negotiating Committee (INC) adopted the decision guidance document for monocrotophos (Decision INC-9/1) with the effect that all formulations of this chemical became subject to the interim PIC procedure.

The Committee also decided that with the circulation of this decision guidance document, countries would be invited to submit a single decision regarding future imports that would apply to all forms of monocrotophos, including the severely hazardous formulations listed in Annex III of the Convention (soluble liquid (SL) formulations of the substance which exceed 600 g a.i./l), unless explicitly exempted in the submitted import response.

At its first meeting, held in Geneva 20 to 24 September 2004, the Conference of the Parties agreed to include monocrotophos in Annex III of the Rotterdam Convention, with the effect that this chemical became subject to the PIC procedure.

The present decision guidance document was communicated to the Designated National Authorities on 1 February 2005 in accordance with Article 7 and 10 of the Rotterdam Convention.

Purpose of the Decision Guidance Document

For each chemical included in Annex III of the Rotterdam Convention a decision guidance document has been approved by the Conference of the Parties. Decision guidance documents are sent to all Parties with a request that they provide a decision regarding future import of the chemical.

The decision guidance document is prepared by the Chemical Review Committee (CRC). The CRC is a group of government designated experts established in line with Article 18 of the Convention, that evaluates candidate chemicals for possible inclusion in the Convention. The decision guidance document reflects the information provided by two or more Parties in support of the national regulatory actions to ban or severely restrict the chemical. It is not intended as the only source of information on a chemical nor is it updated or revised following its adoption by the Conference of the Parties.

There may be additional Parties that have taken regulatory actions to ban or severely restrict the chemical as well as others that have not banned or severely restricted it. Such risk evaluations or information on alternative risk mitigation measures submitted by Parties may be found on the Rotterdam Convention web-site.

Under Article 14 of the Convention, Parties can exchange scientific, technical, economic and legal information concerning the chemicals under the scope of the Convention including toxicological, ecotoxicological and safety information. This information may be provided directly to other Parties or through the Secretariat. Information provided to the Secretariat will be posted on the Rotterdam Convention website (www.pic.int).

Information on the chemical may also be available from other sources.

DISCLAIMER

The use of trade names in this document is primarily intended to facilitate the correct identification of the chemical. It is not intended to imply any approval or disapproval of any particular company. As it is not possible to include all trade names presently in use, only a number of commonly used and published trade names have been included in this document.

While the information provided is believed to be accurate according to data available at the time of preparation of this Decision Guidance Document, the Food and Agriculture Organization of the United
Nations (FAO) and the United Nations Environment Programme (UNEP) disclaim any responsibility for omissions or any consequences that may flow therefrom. Neither FAO or UNEP shall be liable for any injury, loss, damage or prejudice of any kind that may be suffered as a result of importing or prohibiting the import of this chemical, or of any use of the information in the present document which would be in contravention of the law in force.

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| Abbreviation | Description |
|--------------|-------------|
| <            | less than   |
| ≤            | less than or equal to |
| <<           | much less than |
| >            | greater than |
| ≥            | greater than or equal to |
| >>           | much greater than |
| µg           | microgram   |
| AgDrift      | Spray Drift Taskforce Model |
| a.i.         | active ingredient |
| AchE         | acetylcholinesterase |
| ACGIH        | American Conference of Governmental Industrial Hygienists |
| ADI          | acceptable daily intake |
| ADP          | adenosine diphosphate |
| ALT          | alanine amino-transferase |
| AOEL         | acceptable operator exposure level |
| ARfD         | acute reference dose |
| ATP          | adenosine triphosphate |
| BOEL         | biological operator exposure limit |
| b.p.         | boiling point |
| BSI          | British Standards Institution |
| bw           | body weight |
| °C           | degree celsius (centigrade) |
| CA           | Chemicals Association |
| CAS          | Chemical Abstract Service |
| CCPR         | Codex Committee on Pesticide Residues |
| ChE          | cholinesterase |
| CHO          | Chinese hamster ovary |
| d            | day |
| D            | dust |
| DT₅₀         | period required for 50% dissipation |
| EC           | emulsifiable concentrate |
| EC₅₀         | effect concentration, 50% (median effective concentration) |
| ED₅₀         | effect dose, 50% (median effective dose) |
| EHC          | Environmental Health Criteria |
| FAO          | Food and Agriculture Organization of the United Nations |
| g            | gram |
| GAP          | good agricultural practice(s) |
| GL           | guideline level |
| GR           | granules |
| h            | hour |
| ha           | hectare |
| IARC         | International Agency for Research on Cancer |
| IC₅₀         | inhibition concentration, 50% |
| ICSC         | International Chemical Safety Card |
| Abbreviation | Description |
|--------------|-------------|
| i.m.         | intramuscular |
| i.p.         | intraperitoneal |
| IPCS         | International Programme on Chemical Safety |
| IPM          | integrated pest management |
| ISO          | International Organisation for Standardisation |
| IUPAC        | International Union of Pure and Applied Chemistry |
| JMPR         | Joint FAO/WHO Meeting on Pesticide Residues (Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues) |
| k            | kilo- (x 1000) |
| kg           | kilogram |
| K_{oc}       | organic carbon/water partition coefficient |
| K_{ow}       | octanol/water partition coefficient |
| K_{ow}logP   | logarithim of the octanol/water partition coefficient |
| l            | litre |
| LC_{50}      | lethal concentration, 50% |
| LD_{50}      | lethal dose, 50% |
| LD_{0}       | lethal dose, 0% |
| LD_{100}     | lethal dose, 100% |
| LD_{LO}      | lowest lethal dose |
| LOAEL        | lowest observed adverse effect level |
| LOD          | limit of detection |
| LOEL         | lowest observed effect level |
| m            | metre |
| mg           | milligram |
| ml           | millilitre |
| m.p.         | melting point |
| mPa          | millipascal |
| MRL          | maximum residue limit |
| MTD          | maximum tolerated dose |
| NCI          | National Cancer Institute (United States of America) |
| ng           | nanogram |
| NOAEL        | no-observed-adverse-effect level |
| NOEC         | no observed effect concentration |
| NOEL         | no observed effect level |
| NOHSC        | National Occupational Health and Safety Commission (Australia) |
| NRA          | National Registration Authority for Agricultural and Veterinary Chemicals (Australia) |
| OECD         | Organisation for Economic Co-operation and Development |
| OHS          | Occupational Health and Safety |
| OP           | organophosphorus pesticide |
| p            | same as K_{ow} |
| Pa           | pascal |
| PHI          | pre-harvest interval |
| PIC          | Prior Informed Consent |
| POEM         | predictive operator exposure model |
| POP          | Persistent Organic Pollutant |
| Abbreviation | Description |
|--------------|-------------|
| ppm          | parts per million |
| RfD          | reference dose (for chronic oral exposure. Comparable to ADI) |
| SC           | soluble concentrate |
| SG           | soluble granules |
| SL           | soluble liquid |
| SMR          | standardised mortality ratio |
| STEL         | short term exposure limit |
| SUSDP        | Standard for the Uniform Scheduling of Drugs and Poisons (Australia) |
| TER          | toxicity/exposure ratio |
| TLV          | threshold limit value |
| TMDI         | theoretical maximum daily intake |
| TWA          | time weighted average |
| ULV          | ultra low volume |
| UNEP         | United Nations Environment Programme |
| USEPA        | United States Environmental Protection Agency |
| UV           | ultraviolet |
| Vmd          | volume median diameter |
| VOC          | volatile organic compound |
| WHO          | World Health Organisation |
| WP           | wettable powder |
| wt           | weight |
Monocrotophos

1. Identification and uses (see annex I)

| Common name       | Monocrotophos (BSI, E-ISO) |
|-------------------|---------------------------|
| Chemical name     | Dimethyl (E)-1-methyl-2-(methylcarbamoyl)vinyl phosphate (IUPAC) |
| Other names/      |                           |
| synonyms          |                           |
| CAS-No.(s)        | 6923-22-4 (formerly 919-44-8) |
| Harmonized System |                           |
| Customs Code      |                            |
| Category          | Pesticide                  |
| Regulated Category| Pesticide                  |
| Use(s) in regulated category | An organophosphorus contact and systemic insecticide and acaricide used to control a broad spectrum of pests, including sucking, chewing and boring insects and spider mites on cotton, citrus, olives, rice, maize, sorghum, soybeans and tobacco. |
| Trade names       | Azodrin, Bilobrin, Crisodrin, Crotos, Glore Phos36, Harcros Nuvacron, More-Phos, Monocil, Monocron, Monocrotophos 60 WSC, Nuvacron 600 SCW, Plantdrin, Red Star Monocrotophos, Susvin, Phoskil 400. |
| Formulation types | Available in a variety of soluble, liquid and emulsifiable concentrate formulations including 200, 400, and 600 g a.i./l concentrates, 400, 500, and 600 g a.i./l water-soluble concentrates, and 250 g a.i./litre ULV formulations. Monocrotophos is also available in mixtures with other pesticides. |
| Uses in other categories | No reported uses as an industrial chemical. |
| Basic manufacturers | Agrolinz, Inc.; Bharat Pulverizing Mills Ltd. (India); Cia-Shen Co. Ltd. (China); Comlets Chemical Industrial Co. Ltd. (Taiwan); Cyanamid (Brazil); Hindustan CibáGeigy Ltd. (India); Lupin (India); Nantong Pesticides Factory (China); Hui Kwang (China); National Organic Chemical Industries Ltd. (India); Quimica Estrella SACI el (Argentina); Quingdao Pesticides Factory (China); Sudarshan (India); United Phosphorus (India); Sundat (S) Pte Ltd. (Singapore). |

This is a representative list of current and former manufacturers of monocrotophos. It is not intended to be exhaustive.

2. Reasons for inclusion in the interim PIC procedure

Monocrotophos is included in the interim PIC procedure as a pesticide. It is listed on the basis of the final regulatory actions to ban all uses of monocrotophos reported by Australia and Hungary.

Initially, only formulations of monocrotophos exceeding 600 g a.i./l were included in the interim PIC procedure as severely hazardous pesticide formulations, based on the recommendation of the fifth meeting of the FAO/UNEP Joint Expert Group (October 1992). The action was taken because of their acute hazard classification and concern as to their impact on human health under conditions of use in developing countries.
### 2.1 Final regulatory action

**Australia**

Registration of all monocrotophos products was cancelled from 9 December 1999, with all uses phased out over a year to allow existing stocks to be exhausted. This was seen as the lowest-risk option for disposing of existing stocks of monocrotophos in the light of the risks associated with product recall, storage and disposal. It also allowed users time to change over to other pesticides.

**Reason:** Occupational health* and environmental concerns.

**Hungary**

The registration for monocrotophos was withdrawn in 1996 as the reduction of application rates and the restriction of its uses did not reduce the level of adverse impact on wildlife to an acceptable level.

**Reason:** Environmental concerns.

### 2.2 Risk evaluation

**Australia**

Monocrotophos was applied in Australia using aerial, ground-rig and directed sprays to sorghum, sunflowers, tomatoes, cotton, potato, lucerne, soybean and tobacco to control *Helicoverpa* species, locusts, sorghum midge, western flower thrips, aphids, green vegetable bug, mites, stem borer and potato tuber moth.

On the basis of concerns arising from its risk evaluation and in the absence of a commitment by stakeholders to provide the data necessary to allay these concerns, Australia’s National Registration Authority (NRA) for Agricultural and Veterinary Chemicals concluded that there were reasonable grounds to cancel the registration and approvals for monocrotophos. The key aspects of this evaluation are detailed below.

**Occupational safety and health**

In the absence of measured worker exposure studies for conditions comparable with those for Australian use patterns and conditions for mixer/loader/applicators (M/L/A), the United Kingdom Predictive Operator Exposure Model (POEM) was used, where possible, in the assessment of risk, i.e. exposure and MOE (margins of exposure).

*Exposure was predicted to be high and therefore unacceptable in all usual ground application situations.*

On this basis, it was concluded that data would be required for all registered uses for ground application in Australia, including information on the functional efficacy of lower dose rates, if continued use of monocrotophos were permitted.

**Environmental impact**

The concerns from the environmental assessment are that monocrotophos is very toxic to aquatic invertebrates, birds and mammals and is not compatible with integrated pest management (IPM) programmes. There is a high hazard to birds from uses of monocrotophos when avian food items are sprayed. Spray drift from aerial and orchard air-blast spraying is a significant hazard to aquatic invertebrates. Runoff from recently treated areas was identified as hazardous to aquatic invertebrates from both acute and chronic toxic effects.

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* In the Australian context, “occupational exposure” would include exposure to workers involved in:
  - Manufacture;
  - Formulation and re-packaging;
  - Mixing/loading;
  - Application;
  - Post-application activities such as cleaning of equipment; and
  - Re-entry following application for trimming/maintenance, bug-checking etc.

“Occupational exposure” may even go so far as to take into account exposure to “bystanders” such as fellow workers not directly involved in using the chemical. However, by definition, occupational exposure would not include members of the public. This would be included under “public health”.

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Hungary
Monocrotophos in Hungary was registered for use on sugarbeet, sunflower, *Solanum nigrum*, maize, soybean, and alfalfa to control *Bothynoderes punctiventris*, *Psalidium maxillosum*, *Tanymecus dilaticollis* and *Tanymecus palliatus*.

Monocrotophos was first registered in Hungary in 1971 and the registration was extended in 1975. Registrations for the use of monocrotophos were modified in 1982 because of its observed adverse impacts on wildlife. Further reduction in application rates and restriction of its uses did not reduce the level of adverse impact upon wildlife to an acceptable level, leading to the withdrawal of all registrations in 1996. The key aspects of this evaluation are detailed below.

Environmental impact
The wildlife toxicity studies carried out at pilot and large-scale farms clearly confirmed that the use of Azodrin 40 WSC significantly damaged wildlife, first of all birds. Independently of the age and body weight of the animals and the growth stage of the treated crops, the use of the product caused death to some of the animals and prolonged poisoning in others (6–12 days). The poisoned animals did not respond to stimulus and would not flee, therefore it is probable that most were killed by predators. Additional losses were caused by the fact that the recommended use of the product was at the time of reproduction, thus poisoned animals which survived did not feed for several days, did not return to their nests and so on. In Hungary, in addition to pheasants, field hares (*Lepus europeus*) are the most important small game. In the wildlife toxicity studies carried out at large-scale farms, no hare deaths were observed, though slightly poisoned adults could be seen (3–4 kg). It is therefore probable that Azodrin 40 WSC caused death of young hares of low body weight. Azodrin 40 WSC had been used in Hungary since 1971. The annually treated acreage was 50,000 – 150,000 ha. Considering the very low populations of the dead animals and their unborn progeny, the estimated loss in Hungary amounted to 5 to 10 million pheasants since the use of Azodrin 40 WSC begun (25 years). Losses of other songbirds and granivorous birds of low body weight may be much greater than this figure. No other pesticide has caused damage of this extent in Hungary to the natural wild bird population, and the use of Azodrin 40 WSC has played a significant role in the current very low populations of small game birds and animals in Hungary.

3. Protective measures that have been applied concerning the chemical

3.1 Regulatory measures to reduce exposure

Australia Under the conditions of use in Australia, protective measures, including prohibition of application by back-mounted knapsack sprayers, the use of closed cabins for ground spraying and closed systems for mixer loaders, were not considered sufficient to reduce exposure to an acceptable level. As a result, registration for all monocrotophos products was cancelled.

Hungary Protective measures were taken to reduce exposure, including a reduction in application rates and restriction of uses. They were not considered sufficient to reduce the adverse impacts of monocrotophos on wildlife and the compound was banned.

3.2 Other measures to reduce exposure

*This section should be completed only where a chemical has been subjected to severe restriction and the notifying country or countries has or have allowed continued use of the chemical and associated products.*

Where it has been made available, additional information on protective measures (regulatory and other measures) taken in other countries concerning monocrotophos may be found on the Rotterdam Convention website www.pic.int.
3.3 Alternatives

Monocrotophos is a broad-spectrum contact and systemic insecticide and acaracide used in a wide range of crops. There are a number of alternative products available depending on the individual crop-pest complex under consideration. Limited information on alternatives that have been identified by Australia and Hungary may be found in Annex II.

Where it has been made available, additional information on alternatives to monocrotophos may be found on the Rotterdam Convention website www.pic.int.

It is essential that before a country considers substituting alternatives, it ensures that the use is relevant to its national needs and the anticipated local conditions of use.

3.4 Socio-economic effects

No detailed assessment of socioeconomic effects was undertaken by the notifying countries.
4. Hazards and risks to human health and/or the environment

4.1 Hazard Classification (WHO 1998)

| WHO | Technical product: 1b (highly hazardous), classification based on oral toxicity (WHO, 1999) |
|-----|--------------------------------------------------------------------------------------------|
|     | Classification of formulations                                                               |
|     | **oral toxicity**                                                                             |
|     | LD₅₀: 14 mg/kg bw                                                                             |
|     | **dermal toxicity**                                                                          |
|     | LD₅₀: 112 mg/kg bw                                                                             |
|     | **Formulation**                                                                              |
|     | a.i. (%) | hazard class | a.i. (%) | hazard class |
|     | Liquid   | >70       | 1a       | >25       | 1b       |
|     |          | >5        | 1b       | >1        | 11       |
|     |          | >1        | 11       |           |          |
|     | Solid    | >30       | 1b       | >90       | 1b       |
|     |          | >3        | 11       | >10       | 11       |
| E.C. | Classification of the active substance (E.C. 1998) is:                                       |
|      | Mutagenic category 3 ; R 40: possible risks of irreversible effects;                           |
|      | T+; R 26/28: very toxic by inhalation and if swallowed;                                       |
|      | T; R 24: toxic in contact with skin;                                                         |
|      | N; R 50-53: dangerous to the environment, very toxic to aquatic organisms, may cause long-    |
|      | term effects in the aquatic environment.                                                      |
| USEPA | Category 1 (highly toxic) (USEPA, 1985)                                                        |
| IARC  | Not classified                                                                                |

Notifying countries

Australia – Monocrotophos is listed in the Australian National Occupational Health and Safety Commission (NOHSC) List of Designated Hazardous Substances. All monocrotophos products that were part of the Australian review are determined to be hazardous substances because they contain monocrotophos at 40% (w/v), exceeding the NOHSC cut-off concentration for hazardous substances. It is included in Schedule 7 (Dangerous Poisons) of Australia’s Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP).

Hungary - In compliance with Annex II to Ministerial Decree 6/2001, monocrotophos is on the list of banned active ingredients.

4.2 Exposure limits

Food

The Codex Alimentarius Commission has published maximum residue limits for a range of fruits and vegetables, animal products, grains and edible oils. Maximum residue limits (MRLs) for these commodities range between the limit of analytical quantitation (0.02 to 0.05 mg/kg) and 1.0 mg/kg. These MRLs were recommended by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in 1991 and 1994. JMPR established an acceptable daily intake (ADI) of 0.0006 mg/kg bw in 1993 (FAO/WHO 1993). This value was confirmed in 1995. An acute reference dose of 0.002 mg/kg bw/d was established in 1995 (FAO/WHO 1995).

Drinking water

WHO has not established a drinking-water guideline for monocrotophos.
4.3 Packaging and labelling

The United Nations Committee of Experts on the Transportation of Dangerous Goods classifies the chemical in:

| Hazard Class | 6.1, poisonous substance. |
|-------------|--------------------------|

**Packing**

UN Pack Group II: substances and preparations presenting a serious risk of poisoning, formulations containing 25–100% monocrotophos.

Unbreakable packaging; put breakable packaging into closed unbreakable container. Do not transport with food and feedstuff.

**Internat. Maritime Dangerous Goods (IMDG) Code**

Monocrotophos is classified as a marine pollutant.

For specific guidance on appropriate symbols and label statements regarding formulations of monocrotophos, countries should consult the FAO Revised guidelines on good labelling practice for pesticides (FAO 1995).

4.4 First aid

**NOTE:** The following advice is based on information available from the World Health Organization and the notifying countries and was correct at the time of publication. This advice is provided for information only and is not intended to supersede any national first aid protocols.

The signs and symptoms of acute organophosphate poisoning may occur in various combinations and may become manifest at different times. According to the degree of severity of poisoning, the following signs and symptoms may occur: anorexia, headache, dizziness, weakness, anxiety, miosis, blurred vision, slurred speech, nausea, hypersalivation, stomach pains, diarrhoea, vomiting and excessive sweating. In severe cases, respiratory depression and convulsions may also occur. In the case of monocrotophos, “intermediate syndrome” has been reported: this occurs after initial improvement, approximately one to eight days after poisoning. Muscle weakness leading to paralysis and sudden respiratory arrest occur (WHO 1999).

First aid personnel should wear rubber or plastic gloves to avoid contamination. Contaminated clothing and contact lenses should be removed as quickly as possible to prevent further absorption. If skin contact occurs, the area should be washed with soap and water; wash eyes for 15–20 minutes with running water. In the case of ingestion, the stomach should be emptied as soon as possible by careful gastric lavage, preferably within one hour of ingestion. Do not induce vomiting if the formulation contained hydrocarbon solvents. Activated charcoal may be effective. In massive overdoses, acute respiratory failure may occur. It is important to keep the airway open and to prevent aspiration if nausea and vomiting occur (WHO 1999). Persons who have been poisoned, accidentally or otherwise, must be transported immediately to a hospital and placed under the surveillance of properly trained medical staff. Where possible, show the label of the monocrotophos container when the patient/affected person is presented for medical attention. Antidotes are atropine sulphate and pralidoxime chloride.

Depending on the degree of exposure, periodic medical examination is indicated, particularly since monocrotophos has been known to cause “intermediate syndrome”, which may become manifest some time after acute poisoning effects have worn off. Specific treatment is necessary in the event of poisoning with this substance; the appropriate means, with instructions, must be available.

If the substance is formulated with solvent(s), also consult the International Chemical Safety Cards (ICSC) cards for the solvent(s). Carrier solvents used in commercial formulations may affect the toxicity of the active ingredient by altering the extent of absorption from the gastrointestinal tract or through the skin.
4.5 Waste management

Regulatory actions to ban a chemical should not result in creation of a stockpile requiring waste disposal. For guidance on how to avoid creating stockpiles of obsolete pesticide stocks, the following FAO publications are available: Provisional guidelines on the prevention of accumulation of obsolete pesticide stocks (FAO 1995); Pesticide storage and stock control manual (FAO 1996); and Guidelines for the management of small quantities of unwanted and obsolete pesticides (FAO 1999).

In all cases, wastes should be disposed of in accordance with the provisions of the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal, any technical guidelines thereunder and any other relevant regional agreements.

It should be noted that the disposal/destruction methods recommended in the literature, such as high-temperature incineration, are often not available in, or suitable for, all countries. Consideration should be given to the use of alternative destruction technologies. Further information on possible approaches may be found in the FAO/WHO/UNEP provisional technical guidelines for the disposal of bulk quantities of obsolete pesticides in developing countries (FAO 1996).

**Australia** and **Hungary** avoided creating a stockpile of monocrotophos by taking a step-by-step approach to the phase-out of permitted uses (see Annex II). It was considered that the risk was manageable for this phase-out period.

| Annexes                                      |
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| Annex I                                      |
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INTRODUCTION TO ANNEX I

The information presented in this Annex reflects the conclusions of the two notifying countries, Australia and Hungary. This information is contained in the documents referenced in the notification of regulatory action as supporting their national regulatory actions banning monocrotophos. These notifications of regulatory action were first reported in the PIC Circular of December 2000.

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) reviewed monocrotophos in 1972, 1975, 1991, 1993 and 1994. The conclusions of JMPR were not substantially different from those reported here. Section 2.2.7 includes a brief comparative summary of the conclusions of the two toxicological evaluations.
Annex I – Further information on the substance

1. Physico-chemical properties (Tomlin, 2000)

1.1 Identity Monocrotophos

1.2 Formula C₇H₁₄NO₅P

1.3 Chemical name (IUPAC) Dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate

1.4 Chemical type Organophosphate

Form Pure monocrotophos: colourless hygroscopic crystals. Technical monocrotophos, a reddish-brown semi-solid, is at least 75% pure

1.5 Solubility At 20°C - in water 100%, methanol 100%, acetone 70%, n-octanol 25%, toluene 6%

K_w logP −0.22 (calculated), K_w 0.60 (calculated)

1.6 Vapour pressure 2.9 x 10⁻⁴ Pa at 20°C

1.7 Melting point 54–55°C

1.8 Reactivity Hydrolysis – half-life at 20°C calculated from Arrhenius parameters: 96 days at pH 5, 66 days at pH 7 and 17 days at pH 9.

Corrosive to black iron, drum steel and stainless steel.

1.9 Stability Decomposes above 38°C, thermal runaway reaction can occur above 55°C. Unstable in short-chain alcohols, decomposes on some inert materials.

Decomposes on heating or burning, producing toxic and irritating fumes including nitrogen oxides, phosphorus oxides. Attacks iron, steel, brass.

Storage – monocrotophos technical grade active constituent should be stored out of direct sunlight and under cool and dry conditions to minimize any degradation.

1.10 Molecular Weight 223.2

2. Toxicological properties

2.1 General

2.1.1 Mode of action Monocrotophos affects the nervous system by inhibiting acetylcholinesterase, an enzyme essential for normal nerve impulse transmission. The toxicological profile of monocrotophos is typical of organophosphorus compounds, with cholinergic signs (including tremors, convulsions, salivation and trismus) being similar in experimental mammals and humans.

2.1.2 Symptoms of poisoning Symptoms of monocrotophos poisoning are typical of cholinergic signs seen after exposure to other organophosphorus insecticides and include excess salivation and lachrymation, tremors, convulsions, and miosis (see also Section 3.5).
2.1.3 Absorption, distribution, excretion and metabolism in mammals

Monocrotophos is systemically absorbed if it is swallowed, inhaled or comes in contact with the skin. Dermal absorption of $^{14}$C-labelled monocrotophos in humans was about 22% of a single dose applied (in acetone) to the forearm for 24 h. Oral absorption in experimental animals was effectively 100% of the administered dose.

Monocrotophos was rapidly absorbed and excreted, mainly in the urine, within 24 hours after oral dosing in rodents. Very little residual tissue accumulation of monocrotophos or its metabolites occurred. Unchanged monocrotophos was found in the urine of rats at greater than 30% of the administered dose. After oral administration of monocrotophos to rats and goats, parent compound, N-methyl acetoacetamide and 3-hydroxy-N-methyl butyramide were detected in the urine.

There were variations in the rates of absorption, metabolism and elimination but overall the metabolic path for monocrotophos appeared to be similar between species. The metabolic pathway in mammals was determined to be mainly a detoxification route involving ester cleavage of monocrotophos.

2.2 Toxicology studies

2.2.1 Acute toxicity

**Oral**

Monocrotophos was extremely toxic by oral route for rats and mice, with LD$_{50}$ values of approximately 8 and 10 mg/kg bw respectively.

**Dermal**

The acute dermal toxicity of monocrotophos was solvent-dependent: it was of low to high toxicity in rats (LD$_{50}$ values ranging from 119 to >2,000 mg/kg) and of moderate to high toxicity in rabbits (LD$_{50}$ values ranging from 130 to 709 mg/kg).

**Inhalation**

Monocrotophos had high inhalation toxicity in rats, with an LC$_{50}$ (4 h) of 80 mg/m$^3$.

**Irritation**

In rabbits, monocrotophos was slightly irritating to the eyes and skin but it was not a skin sensitizer in guinea pigs.

**ARfD**

No inhibition of erythrocyte cholinesterase activity or other signs of toxicity were seen in volunteers exposed to single oral doses of monocrotophos at up to 0.0059 mg/kg bw in a 28-day study. Based on this no observed effect level (NOEL), and using a 10-fold safety factor, the acute reference dose (ARfD) for monocrotophos in Australia was established at 0.0006 mg/kg bw.

2.2.2 Short-term toxicity

In short-term studies, the inhibition of cholinesterase activity was the main toxicological effect in experimental animals. When rats were given monocrotophos (technical) in the diet for up to 13 weeks, cholinesterase activity was significantly inhibited, but a 5-week recovery phase following feeding allowed some recovery of cholinesterase activity. In repeat-dose dermal studies, the inhibition of cholinesterase activity was also the main toxicological finding. Even at doses that resulted in clinical signs of intoxication, no significant treatment-related gross or histopathological findings were generally observed.

There did not appear to be any clear difference between monocrotophos binding affinity with plasma (or pseudo- or butyryl-) cholinesterase and with erythrocyte or brain cholinesterase (acetyl- or true cholinesterase). There
was considerable variability in responses to monocrotophos between studies, with brain cholinesterase on occasions being the most sensitive to effects of monocrotophos, while in other studies plasma and/or erythrocyte cholinesterase activities were most sensitive to inhibition by monocrotophos.

The anticipated clinical signs associated with organophosphorus compounds and attributable to an excessive interaction of acetylcholinesterase with muscarinic and nicotinic cholinergic receptors were common to all animal studies using monocrotophos. Measurements of plasma, erythrocyte and brain cholinesterase activity in a variety of studies did not reveal a clear hierarchy of inhibition.

It is Australia’s policy to use human data in preference to animal data where human studies are considered to be adequately conducted and reported according to ethical principles of human experimentation. In two different human studies, volunteers received daily oral doses of monocrotophos at up to 0.0059 mg/kg bw for 28 days. No adverse clinical signs were observed. Erythrocyte acetylcholinesterase activity was not affected at any dose level. Plasma cholinesterase activity was significantly decreased at higher doses but not at the low dose of 0.0036 mg/kg bw/d. The acceptable daily intake (ADI) for monocrotophos in Australia was established as 0.0003 mg/kg bw/d, based on the NOEL of 0.0036 mg/kg bw/d for plasma cholinesterase inhibition and using a 10-fold safety factor.

2.2.3 Genotoxicity (including mutagenicity)

Extensive genotoxicity testing has been conducted with monocrotophos ranging in purity from 36% to 99%. Some in vitro mutagenicity tests in bacteria and in yeast, fungi and mammalian cell cultures showed that monocrotophos and its formulations had weak mutagenic potential, both with and without metabolic activation. Similarly, monocrotophos showed potential to damage chromosomes of human lymphocytes, Chinese hamster ovary cells, and rat tracheal epithelial cells, and to induce unscheduled DNA synthesis in human fibroblasts.

In vivo genotoxicity tests showed predominantly negative results, although a weakly positive result was obtained in a mouse micronucleus assay. Monocrotophos did not induce dominant lethal mutations in mice. The doses at which genotoxic effects were observed in in vivo studies were several orders of magnitude greater than the doses at which cholinesterase inhibition was seen in previous studies.

2.2.4 Long-term toxicity and carcinogenicity

The inhibition of cholinesterase activity was the main toxicological effect in long-term animal studies. A two-year rat study investigated histopathological changes in peripheral and central nerves, and found no evidence for a dose-related increase in abnormalities. Progressive examinations through the two-year period did not provide evidence for any acceleration of normal age-related changes. No other significant pathological findings were observed in long-term studies, even when treatment resulted in clinical signs of intoxication.

There were no carcinogenic effects seen over two years of dosing with monocrotophos at the highest dose tested in CD mice (approximately 1.5 mg/kg bw/d), Charles River rats (approximately 5 mg/kg bw/d), Wistar rats (approximately 0.5 mg/kg bw/d) and Beagle dogs (approximately 0.4 mg/kg bw/d).

2.2.5 Effects on reproduction

Overall, development signs were seen only at doses at or near maternotoxic doses, and there were no significant treatment-related teratogenic findings. A development study using Sprague Dawley rats showed a dose-related decrease in the percentage of male foetuses. However, this effect was not seen in a developmental study using Charles River rats, or in a number of
multi-generation reproduction studies in Wistar or Long-Evans rats. In New Zealand rabbits, there was an increase in the incidence of premature deliveries in one study, but this effect was not seen in a second study using another strain of rabbits. Delayed foetal development, including effects on ossification, were attributed to the maternal toxicity of monocrotophos.

2.2.6 Neurotoxicity/delayed neurotoxicity

There was no evidence for delayed neurotoxicity effects in a range of studies using hens, varying from single oral administration to a 78-day study.

2.2.7 Summary and overall evaluation

Studies in experimental animals indicate that cholinesterase (ChE) inhibition is the major toxic effect of monocrotophos.

In experimental animals, monocrotophos is of high acute toxicity. The lowest oral LD$_{50}$ is 8.4 mg/kg bw in rats (10 mg/kg bw in mice) and lowest inhalation LC$_{50}$ is 80 mg/m$^3$ (4 h) in rats. The acute dermal toxicity of monocrotophos is variable and dependent on the solvent; the lowest dermal LD$_{50}$ is 123 mg/kg (rats). Monocrotophos is a slight skin and eye irritant in rabbits. It is not a skin sensitizer in guinea pigs.

In animal studies, monocrotophos is rapidly excreted mainly in the urine, without evidence of significant accumulation in the body. The metabolic pathway is a detoxification route ultimately involving the ester cleavage of monocrotophos with the formation of N-methyl acetocetamide and 3-hydroxy-N-methyl butyramide as well as dimethyl phosphate and/or monomethyl phosphate.

Single or repeat dose studies (up to 78 days) in hens did not demonstrate delayed neurotoxicity.

It did not have an adverse effect in reproductive parameters in rodent studies. Developmental toxicity was noted only at or near maternotoxic doses in rats and rabbits; however, no teratogenic findings were observed.

Monocrotophos appears to be a weak mutagen at high doses. Metabolic activation was not required for mutagenic or other genotoxic effects of monocrotophos.

Monocrotophos was not found to be carcinogenic. Two-year dietary administration of the chemical in rats did not indicate nerve damage or acceleration of normal age-related changes. The most conservative no observed effect level (NOEL) for monocrotophos established for animal studies was 0.004 mg/kg/d (LOEL 0.04 mg/kg/d) in one- and two-year dog dietary studies for brain ChE depression.

In a number of trials (monocrotophos given in capsule form for 28 days) in human volunteers, a NOEL of 0.0036 mg/kg/d was established based on plasma ChE depression at the next high dose. Red blood cell cholinesterase was not affected. The NOELs established in short-term human studies are similar to the NOEL for long-term animal studies (0.004 mg/kg bw/d).

Acceptable daily intake (ADI) was established at 0.0003 mg/kg bw/d.

The ADI is based on human studies in which volunteers received daily oral doses of monocrotophos at up to 0.0059 mg/kg bw for 28 days. No adverse clinical signs were observed. Erythrocyte acetylcholinesterase activity was not affected at any dose level. Plasma ChE activity was significantly decreased at higher doses but not at the low dose of 0.0036 mg/kg bw/d. The ADI was established as 0.0003 mg/kg bw/d, based on the NOEL of 0.0036 mg/kg bw/d for plasma cholinesterase inhibition (LOEL 0.0057 mg/kg/d) and using a 10-fold safety factor.

The acute reference dose (ARfD) was established at 0.0006 mg/kg bw. The ARfD is based on human studies in which volunteers were exposed to single
oral doses of monocrotophos at up to 0.0059 mg/kg bw in a 28-day study and no inhibition of erythrocyte cholinesterase activity or other signs of toxicity were seen. The ARfD was established based on this no observed-effect level (NOEL) of 0.0059 mg/kg bw and using a 10-fold safety factor.

**FAO/WHO JMPR (1995)**

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) evaluated monocrotophos in 1972, 1975, 1991, 1993 and 1995. Monocrotophos was not found to be carcinogenic or teratogenic and caused no toxicity other than the cholinergic syndrome.

An acceptable daily intake (ADI) of 0.0006 mg/kg bw was allocated in 1993 and confirmed in 1995.

This ADI was established on the basis of a 28-day human volunteer study with an NOAEL for erythrocyte acetylcholinesterase of 0.006 mg/kg bw/d and using a 10-fold safety factor.

An acute reference dose (ARfD) of 0.002 mg/kg bw was established by JMPR in 1995.

It was concluded that the available toxicological data in humans allowed the establishment of an acute reference dose on the basis of erythrocyte acetylcholinesterase inhibition and using a 10-fold safety factor.

### 3. Human exposure/risk evaluation

#### 3.1 Food Australia

An estimate of monocrotophos intake was derived from the Australian Market Basket Survey. This procedure is based on measured monocrotophos residues found in food surveys rather than assuming that the pesticide is present at the MRL. In 1994, the estimated intake in the group with the highest consumption of monocrotophos residues (toddlers aged two) was 7.2 nanograms/kg bw/d. This intake accounts for less than 3% of the ADI.

#### 3.2 Air

Not relevant.

#### 3.3 Water

Not relevant.

#### 3.4 Occupational Australia

In accordance with internationally accepted practice, the occupational risk assessment was based on hazard characterization and worker exposure. The latter took into consideration the mixing, loading and application activities involved in the use of the pesticide.

**End-use applications**

There were no measured worker exposure studies for mixing, loading or application of monocrotophos. Therefore, the UKPOEM was used to estimate exposure from which margins of exposure (MOE) for the Australian use pattern were determined wherever possible.

As a result of the occupational risk assessment, the following conclusions were reached.

Acceptable and supported uses of monocrotophos

**Broadacre crops, potatoes and bananas**

Broadacre crops including tobacco, cereals, wheat, oilseeds and cotton are treated with monocrotophos mainly by aerial spraying, which was the only application method used to treat bananas with this pesticide in Australia. Aerial spraying of monocrotophos may also be used for potatoes. Based on the qualitative risk assessment, continued use of aerial spraying for these crops would be acceptable as long as it remained available only to licensed and authorized personnel.
As the risk could not be quantified, the following control measures are needed for aerial spraying on these crops:

- Essential uses only;
- Development of enclosed mixing/loading systems;
- Farm chemical user training for workers handling monocrotophos;
- Health surveillance to be conducted, when appropriate, for workers handling monocrotophos;
- Human flagging in aerial operations is not acceptable, unless flaggers are protected by engineering controls such as cabs.

**Unacceptable and not-supported uses of monocrotophos**

**Fruit trees and vegetables**

The risk for workers applying monocrotophos by high-volume airblast spraying based on predicted exposure was high and unacceptable, even if mixer/loader exposure was eliminated. Other uses for pome fruit (apples and pears) are not supported as the risk is unacceptable. Measured worker exposure data is needed to quantify risk for these uses.

Monocrotophos use by high-volume or low-volume boom-spraying on tomatoes, French beans and maize is not supported as the risk is unacceptable. Measured worker exposure data is needed to quantify risk for these uses.

Ground-spraying on broadacre crops is not supported as the risk is also unacceptable. Measured worker exposure data is needed to quantify risk for this use.

**Flowers – control of budworms**

The risk for workers applying monocrotophos by high-volume or low-volume boom-spraying based on predicted exposure was high and unacceptable, even if mixer/loader exposure was eliminated in each case, and thus its use was not supported.

**Re-entry**

Overseas studies on dislodgeable foliar residues indicated low levels of residues at 96 hours post-application. The degradation of monocrotophos under aerobic conditions in soil was rapid, with a half-life of between one and seven days, and thus it is unlikely to persist in soil beyond one week following application. It is not expected to bioaccumulate. Based on currently available data, a re-entry period of five days is acceptable.

**Regulatory advice**

It is recommended that appropriate training courses be identified for all workers involved in the use of monocrotophos.

Aerial spraying is the only application method which is supported due to the comparatively minimal exposure likely to users. In general, the use of monocrotophos products should be restricted to emergency-permit use only.

In Australia, organophosphorus pesticides are placed on the National Occupational Health and Safety Commission’s Schedule for Health Surveillance.

**3.5 Medical data**

Several published clinical case studies involving accidental exposure or
15

suicide attempts with monocrotophos have reported the development of “intermediate syndrome”. This condition owes its name to the onset of reversible paralysis of cranial nerves, weakness of thorax muscles and respiratory difficulties occurring after exposure, generally after cholinesterase activity has begun to return to normal. Thus, its onset may be delayed after apparent recovery from the acute effects characteristic of muscarinic, nicotinic and CNS nerve overstimulation.

4. Environmental fate and effects

4.1 Fate

4.1.1 Soil

The degradation of monocrotophos under aerobic conditions in soil is fast, with a half-life of between <1 and 7 days, based on five different soils. The major products were carbon dioxide and non-extractable residues. Some minor metabolites were identified in some soils, with the highest at 3.5% of the applied dose. The major degradation pathway appears to be direct metabolism to carbon dioxide or incorporation into the organic fraction of the soil followed by mineralization.

No studies were presented that determined a half-life or examined whether monocrotophos degrades under anaerobic conditions. The photolysis half-life of monocrotophos on soil was less than seven days.

It is concluded that monocrotophos is mobile in soil and that leaching is possible. However, the rapid degradation will limit the extent of leaching that is likely to occur under field conditions.

4.1.2 Water

No studies were presented that determined a half-life. However, monocrotophos was shown to degrade rapidly under aquatic aerobic conditions (a rice paddy in the tropics) but, by contrast, there was no degradation in natural river water at room temperature, consistent with the hydrolysis experiments. It is concluded that the limited studies show that in aquatic systems with high microbial activity, i.e. with soil/sediment, degradation could be rapid. The lack of a suitable aerobic aquatic metabolism study is a significant data gap.

Hydrolysis is unlikely to be a significant contributor to the overall degradation of monocrotophos within the normal environmental pH range. Direct photolysis in water is not expected but indirect photolysis is possible.

4.1.3 Air

Volatilization from soil, or water, is not expected to be a significant route for dissipation, but volatilization from other non-adsorbing surfaces cannot be ruled out. Significant concentrations in air are not expected.

4.1.4 Bioconcentration

Based on water solubility, low Koc and ready soil degradation, significant bioaccumulation in the aquatic environment is not expected.

4.1.5 Persistence

Does not accumulate in soil because it is biodegradable and photolabile. Its half-life is less than 7 days in soil exposed to natural sunlight. Monocrotophos has a half-life of 1.3 to 3.4 days on plant foliage.

4.2 Ecotoxicity – Effects on non-target organisms

4.2.1 Terrestrial vertebrates

Mammals

Monocrotophos is extremely toxic to laboratory rodents by the oral route of exposure, with LD50s around 10 mg/kg (see Section 2.2.1). The acute dermal toxicity is somewhat less (Section 2.2.1).

In Australia, tests on the native marsupial *Sminthopsis macroura* showed that a single dietary dose at 80–100 mg/kg bw caused death. A lower dose at 2 mg/kg bw at intervals over 18 days did not cause any deaths. The Australian native rodents *Notomys alexis* and *Notomys mitchelli*
when fed monocrotophos at 668 mg/kg for 5 consecutive days showed reduced body weight and all animals were off their feed by the end of the testing period.

In the Hungarian wildlife toxicity studies carried out at large-scale farms using Azodrin 40 WSC at 1.5 l/ha (maximum label rate), no hare deaths were observed, though slightly poisoned adults could be seen. Therefore it is probable that Azodrin 40 WSC causes death of young hares of low body weight.

**Birds**

Monocrotophos is rated (by USEPA) as very highly toxic to birds by both the acute oral (reports for 13 species, LD₅₀ of 0.19 to 6.49 mg/kg) and dietary routes of exposure (3 species, LC₅₀ range 2.4 – 32 ppm). Multi-generation tests (approximately 20 weeks’ exposure) on Japanese quail and Mallard duck showed that effects occurred at low levels, 0.1 and 3.0 mg/kg in feed respectively. [Source: database compiled by the USEPA (Ecological Fate and Effects Division, Office of Pesticide Programs) of studies reviewed by them and judged to meet USEPA guidelines.]

Results in the literature for toxicity also indicate very high toxicity to birds – acute toxicity: 1.0 – 4.21 mg/kg; chronic toxicity: NOEC 0.5 mg/kg/d (Japanese quail, 21 d).

Field reports indicate that monocrotophos has been associated with several incidents of bird kill in the United States of America. These old field studies suggest that where there was either food, i.e. wild seeds, or standing water which attracted birds to either drink or feed in the treated fields, significant mortalities occurred at rates of 1 kg a.i./ha and above, except for one study that showed mortalities at 0.32 kg a.i./ha. Birds entering recently sprayed fields were not affected provided they did not feed or drink in the field. Feeding on sprayed locusts or rodents also led to high mortalities.

There are anecdotal Australian reports of bird kills from label use of Monocrotophos EC, but no reliable reports. There are well-documented reports of monocrotophos causing significant mortalities of Swainson’s hawks in Argentina following use to control grasshoppers.

In Hungary, wildlife toxicity studies at pilot and at large-scale farms clearly confirmed that the use of Azodrin 40 WSC significantly damaged wildlife, mainly birds. Independently of the age and body weight of the animals and the growth stage of the treated crops, the use of the product caused death to some birds and prolonged poisoning to others (6 – 12 days). The poisoned birds did not respond to stimulus and were unable to flee, therefore it is probable that most were killed by predators. Additional losses were caused by the fact that the recommended use of the product in Hungary was at the time of bird reproduction, thus poisoned birds which survived did not feed for several days or return to their nests, and so on.

**4.2.2 Aquatic species**

**Fish**

Fish are the least sensitive aquatic species, with LC₅₀s ranging from 1.9 to 180 mg a.i./l based on 9 species. Monocrotophos is rated as moderately to slightly toxic to fish, again according to USEPA criteria. Several of these values are old, nominal and not considered reliable, but they have been used by NRA in the absence of other data. The USEPA Office of Pesticide Programs database entries show similar sensitivities for fish, with LC₅₀s between 5.2 and 50 mg/l.

**Aquatic invertebrates**

Monocrotophos is rated according to USEPA classifications as very highly to slightly toxic, with invertebrates being the most sensitive class of organisms. The reported acute toxicity to daphnia is given as
between 0.24–20 µg/l but no study meets current requirements.

**Algae**

Monocrotophos is rated as moderately toxic to one species of green alga, *Chlorella vulgaris*, with EC₅₀ of 6.8 mg/l (nominal), but non-toxic to *Scenedesmus subspicatus*, another green alga, where the EC₅₀ was >100 mg/l and NOEC = 100 mg/l. USEPA considers both as insensitive species.

**4.2.3 Honey bees and other arthropods**

Based on the results of 15 reports, monocrotophos is very toxic to all the non-target invertebrates tested, in particular bees, lacewing and a range of other predatory insects. Residues on foliage were very highly toxic to bees 24 hours after application (100% mortality). Some reports show that monocrotophos is more toxic to beneficial insects than to pests.

**4.2.4 Earthworms**

The toxicity to earthworms was 196 mg/kg of soil for one test and 35 mg/kg for another. Tests were stated to be based on OECD Guideline 207. These tests rate monocrotophos as either slightly or moderately toxic to earthworms.

**4.2.5 Soil microorganisms**

No toxicity data were available for these organisms.

**4.2.6 Terrestrial plants**

Direct application to desirable terrestrial plants and vegetation is not expected and monocrotophos is non-phytotoxic when used as directed, although some apple, pear, peach, cherry and sorghum varieties may suffer slight injury. Significant effects on desirable plants are therefore considered unlikely.
5. Environmental exposure/risk evaluation

5.1 Terrestrial vertebrates

Birds
Australia’s environmental assessment calculations using standard methodology show that the overall risk to birds appears high and unacceptable, especially to birds that consume insects, seeds and so on or are directly oversprayed by the chemical. Use of monocrotophos to control locusts at the higher rate is likely to represent a very high risk to avian predators of locusts and is unacceptable. This risk has occurred in Argentina, where large numbers of Swainson’s hawks died following application of monocrotophos to control grasshoppers, and led to use of the chemical being restricted/banned. At the lowest label rate for small locusts, 350 ml/ha, calculations for acute dietary exposure for quail (LC50 = 2.4 ppm, 50% of feed contaminated) for small insects indicate a high risk and for large insects a moderate risk.

5.2 Aquatic species

Fish/aquatic invertebrates
For aerial application, apart from direct overspray the risk to fish is considered to be acceptable. No risk is expected to algae. However, the risk to sensitive aquatic invertebrates was determined to be unacceptable to beyond 300 metres from spray drift at all aerial application rates, based on AgDRIFT (from the USEPA) and literature reports, when used according to current label directions. At the lowest rate examined, 140 g a.i./ha, the risk to less sensitive aquatic invertebrates was acceptable at 300 metres but only with placement spraying (coarse droplets, vmd 350 μm). It should be noted that a high risk exists at high rates from runoff as well.

For orchard applications, AgDRIFT showed that for apple and stone-fruit orchards the risk to aquatic invertebrates from orchard air-blast sprayers was moderate at 50 metres and may be acceptable with additional label restrictions. For larger trees and dormant spraying, the risk was high and extended to beyond 100 metres from the orchard. Information from the agricultural assessment and other sources show that use on pome fruit orchards is declining with the introduction of IPM. Considering the lack of data on degradation, the level of risk and also that use of monocrotophos is declining in favour of chemicals more suitable for IPM, Australia’s assessment favoured the removal of pome fruit use from the label.

The spray-drift risk from boom sprayers (given by AgDRIFT) to aquatic invertebrates was high at 30 metres, especially at the application rate tested, 800g a.i./ha (2 l/ha), and just acceptable at 100 metres. At the lowest rate, 140 g a.i./ha (350 ml/ha), the risk at 30 metres was just acceptable. Runoff remained a potential problem for rates ≥280 g a.i./ha. Australia nor could support the use of monocrotophos by boom spray unless the application rate was significantly reduced.

In the aquatic environment, monocrotophos is not expected to persist for an extended period, but based on very limited data, the degradation rate is considered dependent on the level of microbial activity. The field studies showed that degradation was fast in rice paddies but slow in natural water. There were no data for more typical agricultural sediment/water systems in temperate conditions. Assuming a half-life of two days, calculations showed that chronic and subchronic effects on aquatic invertebrates were possible from aerial spray drift but less likely from other application technologies. Although there are no chronic effect data, it was assumed that chronic effects are approximately one
tenth of the acute effect, a common “rule of thumb”. Chronic effects on aquatic organisms could not be ruled out.

5.3 Honey bees and other arthropods

At the application rate of 720 g a.i./ha (1.5 l/ha, the rate for sunflowers, sorghum, and orchards), the risk to bees was determined to be high. The risk from aerial spray drift to bees is high at the higher rates and likewise for other non-target insects, but is acceptable at rates used for locust control, 280 g a.i./ha at 100 metres. However, spray drift from the lowest rate, 140 g a.i./ha is expected to be toxic to Apanteles spp., the most sensitive insects to topical applications of moncrotophos.

5.4 Earthworms

The risk to earthworms from the use of monocrotophos is expected to be low.

5.5 Soil microorganisms

For other soil invertebrates there may be expected to be a high risk but there are no toxicity data for these organisms.

5.6 Summary

Using standard methodology it was concluded that there was a high risk to birds from the current use of monocrotophos when avian food items were sprayed. There was also a high aquatic risk to sensitive invertebrates from spray drift at all application rates, except for boom-spray applications at 140 g a.i./ha, where, provided suitable measures to reduce spray drift are in place, the risk is moderate. The risk to bees and other non-target insects was high. There is also a potentially high risk to aquatic organisms from runoff if rain occurs within days of application.
### Annex II – Details on final regulatory actions reported

| **Country Name:** Australia |
| --- |

| 1. **Effective date(s) of entry into force of actions** | From 9 December 1999: registration of monocrotophos cancelled, further imports prohibited. Use phased out according to the following schedule: |
| --- | --- |
| **Wholesale supply:** to cease by 30 June 2000; |
| **Retail sale:** to cease by 31 December 2000; and |
| **MRLs withdrawn:** from 30 June 2002. |

| 2. **Succinct details of the final regulatory action(s)** | The decision cancels the registrations and all relevant approvals for monocrotophos, halts further imports and phases out its use over a one-year period. The Australian MRL for monocrotophos to be withdrawn on 30 June 2002. |

| 3. **Reasons for action** | Unacceptable occupational health and safety risks. |

| 4. **Basis for inclusion in Annex III** | Decision follows a review of monocrotophos under the Australian National Registration Authority’s Existing Chemical Review Programme, which failed to satisfy the National Registration Authority that continued use of monocrotophos products, in accordance with the recommendations for its use, would not harm people or the environment. Importantly, there was no commitment by stakeholders to generate the required data to allay concerns about environmental, occupational health and residue impacts. The review identified several areas of concern about the use of monocrotophos relating to environmental and worker exposure, residues, and to its particular toxicity to birds. |

| 4.1 **Risk evaluation** | The review concluded that continued use of monocrotophos would pose an unacceptably high risk to workers, wildlife and trade. |

| 4.2 **Criteria used** | Risks to the environment, occupational health and safety (OHS), public health and trade. |

| **Relevance to other States and regions** | Of special concern to developing countries because of the high risk associated with ground spraying of monocrotophos, even when rigorous OHS practices are employed. |

| 5. **Alternatives** | The following alternatives are considered to pose lower risks to workers and the environment. WHO hazard classifications are provided as an aid to consideration of relative risks. These classifications are for active constituents. Actual hazard depends on formulations. This list is not exhaustive and other alternatives are available. |

| **Moderately hazardous:** | • Chlorpyrifos, diazinon; dimethoate; fenitrothion |
| **Slightly hazardous:** | • Azamethiphos; malathion. |

It is recommended that if any of the above chemicals are to be considered as alternatives, advice should be sought from product manufacturers concerning...
suitability for the proposed use and for local conditions.

6. Waste management

Halting imports followed by phase-out of existing stocks

7. Other

Australia has established a Health Value of 0.001 mg/l for monocrotophos in drinking water. (The “Health Value” is the concentration of contaminant that is not expected to result in any significant health risk to consumers, assuming a lifetime intake of 2 litres of water/day. The derivation of this value assumes a bodyweight of 70 kg and that intake from drinking water will constitute 10% of the ADI (which is 0.0003 mg/kg bw/d).
| **Country Name:** Hungary |
|--------------------------|
| **1. Effective date(s) of entry into force of actions** | Registration of monocrotophos-containing insecticides withdrawn in 1996. |
| **Reference to the regulatory document** | The registration of products with monocrotophos as their active ingredient was reviewed in compliance with Ministerial communiqué 1994/20, by the Plant Protection and Agro-environmental Department of the Ministry of Agriculture and Food, published in the Official Journal of the Ministry. In compliance with Annex II to Ministerial Decree 6/2001 FVM, monocrotophos is on the list of banned active ingredients. |
| **9032/1992; 21175/1996.** |
| **2. Succinct details of the final regulatory action(s)** | Banned for all agricultural uses. |
| **3. Reasons for action** | Unacceptably high adverse impact on wildlife. |
| **4. Basis for inclusion in Annex III** | A review based on field observations and studies which showed that monocrotophos has an unacceptably high adverse impact on the environment. |
| **4.1 Risk evaluation** | Scientific studies carried out at small-scale and large farms indicated extremely high risk to birds and bees during and following the application of monocrotophos-containing products. The review identified concern about environmental impacts resulting from the extreme adverse impacts on wildlife observed under conditions of commercial use, confirmed by toxicity tests at pilot farms and large-scale farms at the Nature and Wildlife Conservation Station (Fácánkert, Hungary) between 1976 and 1980, and reported by users, hunters, and environmentalists. Restrictions on uses and times of application, and of the quantity to be applied per unit area (limited to 0.75-1.0 l/ha to control seedling pests of sugar beet and maize grown in blocks, and crops with poorer wildlife populations) did not reduce the impact on wildlife to an acceptable level. |
| **4.2 Criteria used** | Assessment of impact upon wildlife. |
| **Relevance to other States and regions** | Because of the similar ecological parameters (climate, crops and pests), the action by Hungary is highly relevant to neighbouring States. |
| **5. Alternatives** | The product can be replaced with other organophosphorus compounds and other types of products with lower acute toxicities and lower risk to humans and environment. |
| **6. Waste management** | As monocrotophos has not been used in Hungary since 1996, there are no waste management problems. |
Monocrotophos was registered for use in Hungary in the form of Azodrin 40 WSC (Shell, UK; Agrokémia Szövetkezet, Hungary) at a rate of 0.75 – 1.0 l/ha to control Bothynoderes punctiventris, Psalidium maxillosum, Tanymecus dilaticollis and Tanymecus palliatus in emerging sugar beet and maize grown in blocks if applied within 30 days of the sowing date. Nuvacron 40 WSC (Ciba-Geigy AG, Switzerland; Nitrokémia Ipartelepek, Hungary), with the same active ingredient, was registered for use on sugar beet against Aphis fabae, Bothynoderes punctiventris, Chaetocnema tibialis, Pegomya betae and Lixus scabricollis (rate: 0.75 – 1.25 l/ha); Psalidium maxillosum (rate: 1.0 – 1.25 l/ha); Scrobipalpa ocellatella (rate: 1.5 l/ha); Mamestra brassicae (rate: 1.5 – 2.5 l/ha); and spider mites (Tetranychus urticae) (rate: 1.5 – 2.0 l/ha).

For maize it was registered at rates of 0.75 - 1.25 l/ha and 1.5 l/ha against Tanymecus dilaticollis and Oscinella frit respectively. In maize and soya, the following rates were registered to control various pests: noctuid larvae 1.5 – 2.0 l/ha and spider mites 1.5 - 2.0 l/ha. In sunflower and soya, 1.75 – 1.25 l/ha was the registered rate against Tanymecus spp., Psalidium maxillosum and Sitona spp. For the control of Leptinotarsa decemlineata, 2.4 – 2.8 l/ha was registered in Solanum nigrum. Both products were authorized for large-scale farm use only. Biological efficacy of the products was good against the above pests.

Monocrotophos-containing insecticides were registered for use in Hungary from 1971 until 1996. With their withdrawal, no gaps in the pest management programmes for the concerned crops (sugar beet, maize, sunflower, soya and Solanum nigrum) appeared. For their major uses (to control Bothynoderes punctiventris, Chaetocnema tibialis and Tanymecus dilaticollis), several registered organophosphate insecticides such as Danatox 50 EC, Dimecron 50, Nurelle D 50/500 EC, Pyrinex 48 EC and Ultracid 40 WP, organochlorine insecticides such as Thiodan 35 EC and Thionex 35 EC, and insecticides containing other active ingredients, such as Bancol 50 WP and Padan 50, are available. Regent 80 WG will soon have its registration document, including a very efficient solution for pest management programmes. For sugar beet, maize and sunflower, seed-dressing agents containing chloronicotinyl have recently been registered which can be successfully applied against pests of young plants Bothynoderes punctiventris, Psalidium maxillosum, Tanymecus dilaticollis, Tanymecus palliatus and Chaetocnema tibialis. Other pests such as Aphis fabae, Pegomya betae and Scrobipalpa ocellatella can be well controlled using several registered organophosphates and synthetic pyrethroids with less mammalian toxicity. The replacement of Azodrin 40 WSC has therefore caused no problems in this area either.
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**Industrial chemicals**
**CP** Pesticides and industrial chemicals
**P** Pesticides
Annex IV – References

Regulatory actions

Australia

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Article I

Oriental Pharmacy and Experimental Medicine

Kyung Hee Oriental Medicine Research Center, Kyung Hee University (경희대학교한의학연구소)

Quarterly / 1598-2386(pISSN) / 2211-1069(eISSN)

Domain

Health Sciences > Traditional Korean Medical Science

Aim & Scope

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Article II

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Monocrotophos Pesticide Decreases the Plasma Levels of Total 3,3′,5-Triiodo-L-Thyronine and Alters the Expression of Genes Associated with the Thyroidal Axis in Female Goldfish (Carassius auratus)
Our recent study showed that monocrotophos (MCP) pesticide disrupted the hypothalamic-pituitary-thyroid (HPT) axis in male goldfish (Carassius auratus); however, the effects of MCP on the thyroid system in female goldfish are remain unclear. In the present study, plasma thyroid hormone (TH) and thyroid-stimulating hormone (TSH) levels were evaluated in female goldfish exposed to 0.01, 0.10, and 1.00 mg/L of 40% MCP-based pesticide for 21 days in a semi-static exposure system. Expression profiles of HPT axis-responsive genes, including transthyretin (ttr), deiodinases (d1, d2, and d3), tshβ, thyrotropin-releasing hormone (trh), and corticotrophin-releasing hormone (crh), were determined. The results indicated that MCP decreased the plasma levels of total 3,3′,5-triiodo-L-thyronine (TT3) and the ratio of TT3 to total 3,3′,5,5′-I-thyroxine (TT4), and induced alternative expression of TH-related genes. Exposure to 0.01 and 0.10 mg/L MCP pesticide resulted in the up-regulation of ttr mRNA. The reduction of plasma TT3 levels was partly attributed to an increase in
the metabolism of T3 in the liver, as revealed by the highly elevated hepatic d1 and d3 mRNA levels in the MCP treatment groups, and the expression of hepatic d3 showed a negative correlation with the plasma TT3/TT4 levels in females. Moreover, the plasma TSH levels were lower in females exposed to 0.01 and 0.10 mg/L MCP pesticide, whereas the up-regulation of tshβ mRNA levels was compensated by the decreased plasma TT3 levels. These results indicated that MCP had the potential to influence several pathways of HPT axis homeostasis in female goldfish.

**Article III**

Monocrotophos induced dysfunction on estrous cycle and follicular development in mice.

Rao RP1, Kaliwal BB

Author information

Industrial Health, 01 Jul 2002, 40(3):237-244

DOI: 10.2486/indhealth.40.237 PMID: 12141371

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Abstract

Monocrotophos a organophosphate pesticide was administered orally at doses of 1.6, 3.3, 6.6, 10 and 13 mg/kg body weight/day to normal virgin Swiss albino mice for 30 days. The vaginal smear and body weight of the mice were recorded daily and mice were sacrificed on 31st day. The ovaries from each animal was serially sectioned and stained for follicular studies. Estrous cycle was affected by showing a significant decrease in the number of estrous cycle and duration of proestrus, estrus and metestrus with concomitant significant increase in the duration of diestru in all the treated groups, except with 1.6 mg/kg body weight/day monocrotophos treated group. There were significant decrease in the small, medium, large and total number of healthy follicles and increase in the medium, large and total number of atretic follicles with 6.6, 10 and 13 mg/kg body weight/day monocrotophos treatment. However, there were no significant change in the number of healthy and atretic follicles with 1.6 and 3.3 mg/kg/bodyweight/day monocrotophos treatment. There was no change organs weight except for a significant decrease in weight of the ovary with 3.3, 6.6, 10 and 13 mg/kg body weight/day and uterus and body weight with 10 and 13 mg/kg body weight/day monocrotophos treatment. Interruption in estrous cycle, decrease in healthy follicles and increase in atretic follicles may be due to hormonal imbalance or toxic effects of monocrotophos, which adversely effects reproductive function, as it has also analgesic and sedative action.

**Article IV**

Evaluation of pesticide toxicity at their field recommended doses to honeybees, Apis cerana and A. mellifera through laboratory, semi-field and field studies

Article in Chemosphere 119C:668-674 · August 2014 with 448 Reads

DOI: 10.1016/j.chemosphere.2014.07.039 · Source: PubMed
Field and laboratory studies that try to test lethal doses under field conditions have demonstrated that chronic oral or contact exposure for 10 to 11 days to 1 µg / acetamiprid bee can lead to worker mortality. Other results reveal that a dose of 0.25 g / L of acetamiprid causes 20% mortality in A. mellifera bees 48 hours after treatment (Stanley et al. 2014).

Acetamiprid and its metabolites can also affect the memory process and the metabolism of acetamiprid apparently results in different metabolites in bees, among which 6-chloronicotinic acid is toxic by chronic exposure, but not by acute exposure and remains stable for at least 72 h,
especially in the head and thorax. Given the presence of multiple active metabolites over time, it is very difficult to verify which stages of the memory process (acquisition, consolidation or recovery) are affected by acetamiprid. In addition to the use of synthetic insecticides, it can also cause changes in the social behavior of bees such as increased agitation, aggression, and pollen contamination (Stanley et al. 2014). The toxicity of acetamiprid can also alter the activity of certain key enzymes in the bee’s functional processes. Studies have reported that exposure to a dose of 0.6 mg/L of acetamiprid leads to a decrease in the specific activity of AChE by 0.50 and 0.30 nmol hydrolysis of AChE/mg protein/min in the head and thorax respectively. ...

... Another study shows that exposure to a 2g/L dose of chlorpyrifos causes 100% bee mortality 48 hours after treatment, and this can be explained by the high toxicity of chlorpyrifos to bees (Stanley et al. 2014). ...
## Search active substances

**Search term:** monosodium

| Name       | Status under Reg. (EC) No 1107/2009 | Date of approval | Expiration of approval | Legislation |
|------------|-------------------------------------|------------------|------------------------|-------------|
| monosodium | Not Approved                         |                  |                        | 2002/2076   |

Showing 1 to 1 of 1 entries (Filtered from 1,433 total entries)
Quantitative analysis of in-vivo responses of reproductive and thyroid endpoints in male goldfish exposed to monocrotophos pesticide.

Zhang X\textsuperscript{1}, Liu W\textsuperscript{2}, Wang J\textsuperscript{1}, Tian H\textsuperscript{1}, Wang W\textsuperscript{1}, Ru S\textsuperscript{3}.

Abstract

Cross-regulation occurs at many points between the hypothalamic-pituitary-gonad (HPG) and hypothalamic-pituitary-thyroid (HPT) axes. Monocrotophos (MCP) pesticide could disrupt HPG and HPT axes, but its direct target within the endocrine system is still unclear. In the present study, hormone concentrations and transcriptional profiles of HPG and HPT genes were examined in male goldfish (Carassius auratus) exposed to 0, 4, 40, and 400 μg/L MCP for 2, 4, 8, and 12 d. In vivo data were analyzed by multiple linear regression and correlation analysis, quantitatively indicating that MCP-induced plasma 17β-estradiol (E\textsubscript{2}) levels were most associated with alteration of cyp19a transcription, which was also a potential point indirectly modulated by the MCP-altered thyroid hormones (THs) status; disturbance of THs pathways was most related with effect of MCP on regulation of the hypothalamic-pituitary hormones involved in the thyroid system, and the increased E\textsubscript{2} levels might enhance the impact of MCP on HPT axis by modulating hepatic deiodinase expression. Our finding, based on these correlational data, gave a whole view of the regulations, especially on the cross-talk between sex hormone and thyroid hormone pathways upon exposure to chemicals with unknown direct target in vivo, and cautions should be exercised when developing adverse outcome pathway networks for reproductive and thyroidal endocrine disruption.
Monocrotophos

Protective measures that have been applied concerning the chemical
Annex 1 - further information on the substance
Annex 2 - details on reported control actions
Annex 3 - list of designated national authorities
Annex 4 - References

Published: June 97

Common Name Monocrotophos
CAS-No 6923-22-4
Use Insecticide, acaricide with systemic and contact action
Trade Names Azodrin, Crotos, Bilobrin, Crisodrin, Glore Phos36, Monocil, Monocron, More-Phos, Plantdrin, Susvin, Monocrotophos 60 WSC, Harcros Nuvacon, Nuvacron 600 SCW, Red Star Monocrotophos, Monocron
Formulation Water miscible, soluble concentrate; Ultra Low Volume Spray
Basic Manufacturers Agrolinz, Inc. Bharat Pulverizing Mills Ltd. (India), Cia-Shen Co. Ltd. (China.), Comlets Chemical Industrial Co. Ltd. (R.O.C.), Cyanamid (Brasil), Hindustan Ciba Geigy Ltd (India), Lupin (India), Nantong Pesticides Factory (China), Hui Kwang (China), National Organic Chemical Industries Ltd. (India), Quimica Estrella S.A.C.I.el (Argentina), Quingdao Pesticides Factory (China), Sudarshan (India), United Phosphorus (India), Sundat (S) Pte. Ltd. (Singapore)

Reasons for Inclusion in the PIC Procedure

Formulations of the substance which exceed 500 g a.i./l are included because of their acute hazard classification and concern as to their impact on human health under conditions of use in developing countries. (Fifth meeting of the Joint Expert Group).

Monocrotophos is included in the PIC procedure because of its high toxicity which could cause problems under conditions of use in developing countries.

Registrars need to carefully consider the formulations actually used in each country when determining the risks of continued use of this pesticide. The toxicity of the active ingredient is high, but many formulations will fall into a much lower category of hazard.

Hazard Classification by International Organisms
WHO Technical product.: Ib (highly hazardous), classification based on oral toxicity

Classification of formulations

| formulation | a.i. (%) | hazard class | a.i. (%) | hazard class |
|-------------|---------|--------------|---------|--------------|
| oral toxicity | LD₉₀: 14 mg/kg bw (see Ann. 1) | | LD₉₀: 112 mg/kg bw (see Ann. 1) |
| dermal toxicity | | | | |
liquid >70 la >25 lb
>5 lb >1 ll
>1 ll
solid >30 lb >90 lb
>3 ll >10 ll

**EPA** Category 1 (highly toxic)
**EU** T+ (very toxic), N (dangerous for the environment)
**IARC** not classified

### Protective measures that have been applied concerning the chemical

**Measures to Reduce Exposures**

**Personal**
WHO recommends that for the health and welfare of workers and the general population, the handling and application of monocrotophos should be entrusted only to competently supervised and well-trained applicators, who must follow adequate safety measures and use the chemical according to good application practices. Regularly exposed workers should receive appropriate monitoring and health evaluation. (IPCS, 1993)

In Germany, monocrotophos may not be handled by adolescents and pregnant and nursing women. Before its withdrawal in the United States, monocrotophos was a Restricted Use Pesticide (RUP) which could be used only by certified applicators.

**Protection**
Protective clothing as indicated in the FAO Guidelines for Personal Protection when Working with Pesticides in Tropical Climates (FAO, 1990) is required; a respirator should also be worn by mixers and when spraying tall crops. The use of flaggers should be avoided; if used, they need full protective clothing including a respirator. All equipment and protective clothing should be washed thoroughly after use; clothing should be laundered separately from family clothing.

Unprotected workers should be kept out of treated areas for 48 hours. (FAO, 1990)

**Application**
The manufacture, formulation, agricultural use and disposal of monocrotophos should be carefully managed to minimize contamination of the environment. To minimize risks for all individuals, a 48-hour interval between spraying and re-entry into any sprayed area is recommended.

Pre-harvest intervals have been set in several countries and are generally in the order of 7-15 days for vegetables and potatoes, maize and citrus, and 28-30 days for other crops.

In view of the high toxicity of monocrotophos, this agent should not be considered in hand-applied ULV spraying practices. (IPCS, 1993)

**Regulatory measures**

Although the chemical has been included in the PIC procedure because it is a highly toxic pesticide that is likely to cause problems under conditions of storage, transportation and use in developing countries, some countries have reported control actions that may be of interest when considering its use as a pesticide (see below).

Control actions are reported by 3 countries (Annex 2). The only registered use in Malaysia is application by trunk injection. The two other reporting countries (USA and Sri Lanka) indicated no remaining uses. By a ministerial decree of June 1996, the use of monocrotophos in Indonesia was limited until June 1997.
Not all of the reports have been determined to be of control actions which conform with the FAO/UNEP definitions of banned or severely restricted for health or environmental reasons. However, all reports are provided here since the FAO/UNEP Joint Expert Group on Prior Informed Consent decided that the substance should be included in the PIC procedure due to its potential to cause problems under conditions of use in developing countries regardless of the number of qualifying actions.

For further information on the control actions provided in Annex 2, contact the Designated National Authorities (Annex 3) in the country reporting the control action.

Alternatives

The USA indicated bifenthrin, carbaryl, chlorpyrifos, cyfluthrin, pyrethrins, diazinon and permethrin as alternatives to specific uses of monocrotophos. Alternatives have been reported in literature. (Gips, 1990)

It is essential that before a country considers substituting any of the reported alternatives, it ensures that the use is relevant to its national needs. A first step may be to contact the DNA in the country where the alternative has been reported (see addresses of DNAs in Annex 3). It will then be necessary to determine the compatibility with national crop protection practices.

Packaging and Labelling

Follow FAO Revised Guidelines on Good Labelling Practice for Pesticides (FAO, 1995).

Monocrotophos must be labelled as a marine pollutant.

The United Nations Committee of Experts on the Transportation of Dangerous Goods (IPCS, 1993) classifies the chemical in:

- **Hazard Class 6.1** poisonous substance
- **Packing Group 2** serious risk of poisoning: 25-100 % monocrotophos
- **Packing Group 3** harmful substances: less than 25 % monocrotophos

Waste Disposal

All waste and contaminated material associated with this chemical should be considered hazardous waste. The material should be destroyed by incineration in a special high temperature chemical incinerator facility.

See FAO Guidelines on Prevention of Accumulation of Obsolete Pesticide Stocks and The Pesticide Storage and Stock Control Manual. (FAO, 1996)

It should be noted that the methods recommended in literature are often not suitable in a specific country. High temperature incinerators or secure landfills may not be available.

Exposure Limits

| Type of limit | Type of limit Details | Value |
|---------------|------------------------|-------|
| Food MRLs | (Maximum residue limits in mg/kg) in specified products (FAO/WHO, 1996) | 0.02-1 |
| JMPR ADI | (acceptable daily intake) in mg/kg (FAO/WHO, 1996) | 0.0006 |
| Workplace USA (NIOSH) TLV-TWA | (Threshold Limit Value, Time-weighted average in mg/m³) (Niosh, 1996) | 0.25 mg/m³ |
| First Aid |

First Aid
Early symptoms of poisoning may include excessive sweating, headache, weakness, giddiness, nausea, vomiting, hypersalivation, stomach pains, blurred vision and slurred speech. If these symptoms occur, the person should remove contaminated clothes, wash the affected skin with soap and water and flush with large quantities of water. If in the event of collapse artificial resuscitation is used; vomit may contain toxic amounts of the substance. In case of ingestion, the stomach should be emptied as soon as possible by careful gastric lavage. Do not induce vomiting if the formulation contained hydrocarbon solvents.

Persons who have been poisoned (accidentally or otherwise) must be transported immediately to a hospital and put under the surveillance of properly trained medical staff.

Antidotes are atropine sulphate and pralidoxime chloride.

General surveillance and cardiac monitoring must be maintained for at least 14 days. (IPCS, 1986)

**Annex 1 - further information on the substance**

1. **Chemical and Physical Properties**
   1.1 **Identity**
   Colourless, hygroscopic crystals (tech: dark brown semi-solid). Technical monocrotophos is at least 75% pure

2. **Vapour Pressure**
   0.29 mPa (20°C)

3. **Reactivity**
   Decomposes above 38°C; unstable in short chain alcohols; half-life in aqueous solutions range from 96 days (pH 5) to 17 days (pH 9); monocrotophos is corrosive to black iron, drum steel and stainless steel

   Further information in Tomlin, 1994 and IPCS, 1993

2. **Toxicity**

2.1 **General**

2.1.1 **Mode of action**
Monocrotophos affects the nervous system by inhibiting acetylcholinesterase, an enzyme essential for normal nerve impulse transmission.

2.1.2 **Uptake**
Monocrotophos can be absorbed following ingestion, inhalation and skin contact.

2.1.3 **Metabolism**
In mammals, the primary conversion products of monocrotophos are dimethylphosphate, O-desmethyl monocrotophos and N-desmethyl monocrotophos. N-desmethyl monocrotophos is more toxic than monocrotophos.

2.2 **Known Effects on Human Health**

2.2.1 **Acute Toxicity**

**Symptoms of poisoning**
The organophosphate insecticides are cholinesterase-inhibitors. They are highly toxic by all routes of exposure. When inhaled, the first effects are usually respiratory and may include bloody or runny nose, coughing, chest discomfort, difficult or short breath and wheezing due to constriction or excess fluid in the bronchial tubes. Skin contact with organophosphates may cause localized sweating and involuntary muscle contractions. Eye contact will cause pain, bleeding, tears, pupil constriction and blurred vision. Following exposure by any route, other systemic effects may begin.
within a few minutes or be delayed for up to 12 hours. These may include pallor, nausea, vomiting, diarrhoea, abdominal cramps, headache, dizziness, eye pain, blurred vision, constriction or dilation of the pupils, tears, salivation, sweating and confusion. Severe poisoning will affect the central nervous system, producing incoordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contractions, twitching, tremors of the tongue or eyelids, and eventually paralysis of the body extremities and the respiratory muscles. In severe cases there may also be involuntary defecation or urination, psychosis, irregular heart beat, unconsciousness, convulsions and coma. Respiratory failure or cardiac arrest may cause death.

The ingestion of 120 mg monocrotophos can be fatal.

(IPCS, 1993, Occupational Health Services, 1991; Hayes, W.J. and E.R. Laws, 1991)

2.2.2 Short and long term exposure

Repeated daily high level exposure may gradually lead to poisoning. Several studies on occupationally exposed workers have been conducted in countries with a hot climate where workers usually did not wear protective clothing. In most cases plasma cholinesterase was inhibited. It was extrapolated that absorption of 20 mg of monocrotophos caused inhibition of Plasma AchE. (JMPR, 1993).

Some organophosphates may cause delayed symptoms beginning 1 to 4 weeks after an acute exposure that may or may not have produced immediate symptoms. In such cases, numbness, tingling, weakness and cramping may appear in the lower limbs and progress to incoordination and paralysis. Improvement may occur over months or years, but some residual impairment will remain.

2.3 Toxicity studies with laboratory animals and in vitro systems

2.3.1 Acute Toxicity

| Exposure Type | LD₅₀ (mg/kg; a.i.) | LC₅₀ (mg/m³ air-exposure 4 hrs) |
|--------------|------------------|-------------------------------|
| Oral         | 14 - 20          | 80                            |
| Dermal       | 112 - 250        |                               |
| Inhalation   |                   |                               |
| Irritation   |                   |                               |

Technical monocrotophos is not irritant to skin or eyes. Formulations may be irritant due to the content of organic solvent. (Skripsky and Loosli, 1994)

2.3.2 Short-term exposure

Doses of 10 and 100 mg/kg bw (dermal) given for 4 weeks caused toxic signs and significant inhibition of cholinesterase activities. The dose of 1 mg/kg bw was a NOAEL (no observed adverse effect level). In rats, behavioural tolerance to monocrotophos was observed within 16 days of repeated oral dosing with up to 6 mg/kg bw per day. (Skripsky and Loosli, 1994).

2.3.3 Long term exposure

In a two years study on rats with dietary concentrations from 0.01-10 ppm the NOAEL (no observed adverse effect level) was equivalent to 0.005 mg/kg bw per day. (JMPR, 1991)

2.3.4 Effects on reproduction

The existing data about teratogenic effects are not conclusive. In a teratogenicity study in rabbits, monocrotophos was not teratogenic at doses up to 6 mg/kg bw/day, which was lethal to the mothers. (JMPR, 1993).

2.3.5 Mutagenicity

Weak mutagenicity in vitro. In vivo assays were mostly negative or rarely equivocal. (JMPR, 1993)

2.3.6 Carcinogenicity

Carcinogenicity studies in mice and rats were negative. (JMPR, 1993)

3. Exposure

3.1 Food

Monocrotophos is not usually detected as a residue in food in total diet studies.

3.2 Occupational

In a study conducted by US-EPA regarding acute worker exposure risk assessment under conditions of use in Indonesia for chemicals of concern,
a MOE-value (MOEL = NOEL/anticipated exposure level) of 5 was estimated for monocrotophos. EPA generally considers an MOE of lower than 100 to present an unacceptable risk. (FAO Jakarta, 1996)

In a study conducted in the Philippines, it was demonstrated that in the course of a normal spraying operation, farmers are exposed to contamination of their clothing and potential dermal absorption.

An epidemiological study conducted from 1972 to 1984 in a rural district of central Luzon (Philippines) resulted an increase in mortality of 27 % only in the age and sex classes occupationally (rice growing) exposed. These years were a period of high pesticide use. Among the 4 most commonly used pesticides was monocrotophos. (Loevinsohn, 1987; Forget, 1990)

3.3 Environment
The general population is not generally exposed to monocrotophos from the air or water. (IPCS, 1993)

3.4 Accidental Poisoning
There have been several reports of accidental poisoning with monocrotophos due to occupational use or suicide attempts. (Hayes, 1990; IPCS, 1993)

In Parana State (Brazil) pesticides causing more than 10 incidents were analysed in 1990; monocrotophos caused 107 of the 412 reported incidents. (Dinham, 1993)

4. Effects on the Environment

4.1 Fate

4.1.1 Persistence
Monocrotophos has a low environmental persistence. It does not accumulate in soil because it is biodegradable. Its half-life is less than 7 days in soil exposed to natural sunlight. (Tomlin, 1994; IPCS, 1993; US-EPA, 1985)

4.2.2 Bioconcentration
Monocrotophos and its metabolites are not expected to bioaccumulate. (Farm Chemicals Handbook, 1994)

4.2 Ecotoxicity

4.2.1 Fish
Monocrotophos is moderately toxic to fish
LC50 48 hrs (Rainbow trout) 7 mg/l and bluegill sunfish (23 mg/l) (Tomlin, 1994; Farm Chemicals Handbook, 1994)

4.2.2 Aquatic invertebrates
EC50 48 hrs (Daphnia) 0.023 mg/l (103)

4.2.3 Birds
Acute oral LD50s range from 0.9-6.7 mg/kg bw. Monocrotophos is extremely toxic to birds and is used as an avian poison. Monocrotophos may also kill birds which eat insects poisoned with monocrotophos.
Due to the use of monocrotophos, an estimated 15,000 to 20,000 were killed in Argentina 1995 (Woodbridge, 1996)

4.2.4 Bees
Hazardous to bees (LD50 28-33 µg/bee) (Tomlin, 1994)

Annex 2 - details on reported control actions

KUWAIT
Effective:
Control Action: Severely restricted.
Uses still allowed: Used on plants, only up to the flowering stage.
Reasons:
MALAYSIA
Effective:
Control Action: Registration for use only on coconut and oil palm by means of trunk injection.
Uses still allowed: 
Reasons: Highly toxic and hazardous for use under local conditions.

SRI LANKA
Effective:
Control Action: Severely restricted. Import has been prohibited since July 1995.
Uses still allowed: 
Reasons: High toxicity.

UNITED STATES OF AMERICA
Effective: 1989
Control Action: The substance was voluntarily withdrawn by the registrant, effective July 30, 1989. This represented the deadline for the manufacture, sale and distribution of the product by the registrant. No remaining uses allowed.
Uses still allowed: 
Reasons: EPA's concerns with respect to monocrotophos primarily involved effects of exposure to non-target species, notably birds. Monocrotophos is very highly toxic to birds exposed on an acute oral and sub-acute dietary basis. Monocrotophos was determined to be the cause of mortality or was strongly implicated in a large number of bird kill incidents affecting a wide variety of avian species. Monocrotophos posed serious risks to birds even when application was performed in a manner consistent with label directions. Monocrotophos is also highly toxic to freshwater invertebrates. The chemical is an organophosphate and is determined to be a potent cholinesterase inhibitor. Therefore, applicators and workers are potentially at risk for acutely toxic effects. In laboratory studies on rats and rabbits, monocrotophos was found to induce maternal toxicity and developmentally toxic effects (runting), but no major teratological abnormalities, at low doses.

Annex 3 - list of designated national authorities

KUWAIT
P
Public Authority for Agriculture Affairs & Fish Resources Plant Wealth Department
Safat
13075 Kuwait P. 0. Box 21422
CP
The Secretary General
Environment Protection Council
Safat
13104 Kuwait P.O. Box 24395

MALAYSIA
C
The Director-General
Ministry of Science, Technology and Environment Department of Environment
12th and 13th floor, Wisma Sime Darby Jalan Raja Laut
50662 Kuala Lumpur

Phone 603 2938955
Fax 6032931480
Telex 28154 MOSTEC MA
e-mail

Phone (965) 2427161
Fax
Telex e-mail

Phone (965) 2452790;
2456835/36
Fax (965) 2421993
Telex 46408 EP CNCL KT
e-mail

Phone 60 3 2983077
Annex 4 - References

The information on monocrotophos given in this DGD is mainly based on documents published by WHO, FAO and the International Programme on Chemical Safety (IPCS). If important information from other sources has been used, these references are noted in the text. The following list also includes other publications containing useful information.

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Monocrotophos pesticide affects synthesis and conversion of sex steroids through multiple targets in male goldfish (*Carassius auratus*)

Hua Tian, Yang Sun, Hui Wang, Xin Bing, Wei Wang & Shaoguo Ru

Monocrotophos (MCP) is an organophosphorus pesticide that is median-toxic to fish. MCP pesticide resulted in an increase of 17 beta estradiol following a decrease in testosterone in male goldfish (*Carassius auratus*). To fully understand the mechanism of MCP pesticide that causes the imbalance between male and female hormones, we determined the levels of plasma cholesterol, spermatid steroidogenic acute regulatory protein mRNA, steroidogenesis enzyme mRNA, plasma sex hormone synthesis intermediates, and effectual hormones in male goldfish exposed to MCP pesticide at nominal concentrations of 0.01, 0.10, and 1.00 mg/L for 21 days in a semi-static exposure system. The results indicated that MCP pesticide (a) led to decreased steroidogenic acute regulatory protein mRNA levels; (b) decreased mRNA levels of cholesterol side chain cleavage enzyme and cytochrome P450 17 alpha hydroxylase, which are steroidogenesis enzymes involved in androgen synthesis; and (c) increased cytochrome P450 aromatase mRNA levels, a steroidogenesis enzyme involved in the synthesis of effectual estrogen. The present study provides evidence that MCP pesticide affects synthesis and conversion of sex steroids through multiple targets in male goldfish.

Monocrotophos (MCP, CAS number 6923-22-4) is an organophosphorus pesticide that is high-toxic to birds, median-toxic to fish, and listed as a UNEP Prior Informed Consent chemical. Production, management, and use of MCP pesticides have been comprehensively banned in China since January 1, 2007, but it is still extensively detected in China and some other developing countries. MCP was detected in snow pea samples from western China in 2010. MCP residues in brinjal, okra, cucurbits, crucifers, and green chilies were 0.023–1.140 mg/kg in the Andaman Islands, India, and its concentration in the industrial wastewater near Lucknow City, India, was 8.32 ± 3.9 μg/L.

In our previous studies, it was demonstrated that MCP pesticide was a potential environmental estrogen, and it was furthermore determined that MCP pesticide induced mRNA expression of aromatizing enzyme in the gonads of male goldfish (*Carassius auratus*) and resulted in an increase of 17 beta estradiol (E₂) following a decrease in testosterone (T). The changes in the E₂ and T levels were in good agreement with the increase in aromatase expression; however, this might not be the only mechanism by which MCP pesticide disrupts the balance of sex hormones in male fish because a series of enzymatic reactions are involved in sex hormone synthesis in teleosts.

In theory, the process of sex hormone synthesis provides numerous potential targets for MCP pesticide. First, cholesterol serves as precursor to all steroid hormones, and some xenobiotics disturbed the synthesis of sex hormones by affecting cholesterol levels. Second, the synthesis of sex hormones starts only when cholesterol is transported to the inner mitochondrial membrane. Steroidogenic acute regulatory protein (StAR) plays an important role in this delivery process, and thus, low StAR levels will lead to a sharp decline or even interruption of sex hormone synthesis. For example, beta sitosterol exposure changed gonadal transcript levels of StAR in goldfish, leading to lower concentrations of T. Third, after being transported to the inner mitochondrial membrane, cholesterol is translated into pregnenolone under the catalysis of the cholesterol side chain cleavage enzyme (CYP11A1/P450scc). The conversion from pregnenolone to effectual hormones requires the participation of 3 beta hydroxysteroid dehydrogenase (3 beta HSD), cytochrome P450 17 alpha hydroxylase (CYP17/P450c17/P45017alpha), 17 beta hydroxysteroid dehydrogenase (17 beta HSD), cytochrome P450 aromatase.
(CYP19/P450arom), 20 beta hydroxysteroid dehydrogenase (20 beta HSD), cytochrome P450 11beta (CYP11beta/P45011beta), and 11 beta hydroxysteroid dehydrogenase (11 beta HSD), all of which are potential target sites of xenobiotics. Govoroun et al. demonstrated that E2 inhibited spermatic P450c17, 3 beta HSD, and P450 11beta gene expression in rainbow trout (Oncorhynchus mykiss)10. MCP pesticide might affect synthesis and conversion of sex hormones via a number of pathways, such as changing the contents of synthesis substrates or influencing gene expression and activities of steroidogenesis enzymes.

This study was conducted to fully understand the mechanism by which MCP pesticide causes an imbalance between male and female hormones in male goldfish. First, effects on plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were examined to determine whether a lack of substrates for sex hormone synthesis is responsible for inhibited T levels. Second, spermatic StAR mRNA levels were quantified, which could indicate the influence of MCP on the transport of cholesterol from the outer to the inner mitochondrial membrane. Third, mRNA levels of eight kinds of steroidogenesis enzymes and plasma levels of six kinds of sex hormone synthesis intermediates and four kinds of effectual hormones were determined, to elucidate the effects of MCP pesticide on steroidogenesis in male goldfish.

Results

Effects of MCP pesticide on gonadosomatic index (GSI). The fishes were sampled in summer with degraded gonads. GSI of the control male goldfish was 0.14 ± 0.06%, which was not different from that in any of the MCP pesticide treatments studied (Fig. 1).

Effects of MCP pesticide on cholesterol levels. In the control group, the content of plasma HDL-C was higher than that of LDL-C. TC, HDL-C, and LDL-C levels were not influenced by MCP pesticide exposure (Fig. 2).

Effects of MCP pesticide on gene expression of StAR and steroidogenesis enzymes. Fragments encoding P450scc, 3-beta-HSD, 20-beta-HSD, 17-beta-HSD1, P450 11beta, and 11-beta-HSD2 genes of goldfish were amplified and cloned. The deduced amino acid sequences showed high homology to those of other fish species, containing typical P450 conserved features as indicated in Table 111-14.

Gene expression levels of eight steroidogenesis enzymes, as well as StAR were detected. A significant decrease in StAR gene transcription was caused by MCP pesticide exposure (F < 0.01, η² > 0.15, Fig. 3C). Transcription levels of P450scc, which is related to synthesis of pregnenolone, were significantly down-regulated in all three exposure groups (F < 0.01, η² > 0.15, Fig. 3A). Significant down-regulation of P450c17, which is a key enzyme
Plasma 17 α hydroxypregnenolone (P) levels of plasma androstenedione (P) was not affected.

Effects of MCP pesticide on plasma sex hormone levels. Effects of MCP pesticide on plasma levels of six kinds of intermediates, as well as four kinds of effectual hormones were determined. The highest pesticide up-regulation of its expression (P) was not affected.

Table 1. Alignment of the deduced amino acid sequences of goldfish steroidogenesis enzyme genes with those of other animal species.

### Table 1. Alignment of the deduced amino acid sequences of goldfish steroidogenesis enzyme genes with those of other animal species.

| Gene         | GenBank No. | Amino acid sequence homology | Conserved domain                      |
|--------------|-------------|------------------------------|---------------------------------------|
| P450cc       | JQ340311    | 91% (Gobioicyprio rarus)     |                                       |
|              |             | 84% (Tinngodulbus adspersus) |                                       |
| 3-beta-HSD   | JQ867353    | 91% (Gobioicyprio rarus)     | Partial sequence of cofactor binding  |
|              |             | 87% (Danio rerio)            | motif: Gly-XX-Gly-X-Gly-X-Gly          |
|              |             | 77% (Oncorhynus mykiss)      | Enzyme active site of short-chain     |
|              |             | 76% (Clarias gariepinus)     | alcohol dehydrogenase: Tyr-X-X-X-Lys  |
| 20-beta-HSD  | KC193327    | 97% (Cyprinus carpio)        | Adenine ring binding domain: X-Asp-XX- |
|              |             | 90% (Danio rerio)            | Ap; Sequence for a β-sheet: Gly-Gly- |
|              |             | 80% (Tachysurus fulvidraco)  | X-Asp-X-Ser-Ser-X; Substrate binding |
|              |             | 79% (Oncorhynus mykiss)      | site: X-X-Asn-X-Pro-Gly-X-X-Thr        |
| 17-beta-HSD1 | JX036034    | 86% (Danio rerio)            | Coenzyme binding domain: Thr-Gly-X-X- |
|              |             | 71% (Anguilla japonica)      | X-Gly; Connection between coenzyme   |
|              |             | 70% (Oreochromis niloticus)  | binding domain and substrate          |
| 3-beta-HSD   | KC193328    | 85% (Danio rerio)            | activation site: Asn-Ala-Gly; Enzyme  |
|              |             | 81% (Oreochromis niloticus)  | active site: Tyr-X-X-Ser-Lys with Ser |
|              |             | 80% (Oryzias latipes)       | and Asn upstream; Sequence III directing |
|              |             | 74% (Oncorhynus mykiss)      | reaction 3, 36 |
| 11-beta-HSD2 | KC193326    | 91% (Danio rerio) 82% (Anguilla| Coenzyme I binding domain: Gly-Phe- |
|              |             | 81% (Oncorhynus mykiss)      | Gly; Conserved sequence of 11-beta  |
|              |             | 80% (Oryzias latipes)       | HSDs: Cys-Met-Glu-Val-Asn-Phe-Phe-Gly;|
|              |             | 78% (Oryzias latipes)       | Enzyme active site: Tyr-Gly-X-Ser-Lys |

In bony fish, the substrates of steroid synthesis are mainly provided by exogenous cholesterol 11. For example, climbing perch (Anabas testudineus) used plasma cholesterol as the raw material of sex hormone synthesis 11. Low-density lipoproteins (LDL) play a leading role in mammals, but most cholesterol was transported by high-density lipoproteins (HDL) in fish 17. In this study, plasma levels of LDL-C and HDL-C were both maintained following MCP pesticide exposure, suggesting that the content of raw material for sex hormone synthesis was not affected.

StAR can transfer cholesterol to the inner mitochondrial membrane, providing a reaction substrate for synthesis of pregnenolone, which is catalyzed by P450scc. In rainbow trout, StAR transcriptional levels were closely
related to changes in plasma steroid hormones. P450scc catalyzes the first step of steroidogenesis, making it a key enzyme during this process. MCP pesticide inhibited gene expression levels of StAR in male goldfish testes, leading to the reduction of cholesterol that was transported to the inner mitochondrial membrane, and the decrease in plasma levels of pregnenolone caused by MCP pesticide exposure is consistent with the inhibition of P450scc gene expression.

Pregnenolone, 17α-hydroxypregnenolone, and dehydroepiandrosterone can be converted to progesterone, 17α-hydroxyprogesterone, and androstenedione, respectively. These reactions were all catalyzed by 3-beta-HSD. P450c17 is one of the key enzymes of the steroidogenesis pathway in teleostean gonads. There are two kinds of P450c17 in bony fish: P450c17-I and P450c17-II. P450c17-I shows both 17α-hydroxylase and 17,20-lyase activity, whereas P450c17-II has only 17α-hydroxylase activity. Pregnenolone and progesterone can be converted to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, respectively, by 17α-hydroxylase activity; 17,20-lyase activity can break the chemical bonds between C17 and C20 and transform 17α-hydroxypregnenolone into dehydroepiandrosterone or 17α-hydroxyprogesterone into androstenedione. We found a significant reduction of plasma T in male goldfish after MCP pesticide exposure in our previous study, and we observed that MCP pesticide exposure resulted in decreased plasma androstenedione in this study, which is the substrate of T. The down-regulation of androgens by MCP pesticide corresponded to decreases in P450c17 mRNA levels.

Estradiol is formed stepwise, starting with aromatization of androstenedione to estrone, followed by conversion of estrone to E2 via 17-beta-HSD activity, or conversion of androstenedione to T via 17-beta-HSD, followed by aromatization of T to E2. The intermediates androstenedione and estrone have low bioactivity, whereas T and

Figure 3. Effects of MCP pesticide on testicular transcriptional levels of StAR and steroidogenesis enzymes in male goldfish (Carassius auratus).
E2 are effectual hormones with high bioactivity. MCP pesticide increased P450arom A gene expression in male goldfish gonads, and this is consistent with our previous study5.

P450 11beta catalyzes T into 11-beta-hydroxytestosterone 19, and 11-beta-HSD2 further converts 11-beta-hydroxytestosterone into 11-KT 20. The reduction of P450 11beta gene expression led to inhibition of 11-KT synthesis 10, 21, whereas Kusakabe et al. suggested that plasma 11-KT levels had less relevance to 11-beta-HSD2 gene expression in the testes 22. In this study, we observed significant declines of 11-beta-HSD2 and P45011beta mRNA levels, but there was no significant change in 11-KT.

Androgen is responsible for development of the testes. Based on the appearance and histologic features of the testes, the development process in goldfish can be classified into six periods23. Individuals were sampled in August in this study, when testes had degraded to period III after spermiation, and GSI of the control male goldfish was as low as 0.14 ± 0.06%. Although an abnormally low plasma concentration of T was observed concomitant with an increase in MCP pesticide concentration in our previous study 5, GSI was not affected in this study. This may be explained by the unchanged 11-KT levels, because in general 11-KT, rather than T, is the dominant androgen in most fish species, including goldfish.

Studies on endocrine disruption of exogenous compounds, particularly regarding steroidogenesis, have focused mainly on gene expression and activity of steroidogenesis enzymes 24, 25, especially aromatase 26, 27, although multiple factors could be involved in steroidogenesis. In this research, we demonstrated that MCP pesticide acted on many target sites, affecting the transport of cholesterol and gene expression of steroidogenesis enzymes, leading to abnormal levels of sex hormone synthesis intermediates and effectual hormones, and thus resulting in a proportional imbalance of sex hormones in male goldfish.

Materials and Methods
Fish exposure and sample protocol. Goldfish (8.4 ± 0.6 cm standard length; 17.6 ± 3.6 g wet mass) were obtained from a local dealer in Qingdao, China. Following acclimation in the laboratory at 24–26 °C and under a 16 h light/8 h dark cycle for two weeks, fish were exposed to 0.01, 0.10, and 1.00 mg/L MCP pesticide (40%
water-soluble preparation), the concentrations of which were 1/10000, 1/1000, and 1/100 of the 96-h LC<sub>50</sub> (about 100 mg/L, our unpublished results), respectively<sup>5</sup>. Experiments were conducted in 70 L aquaria containing 50 L dechlorinated tap water using a semi-static toxicity test. Twenty liters of water was renewed daily to keep the MCP concentrations constant. Fish were fed a non-estrogenic pelletized diet daily. Additionally, the fish were handled according to the National Institute of Health Guidelines for the handling and care of experimental animals. The animal utilization protocol was approved by the Institutional Animal Care and Use Committee of the Ocean University of China.

After 21 days of exposure<sup>28–33</sup>, goldfish were anesthetized in 75 mg/L MS-222 (Sigma-Aldrich, St. Louis, MO, USA). Blood was taken from the caudal vein using chilled heparinized syringes. After centrifugation (700g, 15 min, 4 °C or 2810g, 10 min, 4 °C), plasma was frozen at −80 °C for cholesterol or hormone quantification. Individuals were identified as male or female during dissection. The testes were weighed and related to body weight to determine
GSI (GSI = gonad weight/body weight × 100%), and then gonad samples were frozen in liquid nitrogen and stored at −80 °C for steroidogenesis enzyme gene quantification. In addition, ovaries and testes collected from unexposed fish were frozen in liquid nitrogen and stored at −80 °C for steroidogenesis enzyme gene cloning.

**Cholesterol quantification.** After twice dilution with 0.7% saline, plasma was analyzed using a fully automatic biochemistry analyzer to determine the content of TC, HDL-C, and LDL-C, with commercial kits obtained from Shanghai Fenghui Medical Science and Technology Co., LTD, Shanghai Rongsheng Biological Pharmaceutical Co., LTD, Beijing Beihua Kangtai Clinical Reagent Co., LTD, and Shenzhen-Mindray Biological Medical Electronics Co., LTD, China, respectively. The assay detection ranges were 0–20 mmol/L for TC, 0.05–6.0 mmol/L for HDL-C, and 0–9 mmol/L for LDL-C, respectively. The inter- and intra-assay coefficients of variation for TC and LDL-C were ≤5%. The inter- and intra-assay coefficients of variation for HDL-C were ≤2.5% and ≤4.0%, respectively.

**cDNA fragment cloning and mRNA quantification of steroidogenesis enzyme genes.** Isolation of total RNA from gonad tissue was accomplished using phenolic reagent TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Extracted RNA was measured by spectrometry at OD260/280 prior to being treated with DNase I (Promega, Madison, WI, USA). M-MLV first strand reverse transcription reaction I (Invitrogen, Carlsbad, CA, USA) was built with 1.0 μL Oligo(dT)12-18 (500 μg/mL), 5.5 μL total RNA, 1.0 μL

| Gene     | GenBank No. | Primer | Primer sequence (5′-3′) | Annealing temperature (°C) | Amplicon size (bp) |
|----------|-------------|--------|-------------------------|---------------------------|-------------------|
| P450sc   |             | Sense  | GTNYTGATGCGGGAACGCTG    | 50.6                      | ≥500              |
|          |             | Anti-sense | CWWGATGAAATACGATCAGGTT  |                           |                   |
| 3-beta-HSD |            | Sense  | TGGTGTGACAGGGAGAGG      | 60.0                      | 824               |
|          |            | Anti-sense | CCAAGACGCTATAGTTGAAAG  |                           |                   |
| 20-beta-HSD |           | Sense  | TGCCGCGTATGACAGGTTG    | 56.6                      | 690               |
|          |            | Anti-sense | CGTCCTCTGCCCTGTAGCAGGC  |                           |                   |
| 17-beta-HSD1 |          | Sense  | CGATTGACACTACGCTTC      | 58.4                      | 698               |
|          |            | Anti-sense | CACCGCCTCAGATTACACCT   |                           |                   |
| P4501_sus |            | Sense  | GAGGGCTGCGGCCCATTTTA   | 63.5                      | 532               |
|          |            | Anti-sense | GCACGCCGCAAGGTCGCTC    |                           |                   |
| 11-beta-HSD2 |          | Sense  | TTGGATGTTGGAATGCTGACA  | 53.0                      | 697               |
|          |            | Anti-sense | GTCGGATGACAGGACGCTG    |                           |                   |

**Table 2.** Primers for PCR amplification. N = A/T/C/G, W = A/T.

| Gene     | GenBank No. | Primer | Primer sequence (5′-3′) | Amplicon size (bp) |
|----------|-------------|--------|-------------------------|-------------------|
| StAR     | AY877430    | Sense  | CGAGAGTGCACATGGTGAAGGTG | 93                |
|          |             | Anti-sense | TCTGCTCTGCTTCCAGCATCTCC |               |
| P450c17  | FJ809936    | Sense  | CCGACCCTGGGCCACACT     | 193               |
|          |             | Anti-sense | GACACCAAAACATCACCCTC   |                   |
| P450romA | AB009336    | Sense  | TGGTGGGTCTGGTCTCTCTT   | 102               |
|          |             | Anti-sense | CCGACAATGTCTCCGATAT   |                   |
| P450sc   | JQ340311    | Sense  | GGAGCTGGGACGGGATCCTTTA | 107               |
|          |             | Anti-sense | GACCGACACCAGGGTACCTC   |                   |
| 3-beta-HSD | JQ867353   | Sense  | TGGCAAGAGGGTGAAAAGGAC   | 193               |
|          |             | Anti-sense | GGATGGGACCGCCACCATCTC  |                   |
| 17-beta-HSD1 | IX036034 | Sense  | TGGTGTCACTGCGGATGTC    | 163               |
|          |             | Anti-sense | GTCGATATAGACTGCCTGGCT  |                   |
| 20-beta-HSD | KC193327  | Sense  | TGCCGCTTGGTCTGAGTAAAGGC | 173               |
|          |             | Anti-sense | GCTGCTGTGCTGAAATGTGTTGAGAG |           |
| P4501beta | KC193328   | Sense  | ATCCCTGCGGTCTCGGCTGAG   | 145               |
|          |             | Anti-sense | GGTTCCGCTAATCGAAGAGAG  |                   |
| 11-beta-HSD2 | KC193326 | Sense  | AGGTAGACATCACCGCCCTGAC  | 114               |
|          |             | Anti-sense | TGTTCACACACATCCAGCTATT |                   |
| beta-actin | AB039726   | Sense  | GAAACTGAAAGGGAGGTAGC    | 115               |
|          |             | Anti-sense | CTGGAGGGACAGAGTGTGAGA  |                   |
| 18S rRNA | FJ710820    | Sense  | CGGATATCAGGGCGACAG     | 148               |

**Table 3.** Primers for real-time PCR amplification.
To clone cDNA fragments of steroidogenesis enzyme genes, including P450scc, 3-beta-HSD, 20-beta-HSD, 17-beta-HSD1, P450 11beta, and 11-beta-HSD2, from goldfish gonads, primers were designed for conserved amino acid sequence regions based on alignments of related gene sequences in other fish species that are closely related to goldfish (Table 2). Primers designed by Primer Premier 5.0 were compounded by Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China. The polymerase chain reaction (PCR) was conducted with an initial denaturation step at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 s, annealing temperature (Table 2) for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Amplified PCR products were cloned in pGM-T easy vector (Takara Bio Inc., Shiga, Japan) and sequenced (Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China). MegAlign and GeneDoc programs in DNAStar were used to analyze the homology of the deduced amino acid sequences of goldfish steroidogenesis enzymes with those of other vertebrates.

Quantitative real-time PCR assays were developed to examine the expression patterns of steroidogenesis enzyme genes and StAR in response to MCP pesticide based on the cloned goldfish steroidogenesis enzyme cDNA fragments and StAR, P450scc, P450arom A, beta actin (internal control 1), and 185 rRNA (internal control 2) sequences for goldfish published in GenBank. Primers designed by Primer Premier 5.0 were compounded by Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China (Table 3). Real-time PCR was performed in 20 μL reaction mixtures consisting of SYBR® Premix Ex Taq™ II (Takara Bio Inc., Dalian, China), 0.4 μL ROX Reference Dye (Takara Bio Inc., Dalian, China), 0.8 μL sense primer (10 μM), 0.8 μL anti-sense primer (10 μM), 4.0 μL cDNA, and 4.0 μL ultrapure water (sterile). The real-time PCR reactions were incubated at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 30 s. Melting curve analyses were conducted to differentiate between the desired PCR products and primer-dimers or DNA contaminants. In addition, 2% agarose gel electrophoresis of the PCR products was performed to confirm the presence of single amplicons of the correct predicted size (data not shown). Gene expression was measured in duplicate. Neither beta actin nor 18S rRNA levels were affected by any of the experimental conditions in the study. The relative target gene mRNA levels were normalized using the geometric mean of the beta actin and 185 rRNA gene expression levels following the formula $2^{-\Delta \Delta Ct}$ and plotted on a logarithmic scale.

**Hormone quantification.** Fish hormone ELISA Kits (R&D Systems, Inc., Minneapolis, MN, USA) were used to quantify plasma pregnenolone, progesterone, 17 alpha hydroxyprogrenolone, 17 alpha, 20 beta double hydroxyprogestrone, dehydroepiandrosterone, androstenedione, estrone, 11 beta hydroxytestosterone, and 11-KT levels according to the manufacturer's instructions. The concentration of each hormone was calculated from the linear part of the standard curve, and plasma was diluted when the hormone concentration exceeded the linear range.

**Statistics.** All data were tested for normality and homoscedasticity. Kruskal-Wallis H test followed by Dunnett's correction was used to analyze GSI, TC, HDL-C, LDL-C, dehydroepiandrosterone, 17 alpha hydroxyprogesterone, androstenedione, estrone, 11 beta hydroxytestosterone, and 11-KT levels according to the manufacturer's instructions. The concentration of each hormone was calculated from the linear part of the standard curve, and plasma was diluted when the hormone concentration exceeded the linear range.

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Author Contributions
Hua Tian and Shaoguo Ru designed the experiment and wrote the manuscript. Yang Sun and Hui Wang undertook the experimental tasks and data processing work. Xin Bing and Wei Wang offered advice on this study. All authors reviewed the manuscript.

Additional Information
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