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Item 6 (c) of the provisional agenda*

Consideration of draft risk profiles: adverse effects of endosulfan on human health

Adverse effects of endosulfan on human health

Note by the Secretariat

1. By its decision POPRC-5/5, the Persistent Organic Pollutants Review Committee adopted a risk profile on endosulfan (UNEP/POPS/POPRC.5/10/Add.2). By paragraph 2 of that decision, the Committee decided to invite the ad hoc working group on endosulfan that prepared the risk profile to explore any further information on adverse human health effects and, if appropriate, to revise the risk profile for consideration by the Committee at its sixth meeting.

2. The annex to the present note contains the information submitted in response to that invitation. The information is presented as received and has not been formally edited.

Possible action by the Committee

3. The Committee may wish to revise the risk profile, with any amendments that it deems appropriate, taking into consideration the information set forth in the annex to the present note.

* UNEP/POPS/POPRC.6/1/Rev.1.

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Annex

1. Proposal by the United States of America

Hazard profile

"Endosulfan is not carcinogenic and does not show any mutagenic potential. There was no increase in the frequency of tumors in either the rat or mouse carcinogenicity studies. Endosulfan is classified as having no evidence of carcinogenicity for humans. The submitted mutagenicity studies have satisfied the data requirements for mutagenicity testing, and there is no concern for a mutagenic effect in somatic cells. In the in vitro or in vivo mutagenicity studies, both the mouse lymphoma forward mutation assay and the unscheduled DNA synthesis assay were negative."

2. Proposal by Pesticide Action Network International (PAN Int) and International POPs Elimination Network (IPEN)

Revise page 16 of UNEP/POPS/POPRC.5/10/Add.2. as follows:

Adverse effects on human health

There is considerable evidence that endosulfan can be genotoxic. The assessments conducted by the EU, Canada or the USA concluded that endosulfan is not carcinogenic. However, Bajpayee et al., (2006) found that exposure to sublethal doses of endosulfan and its metabolites induce DNA damage and mutation. Although the contribution of the metabolites to the genotoxicity of the parent compound in bacteria (Salmonella spp.) and mammalian cells was unclear, and the pathways leading to bacterial mutation and mammalian cell DNA damage appeared to differ. Genotoxicity has also been demonstrated in earthworms and white clover (Liu et al 2009),[1] the dinoflagellates, Karenia mikimotoi and Alexandrium minutum, and the diatom, Chaetoceros gracilis (Akcha et al 2008),[2] root tips of the wetland macrophyte Bidens laevis (Pérez et al 2008),3[3] hepatocyte-derived transformants (Hashizume et al 2010),4[4] and Hep G2 cells (Li et al 2010).5[5] DNA damage was induced in the earthworms, white clover and phytoplankton; and micronuclei induction in Bidens laevis and hepatocyte-derived transformants. Silva & Beauvais (2010), concluded that endosulfan is considered to be genotoxic on the basis of evidence of genotoxicity in tests for gene mutation, chromosomal aberration and DNA damage in open literature studies, despite other tests being negative.6[6]

Contradictory opinions on the potential for endocrine disruption have been presented. Recent information indicates that endosulfan mimics non-uterotrophic E(2) actions, strengthening the hypothesis that endosulfan is a widespread xenoestrogen (Varayoud et al., 2008), acts via a membrane version of the estrogen receptor-α on pituitary cells and can provoke Ca++ influx via L-type channels, leading to prolactin (PRL) secretion (Watson et al., 2007), and is also anti-progestative (Chatterjee et al., 2008), and alters circulating levels of prolactin, luteinizing hormone, growth hormone, and thyroid stimulating hormone (Caride et al 2010).[7]

[1] Liu W, Zhu L-S, Wang J, Wang J-H, Xie H, Yan Song Y. 2009. Assessment of the genotoxicity of endosulfan in earthworm and white clover plants using the comet cssay. Arch Environ Contam Toxicol (2009) 56:742–746

[2] Akcha F, Arzul G, Rousseau S, Bardouil M. 2008. Comet assay in phytoplankton as biomarker of genotoxic effects of environmental pollution. Mar Environ Res. 2008 Jul;66(1):59-61.

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[4] Hashizume T, Yoshitomi S, Asahi S, Uematsu R, Matsumura S, Chatani F, Oda H. 2010. Advantages of human hepatocyte-derived transformants expressing a series of human cytochrome P450 isoforms for genotoxicity examination. Tox Sci, online May 27, doi:10.1093/toxsci/kfq154.

[5] Li D, Liu J, Li J. 2010. Genotoxic evaluation of the insecticide endosulfan based on the induced GADD153-GFP reporter gene expression. Environ Monit Assess. 2010 Jul 14. [Epub ahead of print].

[6] Silva MH, Beauvais SL. 2010. Human health risk assessment of endosulfan. I: Toxicology and hazard identification. Regulatory Toxicology and Pharmacology 56 4–17.

[7] Caride A, Lafuente A, Cabaleiro T. 2010. Endosulfan effects on pituitary hormone and both nitrosative and oxidative stress in pubertal male rats. Toxciol Letts [Epub May 12].

3. Proposal by CropLife

Genotoxicity:

Pesticide registrants such as MAI must comply with rigorous standards for the conduct of mandated studies that deal with mutagenicity. Other parties, interested in pesticide toxicity, may also conduct tests, but these may or may not comply with the appropriate Test Guidelines, and they may or may not be conducted under Good Laboratory Practice regulations (GLPs).

Guideline compliant GLP studies show endosulfan is not mutagenic when studied in yeast (both a gene conversion DNA repair assay and forward mutations), mouse lymphoma forward mutation assay, primary rat hepatocytes for unscheduled DNA synthesis, or micronuclei in both male and female mice (Cifone and Myhr 1984a, Cifone and Myhr 1984b, Jung et al. 1983, Mellano and Milone 1984a, Mellano and Milone 1984b).

The US EPA has judged endosulfan as not mutagenic (*Endosulfan is neither mutagenic or carcinogenic,* US EPA 2002). *The submitted mutagenicity studies have satisfied the data requirements for mutagenicity testing, and there is no concern for a mutagenic effect in somatic cells. In the in vitro or in vivo mutagenicity studies, both the mouse lymphoma forward muation assay and the unscheduled DNA synthesis assay were negative* (US EPA 2010).

The FAO/WHO Joint Meeting on Pesticide Residues (JMPR) notes that in a wide range of assays for genotoxicity, both *in vitro* and *in vivo, there was no evidence of genotoxicity in most …assays* (McGregor 1998, Table 1).

The European Union (EU 1999, EU 2001) concluded based on data from studies carried out with technical material of clearly defined specifications that endosulfan is not a mutagen (*It can be said endosulfan is not mutagenic in vitro and in vivo for somatic cells. Never the less some positive results obtained in studies in vivo with germ cells suggest mutations specifically in spermatogonia).* This claim could not be confirmed in the recently evaluated developmental neurotoxicity study, where no effects on reproduction parameters (sperm production - count, motility, morphology) were observed at any dose level (Anderson and Facey 2007).

Most Regulatory Agencies, such as the EU, US EPA, PMRA and JMPR attempt to integrate the collective data and provide a reasoned summation that reflects the actual hazards. When these collective endosulfan data are analyzed for causality, it can be concluded that there is no inherent mutagenic hazard from the exposure to endosulfan.

Table 1: Results of assays for the genotoxicity of endosulfan (McGregor 1998).

In Vitro				
End-point	**Test Objective**	**Dose (LED or HID) a**	**Result**	**Reference**
Differential toxicity	*B. subtilis* rec strains H17 and M45	2000 µg/disc	Negative a	(Shirasu 1978)
Reverse mutation	*S typhimurium* TA100, TA155, TA1537, TA1538, TA98; E. coli WP2 uvrA	5000 µg/ml	Negative b	(Shirasu 1978)
Gene conversion	S. *cerevisiae*, D4	5000 µg/ml	Negative b	(Mellano and Milone 1984a)
Forward mutation	*S. pombe*	500 µg/ml	Negative b	(Mellano and Milone 1984b)
Unscheduled DAN synthesis	Male F344 rat primary hepatocytes	51 µg/ml	Negative a	(Cifone and Myhr 1984a)
Gene mutation	Mouse lymphoma L5178Y cells, tk locus	75 µg/ml	Negative b	(Cifone and Myhr 1984b)
Chromosomal aberration	Human lymphocytes	200 µg/ml	Negative b	(Asquith and Baillie 1989)

Chromosomal aberration	Human lymphocytes	200 µg/ml	Negative	(Pirovano and Milone 1986)
In vivo				
End-point	**Test Objective**	**Dose (LED or HID) a**	**Result**	**Reference**
Micronucleus formation	NMRI mouse bone-marrow cells	5 mg/kg bw, po x 1	Negative	(Jung et al. 1983)
Micronucleus formation	NMRI mouse bone-marrow cells	10 mg/kg bw, po x 1	Negative	(Müller 1988)
Chromosomal aberration	Albino rat bone-marrow cells	55 mg/kg, po x 5	Negative	(Dikshith and Datta 1978)
Dominant lethal mutation	Male Swiss mice	16.6 mg/kg bw, ip x 5	Equivocal	(Pandey et al. 1990)
Dominant lethal mutation	Male Balb/c mice	0.64 mg/kg bw, ip x 1 and ip x 5	Negative	(Dzwonkowska and Hubner 1991)
Sperm morphology	Mice	16.6 mg/kg bw, ip x 5	Positive	(Pandey et al. 1990)
Sperm morphology	Mice *in vivo*	3 mg/kg bw, ip x 35	Positive	(Khan and Sinha 1996)

LED: lowest effective dose; HID: highest ineffective dose; po: oral; ip: intraperitoneal.
a: In the absence of exogenous metabolic activation; not tested in the presence of exogenous metabolic activation.
b: In the absence and presence of exogenous metabolic activation

Endocrine Disruption:
Endocrine disruptors have been a focus of many Regulatory Agencies, e.g. the US EPA has developed an Endocrine Disruptor Screening Program (EDSP) to determine whether certain chemicals may have an effect in humans or wildlife similar to an effect produced by a "naturally occurring estrogen". The EDSP employs a 2-tiered approach: Tier 1 screening, and Tier 2 testing. Endosulfan is among the group of 58 pesticides on the initial EDSP list. MAI submitted a comprehensive response, "other scientifically relevant information", to EPA's test orders, which is presently under review by the Agency.

It should be noted that many of the endpoints of concern in the EDSP are already being addressed by the existing guideline studies, e.g. multi-generation reproductive and developmental toxicity testing evaluate these same endocrine tissues in animals exposed at various life stages (*in utero*, post-natally and adulthood). Therefore, even without data collected from validated endocrine disruptor screening tests (Tier 1 screens and Tier 2 tests), there is a good deal of relevant toxicological data collected from animal testing for endosulfan that has been already performed and evaluated by regulatory bodies as well as independent scientists.

In a weight-of-the-evidence evaluation by Plunkett (2008) considering all of the available data (published *in vitro* and *in vivo* data, published human data, and unpublished toxicological data submitted as part of the pesticide registration process), it was demonstrated that the potency of endosulfan in the available *in vitro* studies was very low, with potencies in the range of 10^5 to 10^6 times less than the naturally occurring hormones and even natural phytoestrogens that are present in the human diet. In addition, the *in vivo* toxicological guideline studies showed consistently that endosulfan was not toxic to endocrine organs, even following lifetime exposures, and that effects observed were limited to situations where exposure conditions were unrealistic as compared to human exposures.

The European Union concluded (EU 1999, EU 2001) that endosulfan does not meet the criteria of an endocrine disruptor: *No effects were found on endocrine, reproductive or sexually regulated systems in vivo at doses causing clear toxicity.* Furthermore, it was stated that a full evaluation of endosulfan as an endocrine disruptor cannot be performed until commonly accepted test procedures have been established and validated.

The Australian Pesticides and Veterinary Medicines Authority found in its toxicological evaluation of endosulfan (APVMA 2003) that the endocrine disrupting potential is not a significant risk to human health under the existing management control and health standards.

In conclusion, the toxicological profile of endosulfan is defined and very well known. After consideration of all of the available data, the weight-of-the-evidence indicates that endosulfan is not an endocrine-disrupting compound at environmentally relevant concentrations.

MAI appreciates the ad hoc working group's effort and consideration taking the provided comments into consideration when drafting an objective, robust and science-based risk profile.

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