

Methoprene in Drinking-water:
Use for Vector Control in Drinking-water Sources and Containers

Background document for development of
WHO Guidelines for Drinking-water Quality

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Preface

One of the primary goals of WHO and its member states is that “all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water.” A major WHO function to achieve such goals is the responsibility “to propose ... regulations, and to make recommendations with respect to international health matters”

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbial aspects in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2005, and the second addendum to the third edition was published in 2008.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America (USA) prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health

Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meeting on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

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The work of the following working group coordinators was crucial in the development of this document and others contributing to the second addendum to the third edition:

Dr J. Cotruvo, Joseph Cotruvo & Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Professor Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Dr A.V. Festo Ngowi, Tropical Pesticides Research Institute, United Republic of Tanzania (*Pesticides*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)

The draft text was discussed at the Working Group Meeting for the second addendum to the third edition of the GDWQ, held on 15–19 May 2006. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinators were Dr J. Bartram and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the Programme on Chemical Safety, WHO Headquarters. Mr R. Bos, Assessing and Managing Environmental Risks to Health, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

ADI	acceptable daily intake
CAS	Chemical Abstracts Service
FAO	Food and Agriculture Organization of the United Nations
GDWQ	<i>Guidelines for Drinking-water Quality</i>
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_{oc}	soil sorption coefficient
K_{ow}	octanol–water partition coefficient
LD ₅₀	median lethal dose
NOAEL	no-observed-adverse-effect level
WHO	World Health Organization

Table of contents

1. GENERAL DESCRIPTION	1
1.1 Identity	1
1.2 Physicochemical properties	1
1.3 Major uses and sources in drinking-water	1
1.4 Environmental fate	1
2. HUMAN EXPOSURE.....	1
3. TOXICOLOGICAL SUMMARY	2
4. PRACTICAL ASPECTS	4
4.1 Analytical methods and analytical achievability	4
4.2 Use for vector control in drinking-water sources	5
5. CONCLUSIONS.....	5
6. RECOMMENDATIONS.....	5
7. REFERENCES	6

1. GENERAL DESCRIPTION

1.1 Identity

CAS No.: 40596-69-8
Molecular formula: $C_{19}H_{34}O_3$

The IUPAC name for methoprene is isopropyl (2E, 4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate. The ISO common name is methoprene, and this is a racemic mixture of R and S enantiomers. Reports and studies in this document relate to the mixture unless otherwise stated.

1.2 Physicochemical properties (WHO/FAO, 1996)

<i>Property</i>	<i>Value</i>
Melting point	100 °C
Density	0.261
Water solubility	1.39 mg/l at 20 °C
Log octanol–water partition coefficient (log K_{ow})	5.50
Vapour pressure	3.15×10^{-6} kPa at 20 °C

1.3 Major uses and sources in drinking-water

Methoprene is a stable juvenile hormone analogue that interferes with metamorphosis in insects. There is no equivalent process in mammals. It is a racemic mixture of two enantiomers (R and S in a ratio of 1:1), but the activity of the compound as a juvenile hormone is restricted to the S enantiomer. Methoprene is used to control a range of pests in food production and public health. WHO has assessed methoprene for use as a mosquito larvicide in drinking-water in containers, particularly to control dengue fever. The recommended dosage of methoprene in potable water in containers should not exceed 1 mg/l under the WHO Pesticides Evaluation Scheme (WHO, 2006).

1.4 Environmental fate

Extensive studies have shown that methoprene breaks down rapidly in the environment (USEPA, 2001). It undergoes demethylation, hydrolysis and oxidative cleavage in microbes, insects and plants and is rapidly metabolized in fish, birds and mammals (Glare & O'Callaghan, 1999). It has an estimated K_{oc} of 23 000 and is expected to be immobile in soil. In water, it would be expected to adsorb to suspended solids. It is fairly rapidly biodegraded in both soil and water and rapidly degraded when exposed to sunlight (WHO/FAO, 1996).

2. HUMAN EXPOSURE

It is expected that exposure of the public through either food or drinking-water would be low. However, there is a potential for direct exposure through drinking-water when methoprene is directly applied to drinking-water storage containers.

3. TOXICOLOGICAL SUMMARY¹

The absorption, distribution, excretion and metabolism of racemic methoprene have been studied in mice, rats, guinea-pigs, cows and chickens given single doses. No study of metabolism after repeated doses was available. After administration of single oral doses of methoprene, the radiolabel was relatively rapidly absorbed and excreted in urine, faeces and expired air. Further, about 8% of the radiolabel administered to a cow was excreted in milk 7 days after dosing, and up to 19% of radiolabel administered to chickens was excreted in eggs 14 days after dosing. In most species investigated, the bulk of the radiolabel was excreted within 5 days or less, and the remainder was incorporated into tissues.

Substantial enterohepatic circulation occurs in rats, and a small percentage of intact, unabsorbed methoprene was found in faeces, with none in urine or bile, after its administration. Methoprene is probably extensively metabolized in rats, as a large portion of the radiolabel was excreted with carbon dioxide. After a single oral dose to rats, the peak plasma concentration, 1.6% of the administered radiolabel, was reached by 6 h; the level declined slowly, with a half-time of about 48 h. Whole-body autoradiography and tissue analysis showed that most of a single labelled dose was located in organs concerned with absorption, biotransformation and excretion. A relatively high concentration of radiolabel was found in adrenal cortex, lachrymal glands and adipose tissue after 48 h.

Studies in guinea-pigs, cattle and chickens showed that racemic methoprene was extensively metabolized to polar conjugates (glucuronides), which were excreted in the urine and faeces, and that the C5-labelled molecule underwent rapid α - and β -oxidation to produce carbon dioxide and acetate, which was incorporated into natural products such as triglycerides, bile acids and cholesterol found in tissues, milk and eggs.

The pharmacokinetics of (*S*)-methoprene were investigated for 7–8 h in blood and fat of rats given a single oral or intravenous dose. The clearance of (*S*)-methoprene was relatively rapid after intravenous administration of 10 mg/kg of body weight. After oral administration of 10 or 100 mg/kg of body weight, (*S*)-methoprene was rapidly absorbed, and the maximum concentration of parent compound in blood was reached 2 h after dosing. In fat, the concentration of unchanged methoprene reached a plateau 3–4 h after intravenous and 4–6 h after oral administration, and then very slowly declined. Because of this slow decline, methoprene may build up in fat after repeated dosing. Most of the radiolabel in fat was unchanged methoprene, whereas in blood, methoprene was degraded rapidly to other radiolabelled compounds.

The racemate and (*S*)-methoprene showed little acute toxicity. The LD₅₀ values for (*S*)-methoprene were >5000 mg/kg of body weight (oral, rat) and >2000 mg/kg of body weight (dermal, rabbit), and those of the racemate were >24 000 mg/kg of body weight (oral, rat) and >3400 mg/kg of body weight (oral, dog). The LD₅₀ for the racemate after intraperitoneal administration in rats was 3300 mg/kg of body weight. WHO (1999) has classified methoprene as “unlikely to present acute hazard in normal use”. The racemate and (*S*)-methoprene were not irritating to the eye or skin of

¹ After FAO/WHO (2002).

rabbits. In a limited test in guinea-pigs, the racemate appeared to have no sensitizing properties. In a study with a formulation containing 20% (*S*)-methoprene, skin sensitization was seen; however, it was unclear whether the effect was due to (*S*)-methoprene or to another compound in the formulation.

Several studies of the toxicity of repeated doses of racemic methoprene given by oral or dermal application or inhalation were available. The design and reporting of these studies did not meet current guidelines, and the studies of dermal application or inhalation were considered inadequate for evaluation. The studies by oral administration could be used to deduce the toxicological profile of methoprene, and, despite their shortcomings, most were considered suitable for use in risk assessment.

Studies in which mice (78 weeks), rats (14 or 90 days, 104 weeks) and dogs (14 or 90 days) were given racemic methoprene in the diet showed that the compound has little toxic potential. Some effects on food intake and body weight were found, but the main effect was to increase the weight of the liver relative to body weight (in rats at doses ≥ 5000 mg/kg; in dogs at doses ≥ 1000 mg/kg). This effect was not always associated with histopathological changes. In the 90-day study in dogs treated with methoprene in the diet, the NOAEL was 500 mg/kg, equivalent to 8.6 mg/kg of body weight per day. In the 2-year study in rats treated with methoprene in the diet at 5000 mg/kg, the highest dose tested, increased absolute and relative liver weights and an increased incidence of hepatic lesions such as bile duct proliferation and portal lymphocyte infiltration were observed in male rats. The NOAEL was 1000 mg/kg, equivalent to 44 mg/kg of body weight per day. Minor histopathological changes in the kidneys observed in this study were considered of no significance for human risk assessment. In the 78-week study of carcinogenicity in mice, hepatic lesions characterized by pigment deposition in the cytoplasm of parenchymal cells were seen at 1000 and 2500 mg/kg, with increased incidence and severity at the highest dose. Focal accumulations of macrophages with brownish foamy cytoplasm were found in the livers of survivors of each sex at 2500 mg/kg, and an increased frequency of amyloidosis of the intestine was seen in females at this dose. No adverse effects (the brownish pigment was considered not to be of toxicological relevance) were observed at 1000 mg/kg, equivalent to 130 mg/kg of body weight per day.

No increase in the incidence of tumours at any site was seen in either the 78-week study of carcinogenicity in mice or the 2-year study of toxicity and carcinogenicity in rats treated with methoprene in the diet.

Racemic methoprene did not induce chromosomal aberrations in Chinese hamster ovary cells in vitro. No increase in the frequency of reverse mutations in *Salmonella typhimurium* was observed. JMPR noted that only a limited range of concentrations were tested. No definitive conclusion can be drawn about the genotoxic potential of the racemate.

(*S*)-Methoprene did not induce reverse mutations in *S. typhimurium* or mitotic crossing-over, gene conversion or reverse mutations in *Saccharomyces cerevisiae*. On the basis of the negative results in a limited range of studies for genotoxicity and the results of the studies of carcinogenicity, JMPR concluded that methoprene was unlikely to pose a carcinogenic risk to humans.

In a three-generation study of reproductive toxicity with racemic methoprene in rats, the total weight gain of animals of each sex in the F₀ and F₁ generations during the growth period was slightly decreased, the mean weight of pups in the F₂ and F₃ litters was reduced, and the mean number of pups born dead per litter in the F₃ litters was increased at a dose of 2500 mg/kg. The NOAEL was 500 mg/kg, equivalent to 29 mg/kg of body weight per day.

In a study of developmental toxicity in which mice were treated on days 7–14 of gestation with racemic methoprene, no toxicologically relevant effects were observed in dams or fetuses at any dose; the NOAEL was 570 mg/kg of body weight per day, the highest dose tested. In the same experiment, several dams were allowed to litter and rear their pups until weaning; the pups of five litters at each dose were killed at weaning, and the remaining litters were observed for 7 additional weeks. Effects on organ weights were observed in pups at the highest dose. The NOAEL for toxicity to offspring was 190 mg/kg of body weight per day. In a study of developmental toxicity in which rabbits were treated on days 7–18 of gestation with racemic methoprene, the NOAEL for maternal toxicity, embryotoxicity and fetotoxicity was 190 mg/kg of body weight per day, on the basis of reduced weight gain and an increased frequency of abortions among the does and an increased percentage of fetal deaths at 1900 mg/kg of body weight per day, the highest dose tested. The shortcoming of the studies — mice and rabbits were treated for 2 and 1 day less than that required in the relevant test guideline — was not considered critical for evaluating end-points of developmental toxicity. All the developmental effects were seen at very high doses or only postnatally. JMPR considered that additional studies were not necessary and concluded that methoprene is not teratogenic.

JMPR reaffirmed the basis of the ADI for racemic methoprene established in 1987, but lowered the value to 0–0.09 mg/kg of body weight to correct for the purity of the racemate tested. The basis for the ADI was the NOAEL of 500 mg/kg, equivalent to 8.6 mg/kg of body weight per day (corrected for purity), in the 90-day study in dogs and a safety factor of 100. As no bridging studies with repeated doses were available for (*S*)-methoprene, JMPR made the conservative assumption that, in the absence of any information to the contrary, all the toxicity of the racemate was due to the *S* enantiomer. On this basis, JMPR established an ADI for (*S*)-methoprene of 0–0.05 mg/kg of body weight, equal to one-half the ADI for the racemate (which is a 1:1 mixture of the *R* and *S* enantiomers).

As the LD₅₀ for racemic methoprene given orally was >2000 mg/kg of body weight, with no toxic signs seen at this dose and no signs indicative of acute toxicity seen in studies with repeated oral doses (including studies of teratogenicity), JMPR concluded that allocation of an acute reference dose was unnecessary.

4. PRACTICAL ASPECTS

4.1 Analytical methods and analytical achievability

Methoprene can be analysed by reversed-phase high-performance liquid chromatography using solid-phase extraction (detection limit between 50 and 100 ng/ml in water; Abu-Qare & Abou-Donia, 2001) or by liquid chromatography using

electrospray ionization with tandem mass spectrometry (detection limit 20 ng/l in water; Aronov et al., 2005).

4.2 Use for vector control in drinking-water sources

Methoprene is used as a larvicide for control of disease-carrying mosquitoes that breed in drinking-water containers at a dosage not exceeding 1 mg/l (WHO, 2006).

Formulations of pesticides used for vector control in drinking-water should strictly follow the label recommendations and should only be those approved for such a use by national authorities, taking into consideration the ingredients and formulants used in making the final product.

5. CONCLUSIONS

It is not considered appropriate to set a formal guideline value for methoprene used as a vector control agent in drinking-water. The ADI for methoprene, determined by JMPR in 2001 (FAO/WHO, 2002), is 0–0.05 mg/kg of body weight. Young animals do not appear to be significantly more sensitive than adults, and exposure from food is considered to be low. Where methoprene is used for vector control in potable water, this will involve less than lifetime exposure. The maximum dosage in drinking-water of 1 mg/l would be equivalent to approximately 66% of the ADI (0.033 mg/kg of body weight) for a 60-kg adult drinking 2 litres of water per day. The exposure for a 10-kg child drinking 1 litre of water would be approximately 0.1 mg/kg of body weight, compared with the ADI of 0–0.05 mg/kg of body weight; for a 5-kg bottle-fed infant, the exposure would be approximately 0.15 mg/kg of body weight, compared with the ADI of 0–0.05 mg/kg of body weight. However, the low solubility and the high log K_{ow} of methoprene indicate that it is unlikely to remain in solution at the maximum recommended applied dose, and the actual levels of exposure are likely to be much lower than those calculated.

National authorities should note that this document refers only to the active ingredient and does not consider the additives in different formulations.

6. RECOMMENDATIONS

In setting local guidelines or standards, health authorities should take into consideration the potential for higher rates of water consumption in the area or region under consideration. Consideration should be given to using alternative sources of water for small children and bottle-fed infants for a period after an application of methoprene, where this is practical. However, exceeding the ADI will not necessarily result in adverse effects.

The diseases spread by vectors are significant causes of morbidity and mortality. It is therefore important to achieve an appropriate balance between the intake of the pesticide from drinking-water and the control of disease-carrying insects. Better than establishing guideline values are the formulation and implementation of a comprehensive management plan for household water storage and peridomestic waste management that does not rely exclusively on larviciding by insecticides, but also includes other environmental management measures and social behavioural changes.

7. REFERENCES

Abu-Qare AW, Abou-Donia MB (2001) A solid phase extraction reversed-phase HPLC method for the simultaneous determination of methoprene, permethrin and selected metabolites in rat plasma and urine. *Biomedical Chromatography*, 15:464–470.

Aronov PA et al. (2005) Development of a HPLC/tandem-MS method for the analysis of the larvicides methoprene, hydroprene, and kinoprene at trace levels using Diels-Alder derivatization. *Journal of Agricultural and Food Chemistry*, 4:3306–3312.

FAO/WHO (2002) Methoprene and S-methoprene. In: *Pesticide residues in food — 2001 evaluations. Part II. Toxicological*. Geneva, World Health Organization, Joint FAO/WHO Meeting on Pesticide Residues (WHO/PCS/02.1; <http://www.inchem.org/documents/jmpr/jmpmono/2001pr09.htm>).

Glare TR, O'Callaghan M (1999) *Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene*. Report for the Ministry of Health, Wellington, New Zealand (http://www.smsl.co.nz/biosecure/NZB/Literature/Env%20health%20impacts%20of%20Smeth_glare%20et%20al%20.pdf).

USEPA (2001) *Methoprene. June 2001 update of the March 1991 Methoprene R.E.D. Fact Sheet*. Washington, DC, United States Environmental Protection Agency (Pesticide Fact Sheet; http://www.epa.gov/opbtpd1/biopesticides/ingredients/factsheets/factsheet_105401.pdf).

WHO (1999) *Recommended classification of pesticides by hazard and guidelines to classification 1998–1999*. Geneva, World Health Organization, International Programme on Chemical Safety (WHO/PCS/98.21/Rev. 1).

WHO (2006) *Pesticides and their application for the control of vectors and pests of public health importance*, 6th ed. Geneva, World Health Organization, Department of Control of Neglected Tropical Diseases, Pesticides Evaluation Scheme (WHO/CDS/NTD/WHOPES/GCDPP/2006.1; http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf).

WHO/FAO (1996) *Methoprene*. Geneva, World Health Organization and Food and Agriculture Organization of the United Nations (WHO/FAO Datasheets on Pesticides No. 47; VBC/DS/84.47; http://www.inchem.org/documents/pds/pds/pest47_e.htm).