CHEMICAL MEETING THE CRITIERIA FOR LISTING UNDER PROPOSITION 65 AS KNOWN TO CAUSE REPRODUCTIVE TOXICITY VIA THE AUTHORITATIVE BODIES MECHANISM: AVERMECTIN B1, CHEMICAL IDENTIFIED BY U.S. EPA

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Avermectin B1 (CAS No. 71751-41-2) meets the criteria for listing as known to cause reproductive toxicity under Proposition 65[1] via the authoritative bodies listing mechanism. The regulatory requirements for listing by this mechanism are set forth in Title 22, California Code of Regulations, section 12306[2] and include provisions covering the criteria for evaluating the documentation and scientific findings by the authoritative body to determine whether listing under Proposition 65 is required.

The U.S. Environmental Protection Agency (U.S. EPA) has been identified as an authoritative body for purposes of Proposition 65 in Section 12306(l) and has identified avermectin B1 as causing reproductive toxicity. The identification pertains to the developmental toxicity endpoint.

Formal Identification of Chemicals "as Causing Reproductive Toxicity"

In 2005, avermectin B1 was formally identified as causing reproductive toxicity (developmental toxicity endpoint) in the U.S. EPA document "Avermectin B1 and its delta-8,9-isomer; Pesticide Tolerance. Final Rule." Also, earlier, avermectin B1 was identified as causing reproductive toxicity by U.S. EPA in implementing its Toxic Release Inventory (TRI) program (*i.e.*, Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 (EPCRA), 42 U.S.C. section 11023). In 1994 U.S. EPA added a number of chemicals to the TRI list. In identifying them as causing reproductive and other toxicities, the U.S. EPA published its toxicity findings in the *Federal Register* (**59:**1788-1859, 1994 and **59:**61432-61485, 1994). U.S. EPA stated that a hazard assessment was performed for each candidate, "…in accordance with relevant EPA guidelines for each adverse human health or environmental effect…" (*Federal Register* **59:**1790).

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986, codified at Health and Safety Code section 25249.5

[2] All further references are to Title 22 of the California Code of Regulations, unless otherwise indicated.

OEHHA has found that avermectin B1 has been "formally identified" as causing reproductive toxicity according to Section 12306(d) because this chemical has "been identified as causing … reproductive toxicity by the authoritative body in a document that indicates that such identification is a final action" (*i.e.*, the U.S. EPA TRI *Final Rule* [*Federal Register* **59:**61432]); has "been included on a list of chemicals causing … reproductive toxicity issued by the authoritative body" and the document "specifically and accurately identifies the chemical" (*i.e.* the U.S. EPA TRI Program list); and has been "published by the authoritative body in a publication, such as, but not limited to the federal register…" The U.S. EPA document, "Avermectin B1 and its delta-8,9-isomer; Pesticide Tolerance. Final Rule" (U.S. EPA 2005) also "formally identified" avermectin B1 as causing reproductive toxicity according to Section 12306(d) because the chemical "is the subject of a report which is published by the authoritative body and which concludes that the chemical causes … reproductive toxicity" and, in addition, "has otherwise been identified as causing … reproductive toxicity by the authoritative body in a document that indicates that such identification is a final action."

Evaluation of Scientific Criteria for Listing Avermectin B1

OEHHA finds that the criteria for "as causing reproductive toxicity" in Section 12306(g) have been satisfied for avermectin B1. Although under the TRI program data for Avermectin B1a, Avermectin B1b and Ivermectin were considered due to their chemical similarity, only avermectin B1 (also known as "abamectin") has been formally identified as causing reproductive toxicity for purposes of Proposition 65. In establishing the 2005 Pesticide Tolerance Final Rule for avermectin B1, U.S. EPA relied primarily upon data for avermectin B1, with additional data for the delta-8,9-isomer of avermectin B1.

In evaluating the scientific criteria in Section 12306(g) for avermectin B1, OEHHA relied upon the documents and reports cited by U.S. EPA in making their finding that this chemical causes reproductive toxicity. In some cases, OEHHA consulted additional sources of information on the specific studies cited by U.S. EPA. This was done only where necessary to affirm or clarify details of results and study design for studies cited by U.S. EPA; OEHHA did not review additional studies not relied on by U.S. EPA.

Chemical	**CAS No.**	**Endpoint**	**Pesticide status or usage**	**Reference[a]**
avermectin B1 (abamectin)	71751-41-2	developmental toxicity	Registered in CA	U.S. EPA (1994a,b; 2005)

[a] Formal identification by U.S. EPA of avermectin B1 as causing reproductive toxicity is provided in U.S. EPA (1994b) and U.S. EPA (2005). Additional information on the basis for the 1994 identification is provided in U.S. EPA (1994a).

Studies cited by U.S. EPA in making findings with regard to reproductive toxicity of avermectin B1 are briefly described below. The statements in bold reflect data and conclusions which satisfy the criteria for sufficiency of evidence for reproductive toxicity

in Section 12306(g). Where a notation of "not stated" has been made, OEHHA staff were unable to find an explicit statement of a particular detail such as the number of animals in each dose group. Where NOELs (no-observed-effect-level), LOELs (lowest-observed-effect-level), or LELs (lowest-effect-level) are included in the study descriptions below, they are quoted directly from the cited references.

Avermectin B1 (CAS No. 71751-41-2)

***Developmental toxicity* has been manifested as malformations in experimental animals.**

The U.S. Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that "…there is sufficient evidence for listing abamectin [avermectin B1] on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available developmental toxicity data."

Supporting documentation (U.S. EPA, 1993a) for the TRI listing states, "A peer review evaluation of the developmental and reproductive toxicity of abamectin concluded that this compound induces developmental toxicity in several species with the mouse being the most sensitive species (74 [U.S. EPA, 1993b]). Increased retinal folds in weanlings, decreased viability and lactation indices, decreased body weight, increased number of dead pups at birth (LEL was 0.4 mg/kg/day; NOEL was 0.12 mg/kg/day) were noted in a two-generation rat reproduction study (74 [U.S. EPA, 1993b]). Based on the NOEL, an RfD of 0.0004 mg/kg/day was derived (74 [U.S. EPA, 1993b])."

In the final document adopting the additional chemicals to the TRI list (U.S. EPA 1994b), the Agency notes, "One commenter, Merck, states that primates are less sensitive to the acute effects of abamectin and its analog, ivermectin, than rodents. The commenter implies that because humans are primates, abamectin should be less toxic in humans than in rodents. The commenter further contends that ivermectin and abamectin have been used safely in animals and humans.[3] Abamectin interferes with gamma-aminobutyric acid (GABA) transmission and, as such, produces neurotoxic clinical signs such as tremors, ataxia, convulsions, or coma that are more severe in rodents and dogs than primates. EPA agrees that the available studies indicate that the sensitivity as well as doses required to produce neurotoxic effects vary from rodents to primates by a 20-fold factor. However, abamectin was proposed for addition to the EPCRA section 313 list based on developmental effects rather than neurotoxicity. There are no developmental studies with abamectin in primates. Therefore, EPA believes that the rodent studies cited in the proposed rule provide sufficient evidence that abamectin can reasonably be anticipated to cause developmental toxicity in humans. When administered in

[3] OEHHA notes that the package insert required by the U.S. Food and Drug Administration for the drug ivermectin states that "Ivermectin has been shown to be teratogenic in mice, rats, and rabbits when given in repeated doses of 0.2, 8.1 and 4.5 times the maximum recommended human dose…Ivermectin should not be used during pregnancy since safety in pregnancy has not been established."

therapeutic doses, the Agency does not dispute the animal and human safety and efficacy of ivermectin and abamectin, but the safety of a 0.2 to 0.3 mg/kg single therapeutic dose does not diminish the findings of the developmental, reproductive, neurotoxic, chronic, and carcinogenic animal studies with abamectin which in some cases demonstrate compound-related effects at higher than therapeutic doses in all species tested."

In its EPCRA-TRI documentation U.S. EPA identified a total of more than twenty studies in a variety of animal species relevant to its finding of reproductive toxicity. These studies were listed in the previous notice of intent to list, published in the California Regulatory Notice Register on April 23, 1999. On September 7, 1999, U.S. EPA stated publicly that "the CF-1 mouse should not be used for assessing the risk of human exposure to avermectins" (U.S. EPA, 1999). This followed from an internal review conducted by U.S. EPA the previous year (U.S. EPA, 1998). Accordingly, the 11 studies in which the CF-1 mouse was used are no longer under consideration by OEHHA in determining whether the criteria specified in Section 12306(g) have been met for avermectin B1. U.S. EPA considered 11 studies in species other than the mouse in making its formal identification of avermectin as causing developmental toxicity in its EPCRA-TRI process. As noted in the supporting document for the previous notice of intent to list, five of these studies did not report developmental toxicity. Developmental toxicity relevant to Proposition 65 was identified by U.S EPA in six of these studies. Information on those six studies is summarized below.

A more recent evaluation of avermectin by the U.S. EPA Hazard Identification Assessment Review Committee discussed developmental toxicity findings in CD-1 mice, a strain of mouse considered suitable for risk assessment of avermectin B1. In that evaluation U.S. EPA stated that "[d]evelopmental toxicity was also seen in … species [other than the CF-1 mouse], notably in the rabbit and in the rat reproduction study" of avermectin (U.S. EPA, 2000). In the U.S EPA document that concluded that CF-1 mice should not be used for assessing risk of human exposure to avermectin (U.S. EPA, 1999), a chronic reference dose (RfD) was calculated on the basis of developmental toxicity to rat pups in the two-generation reproduction study (identified as study d below). Similarly, as discussed more fully below, a recent Final Rule on pesticide tolerances for avermectin B1 notes that "increased susceptibility (qualitative and/or quantitative) was seen in prenatal developmental toxicity studies in CD-1 mice and rabbits following in utero exposure to avermectin B1" (U.S. EPA, 2005). The Final Rule thus provides an additional basis for listing.

With regard to the studies cited by U.S. EPA t support adding this chemical to the EPCRA-TRI list, OEHHA finds that the evidence for developmental and reproductive toxicity effects meet the criteria of Section 12306 on the basis of studies of avermectin B1 in rat and rabbit (studies a and d below) supported by studies of the structural isomers avermectin B1a and ivermectin in rats and rabbits. OEHHA also notes the following:

1. **Adequacy of the experimental design:** All studies listed below were discussed in U.S. EPA's peer review of the developmental and reproductive toxicity of

avermectin (U.S. EPA, 1993b), and all were considered in the Agency's weight-of-evidence determination. OEHHA notes that although the effects on postnatal growth and pup death observed in a two-generation reproductive toxicity study conducted in rats (study "d" below) appear to have been the result of exposure to avermectin during the postnatal period, which is not considered relevant to Proposition 65, the retinal abnormalities are the result of prenatal exposures.

Study a) developmental toxicity study in rabbits ("MK-0936 avermectin B1; approximately 94% pure"),
Study b) one-generation reproduction toxicity study in rats (avermectin B1a),
Study c) one-generation reproduction toxicity study in rats (avermectin B1a),
Study d) reproduction toxicity study in rats (avermectin B1),
Study e) developmental toxicity study in rats (ivermectin),
Study f) developmental toxicity study in rabbits (ivermectin),

2. **Route of administration:**
   Study a) oral, presumed gavage,
   Study b) oral, presumed gavage,
   Study c) oral, presumed gavage,
   Study d) oral, gavage,
   Study e) not stated,
   Study f) not stated,
3. **The frequency and duration of exposure:**
   Study a) daily on gestation days 6-27,
   Study b) from prior to mating, though gestation and lactation,
   Study c) from prior to mating, though gestation and lactation,
   Study d) from prior to mating, though gestation and lactation, for two generations,
   Study e) not stated,
   Study f) not stated,
4. **The numbers of test animals:**
   Study a) not stated,
   Study b) 15 mated females per group,
   Study c) 12 mated females per group,
   Study d) 30 animals/sex/dose,
   Study e) 25 per group,
   Study f) 16 per group,
5. **The choice of species:** Rats and rabbits are standard species used in reproductive toxicity studies.
6. **The choice of dosage levels:**
   Study a) 0, 0.5, 1.0, 2.0 mg/kg/day,
   Study b) 0, 0.1, 0.2, 0.4 mg/kg/day,
   Study c) 0, 0.5, 1.0, and 2.0 (reduced to 1.5 mg/kg/day during the course of the study) mg/kg/day,
   Study d) 0, 0.05, 0.12, 0.40 mg/kg/day,
   Study e) 0, 2.5, 5, 10 mg/kg/day,
   Study f) 0, 1.5, 3, 6 mg/kg/day,

7. **Maternal toxicity:**
   Study a) maternal LOEL=2.0 mg/kg/day (decreased body weight gain) NOEL=1.0 mg/kg/day; developmental LOEL=2.0 mg/kg/day (malformations and incomplete ossification), NOEL=1.0 mg/kg/day,
   Study b) maternal NOEL > 0.4 mg/kg/day (high dose tested); developmental LOEL 0.2 mg/kg/day (increased spastic movements, decreased pup body weights, decreased litter size on lactation day 1), NOEL 0.1 mg/kg/day,
   Study c) maternal LOEL=1.5-2.0 mg/kg/day (tremors, clinical symptoms, mortality), NOEL 1.0=mg/kg/day; developmental LOEL < 0.5 mg/kg/day (ldt) (decreased pup weights on pnd 1, as well as later time points; and decreased postnatal survival),
   Study d) NOELs for systemic and reproductive toxicity stated to be $\geq$0.40 mg/kg/day (effects on adult body weights); developmental NOEL=0.12 mg/kg/day, LOEL=0.40 (reduced postnatal viability and growth, retinal abnormalities),
   Study e) maternal NOEL=5 mg/kg/day, LOEL=10 mg/kg/day (3 high-dose dams sacrificed moribund); developmental NOEL=5 mg/kg/day, LOEL=10 mg/kg/day (cleft palate),
   Study f) maternal NOEL=3 mg/kg/day, LOEL=6 mg/kg/day ("sedation," decreased body weights); developmental NOEL=1.5 mg/kg/day, LOEL=3 mg/kg/day (cleft palate, clubbed forepaw; aborted litters at 6 mg/kg/day)

Increased susceptibility **(qualitative and/or quantitative) was seen in** ***prenatal developmental toxicity*** **studies in CD-1 mice and rabbits following in utero exposure to avermectin B1. There was also an increase in quantitative and qualitative susceptibility in the rat reproductive toxicity study.**

U.S. EPA (2005) identified three studies in mice, rabbits and rats as demonstrating developmental toxicity, and used the NOEL for developmental toxicity in the rat reproductive toxicity study as the basis for the reference doses (RfDs) for chronic dietary, short-term and intermediate-term incidental oral, dermal, and inhalation exposures in humans.

With regard to the studies cited by U.S. EPA supporting the establishment of a pesticide tolerance for avermectin B1, OEHHA finds that the evidence of developmental and reproductive toxicity effects meet the criteria of Section 12306 on the basis of studies of avermectin B1 in rat and rabbit and a study of the delta 8,9-isomer of avermectin B1 in CD-1 mouse. The rabbit and rat studies cited in this regard were also cited in the addition of avermectin B1 to the EPCRA-TRI list, and are identified above as studies a and d. The study in CD-1 mice conducted using the delta-8,9 isomer of avermectin is described below as study g. OEHHA notes the following:

1. **Adequacy of the experimental design:** The study listed below was discussed in U.S. EPA's pesticide tolerance final rule for avermectin B1 and its delta-8,9 isomer (U.S. EPA, 2005).

Study g) developmental toxicity study in CD-1 mice delta-8,9 isomer of avermectin B1; 98.1% pure)

2. **Route of administration:**
   Study g) oral gavage
3. **The frequency and duration of exposure:**
   Study g) daily on gestation days 6-15
4. **The numbers of test animals:**
   Study g) 22 per group
5. **The choice of species:** Mouse is standard species used in reproductive toxicity studies.
6. **The choice of dosage levels:**
   Study g) 0, 0.75, 1.5, 3.0 mg/kg/day
7. **Maternal toxicity:**
   Study g) maternal LOEL=3.0 mg/kg/day (hind limb splay) NOEL=1.5 mg/kg/day; developmental LOEL=0.75 mg/kg/day cleft palate and hindlimb extension), NOEL<0.75 mg/kg/day

References

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