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

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Molinate (CAS No. 2212-67-1) 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 molinate as causing reproductive toxicity. The identification pertains to the developmental and male and female reproductive toxicity endpoints.

Formal Identification of Chemicals "as Causing Reproductive Toxicity"

The 2002 U.S. EPA document "Molinate – Review of 60-Day Comments and Revised Human Health Risk Assessment" identifies molinate as causing reproductive toxicity (developmental and male and female reproductive toxicity endpoints). Also, earlier, molinate 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).

OEHHA has found that molinate 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

[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.

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 "Molinate – Review of 60-Day Comments and Revised Human Health Risk Assessment" (U.S. EPA 2002e) also "formally identified" molinate 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."

Evaluation of Scientific Criteria for Listing Molinate

OEHHA finds that the criteria for "as causing reproductive toxicity" in Section 12306(g) have been satisfied for molinate. In evaluating the scientific criteria in Section 12306(g) for molinate, 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.

Since OEHHA's April 1998 Request for Information, the U.S. EPA has considered molinate's toxicity in order to assess the re-registration of the chemical as an herbicide for use in the U.S. A number of memoranda and documents have been produced, none of which indicate the U.S. EPA no longer considers molinate to cause developmental or male or female reproductive toxicity in animals or that the overall findings are not relevant to humans (See references U.S. EPA 1998; 2000; 2001a,b,c; 2002a,b,c,d,e; 2003).

Chemical	**CAS No.**	**Endpoint**	**Pesticide status or usage**	**Reference[a]**
molinate	2212-67-1	developmental toxicity male reproductive toxicity female reproductive toxicity	Registered in CA	U.S. EPA (1994a,b; 2002e)

[a] Formal identification of molinate in U.S. EPA (1994b) and U.S. EPA (2002e). Additional information on the basis for the 1994 identifications is provided in U.S. EPA (1994a).

Studies cited by U.S. EPA in making findings with regard to reproductive toxicity are briefly described below for molinate. 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.

Molinate (CAS No. 2212-67-1)

In molinate exposed animals, *developmental toxicity* has been manifested as reduced viability, reduced body weights, and an increased frequency of morphological variations. *Male reproductive toxicity* has been manifested as reduced fertility, testicular degeneration, and sperm abnormalities. *Female reproductive toxicity* has been manifested as reduced fertility and fecundity, and abnormal histopathology of ovarian tissues.

U.S. EPA (1994a and 1994b) concluded that: ". . . there is sufficient evidence for listing molinate on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available developmental, [and] reproductive . . . toxicity data for this chemical." Supporting documentation for the TRI listing (U.S. EPA, 1993) discusses data on developmental toxicity, and male and female reproductive toxicity:

> "In a rat developmental toxicity study, adverse effects observed at 35 mg/kg/day (LOEL) included increased post-implantation loss, lower fetal body weight, increased incidence of runts, and external/soft tissue/skeletal variants . . . In a rabbit developmental study, adverse effects such as an increase in the number of abortions, and a decrease in the number of does with live fetuses were noted at 200 mg/kg/day."
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> "In a rat fertility test, reductions in fertility, dose-related altered sperm morphology, and a reduction in the number of viable fetuses were observed . . . In a 90-day study in male rats, the lowest toxic oral dose of 324 mg/kg[3] produced adverse effects on spermatogenesis, male fertility, and viability index (9 [RTECS, 1993]). The 20-day inhalation male rat TCLo is 0.0006 mg/L. At this exposure level adverse effects on spermatogenesis and male fertility index were reported (9 [RTECS, 1993]) . . . In a 3-month rat inhalation study, testicular degeneration and abnormal spermatozoa were observed at 0.002 mg/L (LOEL). No NOEL was determined (71 [U.S. EPA, 1992])."

[3] This appears to be a verbatim citation of RTECS. RTECS expresses oral doses as summed over the entire treatment period. Hence 324 mg/kg was the total given over a period of 90 days, and the daily dose would actually have been 3.6 mg/kg.

> "In a 2-generation rat reproduction study, the reproductive NOEL was 0.3 mg/kg/day and the LOEL was 2.5 mg/kg/day based on reduced fecundity, and increased incidence of ovarian vacuolation/hypertrophy (71 [U.S. EPA, 1992])."

In the final rule document (U.S. EPA, 1994b), the Agency responded to comments from Zeneca Inc. which argued against including molinate as a reportable compound under TRI. The specifics of these comments and the Agency's response were:

> "Zeneca Incorporated contends that the observations attributed to the 35 mg/kg/day dose level in the rat developmental toxicity study 'in fact occurred at 140 mg/kg/day, the highest dose tested and were thus a consequence of maternal toxicity.' The commentor states that the NOEL for that study was 35 mg/kg/day. The Agency does not agree that the NOEL for this study was 35 mg/kg/day. The NOEL for developmental toxicity was 2.2 mg/kg/day based on an increase in runting at the next highest doses, 35 and 140 mg/kg/day . . . The NOEL for maternal toxicity was 35 mg/kg/day and that the effects on the pups (runting) occurred at a dose level lower than the dose level found to be maternally toxic."
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> The same commentor stated that the evidence for the reproductive toxicity of molinate rests solely on studies in rodents, and that studies in other species "have shown 'conclusively that the effects seen in rodents is [sic] not relevant to man.'" The Agency countered that "data on the rabbit and dog do not support the commentor's contention that the effects seen in rodents are specific only to rodents. For example, in each of the fertility studies in rabbits, both an increase in pre-implantation loss and abnormal sperm were observed. These two consistent [reproducible] observations are suggestive of fertility effects, are two of the same observations found in rats and although not as dramatic as observed in rats, cannot be negated. In the chronic dog study, lesions in male reproduction organs and effects on sperm were observed, which demonstrated that, at least in the males, the gonads are target organs for molinate . . . Since molinate is reaching the gonads in all species, not only in rodents as the commentor claims, molinate can reasonably be anticipated to cause fertility/reproductive effects in humans. Further, animals are accepted as surrogates for toxicity testing to predict potential hazard to humans, except in a few rare cases where effects have been determined to be species-specific [e.g., $\alpha 2\mu$-globulin]."

With regard to the studies cited as supporting U.S. EPA's action in adding a chemical to the EPCRA-TRI list, OEHHA finds that the evidence for developmental and reproductive toxicity (DART) effects appears to meet the criteria of Section 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rat developmental toxicity study was classified as acceptable by the California Department of Pesticide Regulation (CDPR, 1997)
   Study b) rabbit developmental toxicity study was classified as acceptable by CDPR (1997)

Study c) rat fertility study - study was rated as core grade supplementary. This study was used as the principal study in calculating the oral RfD for molinate. For this purpose, the study was considered to be of fair quality and was given a medium confidence rating

Study d) 90-day rat study - not a standard reproductive toxicity protocol. Original study report in Russian language

Study e) 20-day inhalation rat study - not a standard reproductive toxicity protocol

Study f) 2-generation rat reproduction study - females only treated. This study was considered to be acceptable as a supplemental study by CDPR (1997)

Study g) 3-month rat inhalation study

2. **Route of administration:**

Study a) gavage
Study b) gavage
Study c) gavage
Study d) oral
Study e) inhalation
Study f) oral, diet
Study g) inhalation

3. **The frequency and duration of exposure:**

Study a) daily on gestation days 6-15

Study b) not stated, but guidelines specify treatment on each of gestation days 6 - 18

Study c) 0, 12, or 30 mg/kg/day for 30 days or; 0, 0.2, 4, 12, or 30 mg/kg/day for 5 or 10 weeks

Study d) 0, 3.6, or 18 mg/kg/day for 90 days

Study e) 0, 0.1, 0.6, 1.8, 4.0 mg/m3, 6 hrs/day, 5 days/week for 13 weeks or; 0, 0.07, 0.16, 0.30, or 1.6 mg/m3, 6 hrs/day, 5 days/week for 4 weeks

Study f) prior to mating and continuously throughout gestation, lactation, and weaning of their offspring for 2 generations

Study g) 6 hours/day, 5 days/week for 3 months

4. **The numbers of test animals:**

Study a) 26 pregnant females per dose group

Study b) not specifically stated, but study was generally considered to be of acceptable quality

Study c) not stated
Study d) not known
Study e) not stated
Study f) 25 females per dose group,
Study g) 10 rats/sex/group

5. **The choice of species:**

The rat and rabbit are standard species in toxicity testing.

6. **The choice of dosage levels:**

Study a) 0, 2.2, 35, 140 mg/kg/day
Study b) 0, 2, 20, 200 mg/kg/day

Study c) 0, 0.2, 4, 12, 30, and 60 mg/kg/day
Study d) 0, 6, 50, and 450 ppm in the feed
Study e) see # 3, above
Study f) 0, 6, 50, 450 ppm (0, 0.34, 2.9, 28 mg/kg/day)
Study g) 0, 2, 10, 50 mg/m^3

7. **Maternal toxicity:**
   Study a) The NOEL for maternal toxicity was 35 mg/kg/day, the NOEL for developmental toxicity was 2.2 mg/kg/day
   Study b) Maternal NOEL = 20 mg/kg/day, developmental NOEL = 20 mg/kg/day
   Study c) not relevant
   Study d) not relevant
   Study e) not relevant
   Study f) adult female systemic toxicity NOEL = 6 ppm (0.34 mg/kg) reproductive toxicity NOEL = 6 ppm (0.34 mg/kg/day)
   Study g) not relevant.

Molinate is a *reproductive toxicant*.
***Increase susceptibility of offspring* was observed in the *prenatal developmental toxicity* study and the neurotoxicity study in rats.**

U.S. EPA (2002e) identified fifteen studies in rats, mice, rabbits and dogs as demonstrating reproductive and developmental toxicity. U.S. EPA used the LOEL for developmental neurotoxicity in the rat developmental neurotoxicity study as the basis for the reference doses (RfDs) for acute dietary and short-term dermal exposures in humans, and the NOEL for male reproductive toxicity in the five-week male rat fertility study as the basis for the RfD for intermediate-term dermal exposures in humans. This document also addresses the question of specificity of the mechanism of reproductive toxicity to rodents, and notes that **"**the registrant concludes that the reproductive toxicity in the rat is induced by a mechanism that is specific to rodents. Special studies data submitted to establish the proposed mechanism of toxicity were reviewed and evaluated by the HED Mechanism of Toxicity Assessment Review Committee. The Committee concluded that the submitted studies are not adequate to demonstrate the proposed mechanism of toxicity."

With regard to the studies cited as supporting U.S. EPA's formal identification of molinate as causing reproductive and developmental toxicity, OEHHA finds that the evidence for developmental and reproductive toxicity effects meet the criteria of Section 12306 on the basis of studies of molinate in rats, mice, rabbits and dogs. Several of the rabbit and rat studies cited in this regard were also cited in the addition of molinate to the EPCRA-TRI list, and are identified above as studies a, b, c, f and g. The additional studies cited in U.S. EPA (2002e) that were not cited in U.S. EPA (1994a and 1994b) are described below as studies h-q. OEHHA notes the following:

1. **Adequacy of the experimental design:**

Study h) rat 4-week inhalation study (abnormal sperm and decreased implantations)
Study i) rat chronic toxicity study (ovarian lesions; testicular atrophy)
Study j) dog chronic toxicity study (sperm parameters)
Study k) mouse carcinogenicity study (testicular degeneration; non-neoplastic lesions of ovaries)
Study l) rabbit 13-week oral study in males (sperm effects)
Study m) rat 2-generation reproduction study with both sexes dosed (sperm and testis effects; ovarian lesions)
Study n) rat developmental neurotoxicity study (reduced startle amplitude)
Study o) 5-week male fertility study (sperm abnormalities)
Study p) female mechanistic study (microscopic ovarian lesions)
Study q) mouse acute inhalation study (decreased testis weight)

2. **Route of administration:**
Study h) inhalation
Study i) oral, diet
Study j) oral, diet
Study k) oral, diet
Study l) oral
Study m) oral, diet
Study n) oral, diet
Study o) oral gavage
Study p) oral gavage
Study q) inhalation

3. **The frequency and duration of exposure:**
Study h) 6 hours/day, 5 days/week for 4 weeks
Study i) 24 months (12 months at the highest dose tested)
Study j) 1 year (14 weeks at the highest dose tested)
Study k) 18 months
Study l) 13 weeks
Study m) 10 weeks prior to mating and continuously throughout gestation, lactation, and weaning of their offspring for 2 generations
Study n) gestation day 7 through lactation day 11
Study o) daily for 35 consecutive days
Study p) daily on gestation days 7-9
Study q) not stated

4. **The numbers of test animals:**
Study h) 12 males/group
Study i) 50/sex/treatment group
Study j) 4/sex/group
Study k) 50/sex/group
Study l) not stated
Study m) 40/sex/treatment group
Study n) 30 females/group
Study o) 12 males/group
Study p) 10 sperm-positive females/group

Study q) not stated

5. **The choice of species:**
   The rat, mouse and rabbit are standard species in toxicity testing, while dog is also frequently used
6. **The choice of dosage levels:**
   Study h) 0, 0.2, 0.2, 0.4, 0.8, 1.6 mg/m^3
   Study i) 0, 7, 40, 300 ppm (males 0, 0.3, 1.8, 13 / females 0, 0.4, 2, 15 mg/kg/day) [for 24 months]; 600 ppm (30 mg/kg/day) [for 12 months]
   Study j) 0, 1, 10 mg/kg/day [for 1 year]; 100 mg/kg/day [for 14 weeks]
   Study k) 0, 10, 100, 1000, 2000 ppm (males 0, 1, 10.4, 105, 200 / females 0, 1.3, 13.9, 133, 240 mg/kg/day)
   Study l) 0, 10, 100 mg/kg/day [for 49 days]; 200 mg/kg/day [for 16 days]
   Study m) 0, 5, 10, 15 ppm males (0, 0.4, 0.8, 1.3 mg/kg/day) 0, 20, 50, 300 ppm females (0, 1.9, 4.7, 28.8 mg/kg/day)
   Study n) 0, 20, 75, 300 ppm (0, 1.8, 6.9, 26.1 mg/kg/day)
   Study o) 0, 0.5, 1, 2, 3, 4, 8 mg/kg/day
   Study p) 0, 75, 135, 200 mg/kg/day
   Study q) 0, 0.034, 0.09, 0.23, 1.1, 1.8, 2.0, 2.3, 3.2 mg/L)
7. **Maternal toxicity:**
   Study h) not relevant
   Study i) not relevant
   Study j) not relevant
   Study k) not relevant
   Study l) not relevant
   Study m) Maternal toxicity NOELs/LOELs consistently higher than developmental toxicity NOELs/LOELs
   Study n) Maternal toxicity NOEL 6.9 mg/kg/day, no developmental toxicity NOEL (LOEL 1.8 mg/kg/day)
   Study o) not relevant
   Study p) not relevant
   Study q) not relevant.

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