CHEMICALS MEETING THE CRITERIA FOR LISTING AS DEVELOPMENTAL AND REPRODUCTIVE TOXICANTS (DARTs) VIA THE AUTHORITATIVE BODIES MECHANISM: 5 CHEMICALS IDENTIFIED BY U.S. EPA

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California Environmental Protection Agency

The 5 chemicals listed in the table below meet the criteria for listing under Proposition 65 via the authoritative bodies listing mechanism. The regulatory guidance for listing by this mechanism is set forth in Title 22, California Code of Regulations (CCR), Section 12306. For example, the regulations 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.

U.S. Environmental Protection Agency (U.S. EPA) has been identified as an authoritative body for purposes of Proposition 65 (22 CCR Section 12306(l)) and has identified the chemicals in the table below as causing developmental or reproductive toxicity. This was done by that Agency in implementing its Toxics Release Inventory (TRI) program (*i.e.*, Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986 [EPCRA]). On the basis of identifying chemicals that caused reproductive, developmental and/or other toxicities the U.S. EPA added a number of chemicals to the TRI list. The U.S. EPA published its toxicity findings in the *Federal Register* (**59:**1788-1859, 1994 and **59:**61432-61485, 1994). In proposing specific chemicals for addition to the TRI list, the Agency 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 the chemicals in the table below have been "formally identified" as causing reproductive toxicity according to the regulations covering this issue (22 CCR 12306[d]) because the chemicals have "been identified as causing … reproductive toxicity by the authoritative body" (*i.e.*, U.S. EPA) "in a document that indicates that such identification is a final action" (*i.e.*, the TRI *Final Rule* [*Federal Register* **59:**61432]) and have "been included on a list of chemicals causing … reproductive toxicity issued by the authoritative body" "and the document specifically and accurately identifies the chemical" and has been "published by the authoritative body in a publication, such as, but not limited to the federal register…"

OEHHA also finds that the criteria for "as causing reproductive toxicity" given in regulation (22 CCR 12306[g]) have been satisfied for the chemicals in the table below. In making this evaluation, OEHHA relied upon the documents and reports cited by U.S. EPA in making their finding that the specified chemicals cause 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.

A major source of information used by the U.S. EPA was the "Tox-Oneliner" database maintained by U.S. EPA's Office of Pesticide Programs (OPP). This database consists of brief summaries of (usually unpublished) data submitted to the Agency in compliance with regulatory requirements. Many database entries include a notation of "core grade" – a system formerly used by U.S. EPA to indicate the extent to which a study conformed to published test guidelines (U.S. EPA 1983a and 1983b). Under this scheme, a "core grade guideline" study was considered to meet all guideline requirements; a "core grade minimum" study was considered sufficient for risk assessment; and a "core grade supplementary" study was considered to provide useful supplementary information, but not to be suitable for risk assessment on its own.

Chemical	CAS No.	Endpoints	Pesticide status or usage
Chlorsulfuron	6490-27-23	developmental toxicity female reproductive toxicity male reproductive toxicity	Registered in CA
Dichlorophene	97-23-4	developmental toxicity	Not currently registered in CA
2,4-DP (dichloroprop)	120-36-5	developmental toxicity	Not currently registered in CA
Ethyl dipropyl-thiocarbamate	759-94-4	developmental toxicity	Registered in CA
Quizalofop-ethyl	76578-14-8	male reproductive toxicity	Not currently registered in CA

Studies cited by U.S. EPA in making findings with regard to reproductive toxicity are briefly described below. The statements in bold reflect data and conclusions which satisfy the criteria for sufficiency of evidence for reproductive toxicity in regulation (22 CCR 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 this document, they are quoted directly from the cited references.

Chlorsulfuron (CAS No. 6490-27-23)

Developmental toxicity **was manifested as an increased frequency of fetal resorptions.** ***Male and female reproductive toxicity*** **were manifested as decreased fertility.**

The US Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that: "…there is sufficient evidence for listing chlorsulfuron 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, 1993b) states, "In a rabbit developmental study, an increased incidence of fetal resorptions was observed at the LOEL of 75 mg/kg/day. The NOEL was 25 mg/kg/day… In a 3-generation rat reproduction study, a decrease in [the] fertility index was observed at 125 mg/kg/day (LOEL). The NOEL was 25 mg/kg/day…"

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 DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rabbit developmental toxicity study, graded core minimum (U.S. EPA, 1990).
   Study b) rat 3-generation reproductive toxicity study, graded core guideline (U.S. EPA, 1990).
2. **Route of administration:**
   Study a) not stated directly, but as this study was rated "core grade minimum" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which specify exposure by the oral route.
   Study b) oral, diet.
3. **The frequency and duration of exposure:**
   Study a) gestation days 6-19 (CDFA 1991).
   Study b) daily from 103 days prior to mating, through subsequent study generations (U.S. EPA, 1990).
4. **The numbers of test animals:**
   Study a) not stated directly, but as this study was rated "core grade minimum" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which specify a minimum of 12 pregnant rabbits per dose group.
   Study b) not stated directly, but as this study was rated "core grade guideline" it would have met U.S. EPA test guidelines (U.S. EPA, 1983b) which specify a minimum of 20 animals per dose group.
5. **The choice of species:**
   Rats and rabbits are standard test species used in toxicity testing.
6. **The choice of dosage levels:**
   Study a) 0, 10, 25, 75 mg/kg/day (U.S. EPA, 1990).

Study b) 0, 100, 500, 2500 ppm in the feed (U.S. EPA, 1990). U.S. EPA (1993b and 1994a) expressed the LOEL and NOEL for this study in mg/kg/day, but details of the conversion method were not provided.

7. **Maternal toxicity:**
   Study a) not mentioned.
   Study b) a LEL of 2500 ppm (125 mg/kg/day) and a NOEL of 500 ppm (25 mg/kg/day) were determined for decreased maternal body weight. These levels were the same as those determined for decreased fertility.

Dichlorophene (CAS No. 97-23-4)

***Developmental toxicity* was evidenced by an increased incidence of microphthalmia, delayed ossification, reductions in body weight and length, and increased resorption frequency.**

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

Supporting documentation for the TRI listing (U.S. EPA, 1993b) states, "Increased incidence of microphthalmia was observed in the offspring of rats administered 25 mg/kg/day (teratogenic LOEL). The NOEL was 5.0 mg/kg/day. A dose of 75 mg/kg/day (fetotoxic LOEL) produced delayed ossification of vertebral centra and sternaebra, reduced body weight and length, and increased resorptions in rat fetuses. The fetotoxic NOEL was 5.0 mg/kg/day. The study was classified core minimum…" Further details of the study were obtained from the tox one-liner database (U.S. EPA, 1984), as cited in the TRI supporting documentation (U.S. EPA, 1993b).

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 DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study rated core minimum
2. **Route of administration:**
   Oral, gavage (U.S. EPA, 1984)
3. **The frequency and duration of exposure:**
   Not stated. However, as this study was rated "core grade minimum" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which require treatment on each of gestation days 6 - 15.
4. **The numbers of test animals:**
   Not stated. However, as this study was rated "core grade minimum" it would have met U.S. EPA test guidelines (U.S. EPA, 1983a) which require a minimum of 20 animals per dose group.
5. **The choice of species:**

The rat is a standard test species.

6. **The choice of dosage levels:**
   0, 5, 25, 75 mg/kg/day.
7. **Maternal toxicity:**
   Maternal toxicity was evidenced by reduced body weight gain and food consumption. The LEL for these effects was 25 mg/kg/day, and the NOEL was 5.0 mg/kg/day.

2,4-DP (dichloroprop) (CAS No. 120-36-5)

Developmental toxicity **following prenatal exposure to 2,4-DP has been manifested as reduced viability in fetal rats, and musculoskeletal abnormalities and fetotoxicity in fetal mice.**

The US Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that "…there is sufficient evidence for listing 2,4-DP on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available developmental toxicity data for this chemical."

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "Developmental toxicity has been reported in rats and mice (RTECS, 1993) administered oral doses as low as 20 mg/kg 2,4-DP during gestation days 4-18, with behavioral and/or physical effects in newborn rats, and, in mice, increased postimplantation loss. Exposure of mice to higher doses (3000 and 4000 mg/kg) for shorter durations [sic] (i.e. gestation days 6-15) caused musculoskeletal abnormalities and fetotoxicity (RTECS, 1993). Data from OPP's 'Tox-Oneliner' database support these findings."

OEHHA has retrieved and reviewed the original articles cited in RTECS (1993). It should be noted that RTECS presents dose as a summation of all doses given. That is, a dosing regimen of 10 mg/kg/day repeated on each of 10 days, would be expressed as 100 mg/kg. Of the references cited by RTECS, only one (Roll and Mattiaschk, 1983) was reported in sufficient detail for risk assessment. Roll and Matthiaschk (1983) found adverse effects on fetal weight in mice exposed to doses of 300 mg/kg and above, and skeletal anomalies following prenatal exposure to doses of 400 mg/kg and above.

Of the studies summarized by U.S. EPA's tox one-liner database (U.S. EPA, 1993c), only the rat multi-generation reproduction study reported an adverse effect on exposed offspring. The fetotoxic LEL for this study was stated to be 2000 ppm, based upon findings of an increased number of small litters, and increased mortality. The corresponding NOEL was 1000 ppm.

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 DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

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

Study a) Developmental toxicity studies in rats and mice (Buschmann et al., 1986a). The study is reported in abstract form only, so the adequacy cannot be determined.

Study b) Behavioral developmental toxicity study in rats (Buschmann, et al., 1986b). The study is reported in abstract form only, so the adequacy cannot be determined.

Study c) Developmental toxicity in mice (Roll and Matthiaschk, 1983). The basic protocol and evaluation methods correspond to standard techniques.

Study d) Developmental toxicity study in rats. Range finding study, rated 'core grade guideline' (U.S. EPA, 1993c).

Study e) Developmental toxicity study in rats. Rated 'invalid' (U.S. EPA, 1993c) due to excessive deficiencies.

Study f) Developmental toxicity study in rabbits. Range finding study, rated 'core grade minimum' (U.S. EPA, 1993c).

Study g) 3-generation reproductive toxicity study in rats. Rated 'core grade minimum' (U.S. EPA, 1993c).

2. **Route of administration:**

Study a) Oral, gavage.

Study b) Oral, gavage.

Study c) Oral, gavage.

Study d) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify the gavage route of exposure. As the study received an acceptable grade, it is presumed that guideline requirements were met.

Study e) Not stated,

Study f) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify the gavage route of exposure. As the study received an acceptable grade, it is presumed that guideline requirements were met.

Study g) Oral, diet.

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

Study a) Once on each of 'p.c.' ['p.c.' is not defined, presumably refers to 'post conception] days 4, 10, 13, and 18.

Study b) Once on each of 'p.c.' days 4, 10, 13, and 18.

Study c) Daily on each of gestation days 6-15.

Study d) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify treatment to cover the organogenesis phase of prenatal development, generally days 6-15 for rats. As the study received an acceptable grade, it is presumed that guideline requirements were met.

Study e) Not stated.

Study f) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify treatment to cover the organogenesis phase of prenatal development, generally days 6-18 for rabbits. As the study received an acceptable grade, it is presumed that guideline requirements were met.

Study g) Not stated, but U.S. EPA test guidelines for reproductive toxicity studies (U.S. EPA, 1983b) specify continuous exposure from prior to mating of the parental generation, throughout gestation and lactation, and continuing through postnatal development and reproduction of the F1 generation to produce the F2. As the study was considered to meet guideline requirements, it is presumed that this dosing schedule was adhered to.

4. **The numbers of test animals:**
   Study a) Not stated.
   Study b) Not stated.
   Study c) 21-38 litters per dose group, except for the highest dose group, which had 12 pregnant females.
   Study d) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify a minimum of 20 pregnant rats per dose group. As the study received an acceptable grade, it is presumed that guideline requirements were met.
   Study e) Not stated.
   Study f) Not stated, but U.S. EPA test guidelines for developmental toxicity studies (U.S. EPA, 1983a) specify a minimum of 12 pregnant rabbits per dose group. As the study received an acceptable grade, it is presumed that guideline requirements were met.
   Study g) Not stated, but U.S. EPA test guidelines for reproductive toxicity studies (U.S. EPA, 1983b) specify a minimum of 20 pregnant animals per dose group. As the study received an acceptable grade, it is presumed that guideline requirements were met.
5. **The choice of species:**
   Rats, rabbits, and mice are standard species used in toxicology testing.
6. **The choice of dosage levels:**
   Study a) Rats: 0, 5, 30, 100, 200 mg/kg. Mice: 0, 5, 30, 100, and 150 mg/kg.
   Study b) 0, 5, 30, 100, and 200 mg/kg.
   Study c) 0, 100, 200, 300, 400, 500 mg/kg/day.
   Study d) 0, 25, 100 mg/kg.
   Study e) 0, 10, 30, 100 mg/kg.
   Study f) 0, 25, 100 mg/kg.
   Study g) 0, 1000, 2000 ppm
7. **Maternal toxicity:**
   Study a) Not discussed.
   Study b) Not discussed.
   Study c) Significant decrease in maternal gestational weight gain at the high dose of 500 mg/kg/day.
   Study d) Not discussed.
   Study e) Not discussed.
   Study f) Not discussed.
   Study g) Maternal NOEL=1000 ppm. Maternal LEL=2000 ppm for reduced body weight, and increase in number of small litters.

Ethyl Dipropylthiocarbamate (CAS No. 759-94-4)

Developmental toxicity **has been manifested as increased resorptions, growth retardation, and decreased body weights.**

The US Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that: "…there is sufficient evidence for listing ethyl dipropylthiocarbamate (EPTC) on the EPCRA section 313 list pursuant to EPCRA section 313(d)(2)(B) based on the available …reproductive toxicity data for this chemical."

Supporting documentation for the TRI listing (U.S. EPA, 1993a) states, "An increased incidence of fetal resorptions, increased incidence of fetal retardations, and decreased fetal body weights were observed in rats receiving 300 mg/kg/day on days 6-15 of gestation. The LOEL was 300 mg/kg/day and the NOEL was 100 mg/kg/day (U.S. EPA, 1993[c]). In a 2-generation rat reproduction study, decreased pup weight was observed in both generations at 40 mg/kg/day. The NOEL was 10 mg/kg/day (U.S. EPA, 1993[d])."

The rat teratology study is summarized by both IRIS (U.S. EPA, 1993d) and the Tox-Oneliner database (U.S. EPA, 1993c). The frequencies of fetal resorption and growth retardation were increased, and fetal weights were decreased at a dose of 300 mg/kg/day. No morphological abnormalities were noted at 300 mg/kg/day, and no other adverse developmental effects were noted at doses of 100 or 30 mg/kg/day.

In this instance, review by OEHHA of the original data cited by U.S. EPA (for purposes of response to public comments) revealed that the dose level selected as the LOEL for developmental toxicity (300 mg/kg/day), was also associated with a mortality rate of 60% among maternal animals (Stauffer, 1983). This extent of maternal toxicity is so excessive that evidence for concurrent developmental toxicity cannot be properly evaluated. Therefore, this study should not be used to make a listing determination.

The reproductive toxicity study is summarized by IRIS (U.S. EPA, 1993d), the results for adult toxicity serving as the basis for the oral RfD. Reduced pup weights were noted in both the F1 and F2 generations, at the highest concentration tested of 800 ppm (40 mg/kg/day).

In the course of reviewing public comments, OEHHA has also reviewed the original data for this study (Hazelton Laboratories, 1986), and notes that in the F1a generation, mean birth weight was significantly lower in the high dose (800 ppm) group than in controls, and pup weights for this group remained significantly depressed at all time points evaluated until weaning at postnatal day 21. In the F2a generation, mean birth weights for the high dose group were lower than controls (6.2 ± 0.92 and 6.9 ± 1.53, respectively), but this difference was not statistically significant. At all subsequent time points measured until weaning, pup weights of the high-dose group were significantly lower than those of controls.

It should be noted that, while U.S. EPA cites reproductive toxicity as the basis for addition to the TRI list, developmental toxicity is the appropriate basis for addition to the Proposition 65 list.

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 DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   Study a) rat teratology study - stated to be core grade minimum (U.S. EPA, 1985; U.S. EPA, 1997b). OEHHA considers this study to be confounded by excessive maternal mortality.
   Study b) rat 2-generation study - U.S. EPA (1997b) lists the study as core grade minimum.
2. **Route of administration:**
   Study a) oral gavage.
   Study b) oral, in diet.
3. **The frequency and duration of exposure:**
   Study a) each of gestation days 6-15.
   Study b) continuous, in diet.
4. **The numbers of test animals:**
   Study a) not stated. However, U.S. EPA test guidelines (1983a) require a minimum of 20 rats per dose group.
   Study b) 30 per sex in each dose group.
5. **The choice of species:**
   The rat is a standard species used in toxicology testing.
6. **The choice of dosage levels:**
   Study a) 0, 30, 100, 300 mg/kg/day.
   Study b) 0, 50, 200, 800 ppm (0, 2.5, 10, 40 mg/kg/day).
7. **Maternal toxicity:**
   Study a) maternal toxicity, manifested as increased mortality, reduced body weight gain, and reduced food consumption, occurred at the same dose as developmental toxicity. At the LOEL dose for developmental toxicity, maternal mortality reached a frequency of 60%.
   Study b) F0 females given 800 ppm EPTC demonstrated significantly reduced body weights prior to and during gestation; but their mean gestational weight gains, as well as feed consumption, were not different from controls. A similar pattern of effects was observed among F1a females, excepting that feed consumption at the high dose did show significant reductions on gestation day 7 (but not days 14 or 20) and over the interval of gestation days 0-20.

   At necropsy, microscopic evidence of cardiomyopathy was described as occurring in all rats fed 800 ppm EPTC. While this pathology is also reported among control animals, and stated to be "not uncommon" for rats of the strain used in this study, the frequency and severity among treated animals was taken to indicate an effect of treatment. A dose-response relationship was considered to have been demonstrated, with treatment-dependent effects also occurring at 200 ppm EPTC.

Quizalofop-ethyl (CAS No. 76578-14-8)

Male reproductive toxicity **has been manifested as testicular atrophy in dogs.**

The US Environmental Protection Agency (U.S. EPA, 1994a and 1994b) concluded that "…there is sufficient evidence for listing quizalofop-ethyl on EPCRA section 313 pursuant to EPCRA section 313(d)(2)(B) based on the available reproductive … toxicity data for this chemical."

Supporting documentation for the TRI listing (U.S. EPA, 1993b) states, "In a 6-month dietary dog study, 10 mg/kg/day produced testicular atrophy in males. The NOEL was 2.5 mg/kg/day."

U.S. EPA (1993b) cites the 1993 tox-oneliner for the dog study. OEHHA obtained a more recent version of the tox-oneliner on quizalofop-ethyl (U.S. EPA, 1996), which includes the Agency's previous assessment of the dog study.

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 DART effects appears to meet the criteria of 22 CCR 12306, and notes the following:

1. **Adequacy of the experimental design:**
   The 6-month feeding study conducted in dogs was considered to have met guideline requirements (U.S. EPA, 1996).
2. **Route of administration:**
   Oral, diet.
3. **The frequency and duration of exposure:**
   Continuous for 6 months.
4. **The numbers of test animals:**
   Not directly stated, but study was considered to meet guideline requirements.
5. **The choice of species:**
   Dogs are a standard species used in toxicology testing.
6. **The choice of dosage levels:**
   0, 25, 100, and 400 ppm in the diet.
7. **Maternal toxicity:**
   Not relevant.

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