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MEMORANDUM

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FROM: Anna M. Fan, Ph.D., Chief M Marty for A Fan
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DATE: July 22, 2013

SUBJECT: COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR CARBARYL

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the draft Risk Characterization Document (RCD) for occupational and ambient air exposure to carbaryl, prepared by the Department of Pesticide Regulation (DPR), dated July 12, 2012. Our comments are provided in the attachment. OEHHA is providing comments on the Exposure Assessment Document for Carbaryl separately. OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agriculture Code section 11454.1.

OEHHA has provided a number of comments on the risk characterization methodology and conclusions of the draft RCD. These comments and our recommendations, as well as suggested clarifications, additions and corrections, are contained in the attachment.

Thank you for providing this draft document for our review. If you have any questions regarding OEHHA's comments, please contact Dr. David Ting at (510) 622-3226 or me at (510) 622-3200.

Attachment

cc: David Ting, Ph.D., D.A.B.T.
Chief, Pesticide and Food Toxicology Section
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Office of Environmental Health Hazard Assessment

OEHHA's Comments on DPR's Draft
Risk Characterization Document for Carbaryl
(Occupational and Bystander Exposures)

The Office of Environmental Health Hazard Assessment (OEHHA) is responding to a request from the Department of Pesticide Regulation (DPR) to comment on the draft Risk Characterization Document (RCD) for carbaryl dated July 12, 2012.

OEHHA reviews risk assessments prepared by DPR under the authority of the Food and Agricultural Code Section 11454.1, which requires OEHHA to conduct scientific peer reviews of DPR risk assessments.

SUMMARY

The RCD was well-written and comprehensive in the presentation of the toxicological studies, analysis of weight of evidence, and approaches used to derive the critical endpoints and points of departure (PODs) for the calculation of margins of exposure (MOEs) and cancer risk. It included a thorough discussion on the uncertainty associated with endpoint and PODs selection and the associated impact on the risk values (MOEs and oncogenic risk). Overall, OEHHA concurs with the selection of the studies and endpoints as well as the extrapolation methods. The main concern is with the dose-response analysis of the critical studies.

- For assessing inhalation toxicity hazard, results from oral toxicity studies were used because of inadequacy in the inhalation toxicity database. OEHHA finds this approach scientifically appropriate.

- For assessing dermal toxicity, the same POD was used for all durations. This POD was derived from a subchronic dermal toxicity study. OEHHA concurs with this approach.

- For assessing acute oral and inhalation toxicity, the POD of 1 milligram per kilogram-day (mg/kg-day), based on observation of the pregnant rats in a developmental neurotoxicity study of Robinson and Broxup (1997), was selected. OEHHA has concerns with this choice because there is sufficient evidence to support a POD of less than 1 mg/kg-day for the calculation of acute oral and inhalation MOEs. OEHHA recommends additional data analysis of the clinical signs in this study, and of mortality in pup dogs in a developmental toxicity study reported by Immings et al. (1969).

- Regarding carcinogenicity:
  - The RCD identified carbaryl as a carcinogen because carbaryl induces a number of different tumors in rat and mouse cancer bioassays. OEHHA agrees with the carcinogenicity identification.
  - The combined incidence data for hemangiomas and hemangiosarcomas in male mice were selected for cancer dose-response analysis using the multistage cancer model. OEHHA finds these approaches appropriate.
  - For determining acceptable risk, the RCD used a benchmark probability of cancer of one in a million, as is used by OEHHA.

- Regarding early age susceptibility:
  - For cancer, the RCD did not account for potential heightened sensitivity early in life from exposure to carcinogens. OEHHA recommends the incorporation of age-sensitivity factors (ASFs) to account for increased risk of cancer due to exposure to carbaryl during childhood.
  - For developmental toxicity, a POD was not determined for pup mortality observed in the study with dogs (Immings et al., 1969). A lowest-observed-effect level (LOEL) of 2 mg/kg-day was established. OEHHA is concerned about this endpoint and that a POD, when determined, may be lower than the POD selected for MOE calculations. If the data do not support a POD determination, an additional uncertainty factor should be considered to determine acceptable exposure.

- Regarding non-cancer dose response analysis:
  - The PODs, when derived from benchmark dose (BMD) analysis and used in the MOE calculations, were based on a benchmark response (BMR) of ten percent. OEHHA recommends the use of five percent as the BMR, especially for endpoints related to effects in the brain.
  - The risk of non-cancer effect from exposure was evaluated using the MOE approach and the benchmark was a value of 100 to determine acceptability of exposure. OEHHA agrees with these approaches.

The exposure assessment section of this draft RCD generally reflects the information from the draft Exposure Assessment Document (EAD). The OEHHA review of the draft EAD is provided in a separate memo.

GENERAL COMMENTS

The RCD addressed the following scenarios:

- Workers - inhalation and dermal exposures from agricultural and residential uses;

- Bystanders - inhalation exposure from agricultural and public pest control uses;
- Residents - dermal, inhalation, and oral (toddlers only) exposure from lawn care uses;
- Swimmers - oral and dermal exposures in contaminated water;
- Ambient air- inhalation

The RCD for dietary exposure to carbaryl (DPR, 2010[1]) was previously reviewed by OEHHA (January 29, 2009; OEHHA 2009a). In that review, OEHHA agreed with DPR's choice of critical studies and toxicological endpoints as the basis for all carbaryl risk calculations with one exception, the acute POD of 1 mg/kg-day based on decreased body weight gain and increased cholinergic signs. OEHHA recommended that DPR perform BMD extrapolation with several time points for change in gait and other endpoints such as pinpoint pupils or cholinesterase measures, and then decide on the appropriate POD for the evaluation of acute exposures. For the current draft RCD for occupational and residential exposures, OEHHA remains concerned about the selection of 1 mg/kg-day as the POD. This concern is explained further in this review.

For the endpoints analyzed by BMD in the draft RCD, all BMR were set primarily at 10 percent for both quantal and continuous data. The use of a 10 percent BMR for brain cholinesterase (ChE) inhibition was justified by the lack of overt clinical signs and histopathology, as discussed in a chronic toxicity study review (page 112). While DPR suggested that a lower BMR of 5 percent may be more appropriate for cholinergic signs (page 146), the modeling result using this BMR was not used in the calculation of the MOEs. OEHHA supports the consideration of 5 percent as the BMR for this endpoint. OEHHA typically uses a 5 percent BMR for the dose-response analysis of quantal data (OEHHA, 2008).

SPECIFIC COMMENTS

The following sections present OEHHA's comments relating to specific exposure routes and types of toxicity.

Inhalation Toxicity (Acute, Subchronic, and Chronic)

The draft RCD selected the critical no-observed-effect levels (NOELs) from oral studies to address inhalation exposure to carbaryl due to insufficient inhalation toxicology information. There were no subchronic or chronic inhalation toxicity studies. There was only one inhalation toxicity study with rats exposed to carbaryl for three hours at air concentrations ranging from 10 to 65 milligrams per cubic meters (mg/m^3) (page 40)

[1] The draft RCD for dietary exposure was reviewed by OEHHA in 2009. The draft was finalized in 2010.

(Weinberg, 2008). The lower-bound effective dose for BMR of 10% (LED_{10}) for brain cholinesterase inhibition was 9.81 mg/m^3 (equivalent of 1.18 mg/kg using a DPR default rat inhalation rate of 0.96 cubic meters per kilogram-day (m^3/kg-day). This study was considered inadequate because the rats were exposed for only 3 hours instead of 4 hours as recommended under U.S. Environmental Protection Agency (USEPA) guidelines. DPR used acute oral toxicity data to evaluate acute inhalation exposure and chronic oral data to evaluate subchronic and chronic inhalation exposures.

OEHHA concurs with the reasons stated for the use of oral toxicity studies for assessing inhalation toxicity in this case.

Dermal Toxicity (Acute, Subchronic, and Chronic)

This draft RCD selected a 4-week dermal study in rats (Austin, 2002a) as the critical study. It identified a LOEL of 50 mg/kg-day based on the observed inhibition of brain and red-blood cell (RBC) cholinesterase activities and a NOEL of 20 mg/kg-day. Among the dermal studies on systemic toxicity, it was the only study that was considered adequate, and was used for the evaluation of acute, subchronic, and chronic dermal exposures.

OEHHA concurs with use of the critical subchronic study to address acute, subchronic and chronic dermal exposure to carbaryl. OEHHA suggests using BMD analysis to better characterize the POD for MOE calculation.

Acute Oral Toxicity

For the evaluation of acute oral and inhalation exposures, DPR identified a POD of 1 mg/kg-day based on weight gain deficits, cholinergic signs, and brain and RBC ChE inhibition observed in pregnant rats at a LOEL of 10 mg/kg-day and a NOEL of 1 mg/kg-day from a neurodevelopmental rat study by Robinson and Broxup (1997). The draft RCD also determined an alternative POD of 0.25 mg/kg-day for slight hypotonic gait incidence data gathered during gestation. This value was derived from BMD modeling of the same study that resulted in a LED_{10} of 0.25 mg/kg-day (page108).

OEHHA has concerns about (1) the selection of 1 mg/kg-day as the POD and (2) the approach used to determine the LED_{10} of 0.25 mg/kg-day.

<u>(1) Selection of 1 mg/kg-day as the POD</u>

DPR chose to use the NOEL (1mg/kg-day) over the LED_{10} (0.25 mg/kg) as the POD to calculate the MOEs because according to their evaluation there is a stronger experimental support for the former value (page 112). In addition, the level was supported by the three additional acute oral gavage studies from the same laboratory (Brooks and Broxup, 1995a, b; Brooks et al., 1995). Each of these studies established cholinergic LOELs at 10 mg/kg-day, but did not establish NOELs (page 144). Further support came from:

a) *"The rat acute gavage study of Moser (2007) which established an LED_{10} of 1.1 mg/kg based on brain ChE inhibition (as well as a second LED_{10} of 0.78 mg/kg based on RBC ChE inhibition) in postnatal day 11 animals; and*
b) *The acute inhalation toxicity study of Weinberg (2008), which established an LED_{10} of 1.18 mg/kg based on brain ChE inhibition."*

OEHHA suggests that DPR reconsider the selection of 1 mg/kg-day as the POD because additional data analysis is needed. The calculation of MOEs should be based on the most sensitive endpoint, represented by the lowest POD, which is 0.25 mg/kg-day in this draft of the RCD. PODs need to be determined for the three cited oral gavage studies with only LOELs established, for comparison. Brain and RBC ChE inhibition data should be analyzed using additional, possibly lower, BMRs, instead of a BMR of 10 percent. In addition, the acute inhalation toxicity study of Weinberg (2008) was not properly conducted, as stated in the draft RCD, and therefore this study should not be used to support a POD of 1 mg/kg-day.

(2) Approach used to determine the LED of 0.25 mg/kg-day

In the draft RCD, the value of 0.25 mg/kg-day, as an alternative POD, was obtained by using normalized mean incidence rate for hypotonic gait (Table III-16a, page 99). The following reason was provided for combining the gestational data set from gestation day 6 (GD 6) to gestation day 20 (GD 20):

> *"Because of carbaryl's propensity for clearance from the rat system in less than 24 hours (Struble, 1994) and the relatively rapid decarbamylation reaction (t0.5 = 40 min (cf., Cranmer, 1985)), all of the FOB (Functional Observational Battery) tests conducted during the gestation period were considered to represent separate, but equivalent, acute scenarios. Consequently, the gestational data sets were combined to generate a normalized mean incidence rate, as noted in Table IV-1. Use of mean data in the BMD analysis was preferable to use of data from any day in isolation, as it minimized the random fluctuations noted in single day tests (p. 110)."*

OEHHA notes that the draft RCD also stated that this approach added uncertainty "*because it implied that data from single test days represented fluctuations around a*

mean…it remained possible that it underestimated the sensitivity of the system (page 146)." OEHHA finds no basis for this assertion of random fluctuation and justification for the use of the average incidence. OEHHA agrees with the latter point that the use of the mean incidence rates would underestimate the toxicity of carbaryl on certain days of exposure. Examination of the data in Table III-16a (pages 99 to 100) showed consistent increased incidences of hypotonic gait for three consecutive observation days (GD 12, 15, and 18) for the 1 and 10 mg/kg-day groups. This finding of multiple occurrences increases the significance of the observed clinical sign.

The draft RCD stated the following uncertainty surrounding the 0.25 mg/kg LED_{10} determination (page 145-146):

1. "*The low level of statistical verification of the effect emphasized the possibility that slight hypotonic gait was not a response to carbaryl exposure, at least at 1 mg/kg.*"…" *In six FOB tests conducted during gestation and five conducted within 21days of the end of gestation, statistical significance with respect to controls was achieved only once at 1 mg/kg (GD 12; p<0.05) and once at 10 mg/kg (GD 18; p<0.01). In fact, the statistical significance observed at 1 mg/kg on GD 12 was not supported by an equivalent statistically significant response at 10 mg/kg on the same day.*"
2. "*…The timing of the slight hypotonic gait effect might not be consistent with a classically acute response, if defined as occurring as a result of a single dose.*"…"*An effect of dosing on slight hypotonic gait may not have appeared until GD 9 (i.e., after four applications) or GD 12, when a statistically significant increase was noted at 1 mg/kg. No effect was discernable at 1 mg/kg on GD 6.*"
3. The FOB parameters being used for this evaluation are "*classified by the investigators as "slight" responses ("slight hypotonic gait", "slight ataxic gait", "slight tremors"). This emphasized the subjectivity of the data, since a judgment of "slight" in the hands of one observer either may not have sufficed for a notification or been classified as moderate in the eyes of another evaluator.*"
4. There were uncertainties inherent in the BMD approach such as: (1) Choice of "*benchmark response level of 10% since it was not known if slight hypotonic gait comprised a centrally or peripherally-based response. If centrally-based, for example, the risk represented by slight hypotonic gait might be better characterized by a benchmark response level of 5% rather than 10%; (2) The decision to delete the top dose, which was made in order to generate a curve of appropriate fit added uncertainty since it ignored actual data gathered in the*

experiment; (3) The choice of the probit function over other algorithms added uncertainty because each algorithm generated different LED_{10} and ED_{10} values; and (4) The decision to model the data using normalized mean incidence rates, which was a consequence of considering all of the FOB tests to be acute in nature, added uncertainty because it implied that data from single test days represented fluctuations around a mean. While this was considered the more likely scenario, it remained possible that it underestimated the sensitivity of the system."

OEHHA believes that for the evaluation of acute oral and inhalation exposures, a single-day exposure is relevant and would argue against taking an average of the data collected over six different gestation days (point #2). Effects observed on GD9 and GD12 but not on GD6 may be explained by a higher sensitivity after GD6.

On the concern over the validity of hypotonic gait observation (point #3), this observation was supported by data showing other clinical signs such as pinpoint pupil and slight tremor, which may also be due to effects on the central nervous system, or cholinesterase inhibitory effect on the neuromuscular system. OEHHA recommends additional analysis of data for multiple clinical signs, as presented in Table III-16a (page 100 under "Signs") of the draft RCD.

OEHHA does not agree that each BMD model generating different LED_{10} and effective dose at BMR of 10% (ED_{10}) values adds additional uncertainty (point #4). The best fit model, both statistically and by visual inspection of the graph, should provide the most appropriate POD. The inclusion of results from other models (e.g., other curves or BMR values) would have been helpful for this review.

Thus, OEHHA believes that there is sufficient evidence presented in the draft RCD to support a POD lower than 1 mg/kg to evaluate hazards from acute exposures to carbaryl. OEHHA recommends additional analysis of the data for hypotonic gait and other multiple signs (Robinson and Broxup, 1997) and evaluating the observation for each gestation day independently. For this study and other studies, alternate BMRs other than the default 10 percent (for example, 5 percent as suggested in Point #4) should be considered before selecting the appropriate POD to address acute oral toxicity and as a surrogate value for acute inhalation toxicity.

Subchronic Oral Toxicity

OEHHA notes that the subchronic oral exposure to carbaryl was evaluated by DPR using the critical chronic oral LED_{10} value of 0.5 mg/kg-day for brain ChE inhibition (Hamada, 1987) from the one-year chronic dietary dog study (pages 97, 112). The only subchronic dietary study available (Hamada 1991) had a NOEL (highest dose tested) of

3.83 mg/kg-day (5-week, page 44). According to the draft RCD, this value is considerably higher than the critical acute oral values (1 mg/kg or 0.25 mg/kg) and DPR considered it prudent to base the seasonal risk estimation on a value closer to the acute values.

OEHHA agrees with the use of the chronic oral toxicity study to address subchronic oral exposure, but suggests DPR consider OEHHA's recommendations to use 5 percent BMR for BMD analysis.

Chronic Oral Toxicity

The POD determined for chronic oral exposure was 0.5 mg/kg-day. It was derived from an LED_{10} of 0.5 mg/kg-day based on the brain ChE inhibition reported in a one-year chronic dietary dog study (Hamada, 1987). This value was used by DPR to evaluate the non-cancer risk from annual (i.e., chronic) oral exposure. OEHHA (2009a) has previously provided comments on the chronic oral risk assessment for the use of this LED_{10} value based on the one-year dog study (Hamada, 1987).

There were 3 other chronic/oncogenicity dietary studies with higher NOEL values:

(1) Rat 2-year chronic/oncogenicity study by Hamada (1993a) (NOEL: 10.0-12.6 mg/kg-day based on inhibition of brain AChE and reduced female weight gain)
(2) Mouse 2-year chronic/oncogenicity study by Hamada (1993b) (NOEL: (Male) 14.73 mg/kg-day; (Female) 18.11 mg/kg-day for presence of intracytoplasmic droplets/pigment in the bladders of both sexes, and inhibition of brain and RBC ChE), and
(3) Mouse 180-day oncogenicity study by Chuzel (1999) (NOEL: 5.2 mg/kg-day for globular deposits in the umbrella cell layer of urinary bladder).

OEHHA agrees with the use of the selected toxicity data to address chronic oral exposure, but suggests DPR consider OEHHA's recommendations to use BMR of 5 percent for BMD analysis.

Genotoxicity

In the draft RCD, the genotoxicity database was presented as a summary and in Table III-II (pages 70-71). The reader was referred to DPR's RCD for dietary exposure (DPR, 2010) for more detailed information. Carbaryl was considered a potential genotoxic compound because of positive results for genotoxicity in one of five gene mutation studies, four of six chromosomal aberrations studies and two of four DNA damages studies (page 114). Two metabolites of carbaryl, nitrocarbaryl and 1-naphthol, were

reported to show some genotoxic activity (page 114). No new data were presented in this draft RCD.

OEHHA concurs with DPR's determination that carbaryl is potentially genotoxic. This section should be updated with new information, if any, relevant to the genotoxicity of carbaryl.

Oncogenicity

DPR concluded that carbaryl was carcinogenic because it induced a number of different tumors in rat and mouse cancer bioassays. This conclusion was based on the same database as that presented in the dietary RCD. The potency was estimated using incidences of hemangiomas and hemangiosarcomas in male mice after dietary exposure to carbaryl for two years (Hamada, 1993b; Table III-8c, page 63). The database presented was the same as that in the dietary RCD (DPR, 2010). There was no new oncogenicity information.

In the review of the previous dietary RCD (DPR, 2010), OEHHA (2009a) had concurred with DPR's conclusion that carbaryl is a potential human carcinogen. OEHHA supported the use of the mouse tumor data (Hamada, 1993b) for potency calculation and the cancer potency factor based on the animal doses using the quantal linear model reported in the document. In this draft RCD, the cancer potency factor was recalculated using human equivalent doses in the multistage-cancer model. While OEHHA agrees with the approach, there appeared to be an error in the conversion factor used for converting mouse "internal" doses of 0, 14.73, 145.99 and 1248.93 mg/kg-day to human doses of 0, 2.12, 21.02 and 179.85 mg/kg-day (page 116). According to the equation on page 116, a conversion factor of 0.153 should have been used. However, a factor of 0.144 was actually used instead (e.g., 2.12/14.73 = 0.144). If the latter conversion factor value is correct, the reported human oncogenic potency of $1x10^{-2}$ mg/kg-day^{-1} and oncogenic risk estimates in the draft RCD will have to be revised. In addition, the use of the adjective "internal" to describe the doses may not be appropriate, unless the internal dose of carbaryl were actually measured or estimated by modeling. On page 58, these same doses were referred to as "systemic" doses, corresponding to the concentrations in the diet. There was no explanation how the part- per-million (ppm) values were converted to these dosage terms. It would be helpful to clarify this issue.

Reproductive and Developmental Toxicity

The draft RCD had an extensive discussion of the reproductive and developmental toxicity studies of carbaryl. In the Reproductive Toxicity section (pages 73 to 78), human epidemiologic studies conducted with carbaryl workers in factory and those

exposed as a result of carbaryl use in farms were described. The results showed that carbaryl exposure was associated with increased spermatogenic toxicity (Wyrobek et al., 1981), sperm chromosomal aberrations and DNA damage (Xia et al. 2005), and miscarriages (Savitz et al., 1997). However, the exposure levels of the workers were not reported by the studies. This section also discussed the results of a 2-generation reproductive toxicity study with rats and repeated exposure studies conducted with rats and gerbil (pages 78 to 87). As summarized in Table III-13 (page 88), reproductive toxicity of carbaryl at the LOEL included: increased second generation (F_2) pup mortality (Tyl et al., 2001), decreased sperm motility (Shtenberg and Rybakova, 1968), changes in testicular enzyme levels, sperm and testicular histopathology (Pant et al., 1995), sperm abnormalities (decreased sperm count and motility, abnormal sperm) (Pant et al., 1996), and decreased number of liveborn pups, pup survival, and weaning weight (Collins et al., 1971). The lowest NOEL was less than 7 mg/kg-day for effects in sperm (Shtenberg and Rybakova, 1968).

The Developmental Toxicity section described studies with results on developmental toxicity in several laboratory animals (rats, rabbits, mice, and beagle dogs). As summarized in Table III-15 (page 96), developmental toxicity of carbaryl included: reduced fetal body weights (Murray et al., 1979; Repetto-Larsay, 1998; Tyl et al., 1999), delayed ossification (Repetto-Larsay, 1998), teratogenic abnormalities (Smalley et al., 1968), and increased stillbirths (Immings et al., 1969). The lowest NOEL was less than 2 mg/kg-day for increased stillbirths in a study conducted with dogs (Immings et al., 1969).

Based on results from the reviewed studies, the draft RCD concluded that exposure to carbaryl can potentially lead to reproductive and developmental toxicity (page 156). OEHHA concurs with this conclusion.

In the context of comparison of PODs to address acute oral toxicity, the RCD discussed the results of the two developmental toxicity studies conducted with dogs (Immings et al., 1969; Smalley et al., 1968) (page 111). In the Smalley et al. study (1968), pregnant beagle dogs were exposed to carbaryl in the diet from GD 3 to parturition (GD 62). Increased incidences of pups with various teratogenic abnormalities (abdominal-thoracic fissures, branchygnathia, ecaudate pups, failure of skeletal formation, failure of liver development, and superfluous phalanges) were reported. The LOEL and NOEL were 6.25 mg/kg-day and 3.12 mg/kg-day, respectively.

No developmental toxicity NOEL was established for the second developmental toxicity study conducted in dogs (Immings et al., 1969). Pregnant beagle dogs were given carbaryl (0, 2, 5 or 12.5 mg/kg-day) in the diet from GD 1, continuing through weaning at 6 weeks of age. No treatment-related maternal effects were observed and the maternal NOEL was established at greater than 12.5 mg/kg-day. For the pups, the

significant findings included increased stillborn and pup death (Table III-14). The LOEL was established at 2 mg/kg-day for non-statistically significant increase in stillbirths at that dose. The draft RCD noted that "on a per-litter basis, statistical significance was achieved only at the mid dose, though the incidences at the low and high doses were suggestive of an effect." Furthermore, increased pup death (on per pup basis) at weaning was statistically significant for all doses (page 95). At 2 mg/kg-day, the percentages of pup deaths were 36 to 40 percent, compared to 12 percent for the control.

OEHHA supports DPR's consideration of results from the dog studies for POD determination. However, OEHHA suggests that a POD also be determined for increased pup mortality from the Immings et al. (1969) study. This POD would be compared to other PODs of the same exposure route and duration to insure that the selected POD for MOE calculations would be protective of the reproductive and developmental toxicity from carbaryl exposure.

Developmental Neurotoxicity

In the developmental neurotoxicity study conducted by Robinson and Broxup (1997), pregnant Sprague-Dawley rats were given carbaryl by gavage at 0, 0.1, 1, or 10 mg/kg-day from GD 6 to postpartum day 10 (pages 97-100). In the dams, decreased body weight gain, alterations in FOB measures, and inhibition of plasma, RBC and brain cholinesterase activity were seen at the 10 mg/kg-day dose level. In the offspring, alterations in brain morphometric measurements were observed in the 10 mg/kg-day dose group (page 98). DPR considered the changes in brain morphometry observed in the offspring inconsistent in degree and direction, and therefore established 10 mg/kg-day as the NOEL for developmental effects.

OEHHA disagrees with DPR's analysis of the brain morphometry data. Because of the morphological and physiological differences, different brain regions or different genders should not be expected to react to a toxicant in the same degree or direction. The bilateral decrease in the size of the forebrain (line A) in first generation (F_1) male adults and the bilateral decrease in cerebellar length (line F) in F_1 female pups are indicative of adverse effects of carbaryl on brain development. The appropriate LOEL is 10 mg/kg-day for the toxicity in the offspring, and the appropriate NOEL is 1 mg/kg-day. USEPA also identified 10 mg/kg-day as a LOAEL based on alterations in brain morphometric measurements in the Health Effects Division Chapter of the Reregistration Eligibility Decision Document for carbaryl (USEPA, 2007).

Pre- and Post-natal Sensitivity

DPR did not apply any additional uncertainty factor for potential increased sensitivity of infants and children to carbaryl in the previous dietary assessment (DPR, 2010) or this draft non-dietary risk assessment. In both documents, the rationale given was because the acute critical NOEL of 1 mg/kg-day was similar in magnitude to the POD of 1.1 mg/kg-day developed by USEPA (page 156). The USEPA POD was based on brain cholinesterase inhibition of young rats (postnatal day 11 rats) (Moser, 2007), and thus the sensitivity of young children had been taken into account (USEPA, 2007). However, this draft RCD appeared to suggest that an additional UF is needed. On page 158, it was stated that "*Based on the analysis provided in DPR's dietary assessment of carbaryl (DPR 2010), which showed MOEs of less than 100 for three infant or age 1-2 year subpopulations, it appeared that the extra 10-fold factor should be considered (page 158).*"

In the review of the dietary RCD (DPR, 2010), OEHHA supported DPR's decision not to apply an additional uncertainty factor to the MOE threshold of 100 for determining acceptable exposure, but recommended further investigation of the developmental effects observed in other studies, particularly in the dog (OEHHA, 2009a). In this draft RCD for non-dietary exposure, the results of the dog studies (Smalley et al., 1968 and Immings et al., 1969) were stated to indicate a potential developmental risk (page 156). While a NOEL was established for Smalley et al (1968), only a LOEL (2 mg/kg-day) was established for the Immings et al. (1969) study. OEHHA remains concerned that the PODs selected to calculate the MOEs may not be protective against the fetal mortality observed in the dog study (Immings et al., 1969). If a POD cannot be determined from the reported data in Immings et al (1969), then an additional uncertainty factor may need to be considered to account for the fact that the study only established a LOEL.

Aggregate Exposure Toxicity

The aggregate (multi-route) non-cancer health hazard was evaluated for occupational and residential exposures with the addition of dietary exposures. The only exception is that dietary exposure was not added to the inhalation exposure of the bystanders. The adult bystander exposure was used as the surrogate to represent the upper bound value of the general public's exposure to ambient air concentrations of carbaryl (page 143). For each exposure duration (acute and chronic), the total MOE was calculated using the reciprocal of the sum of the reciprocals of the dermal, inhalation, and oral MOE values:

$$MOE_{total} = 1 / [(1/MOE_{dermal})+(1/MOE_{inhalation})+(1/MOE_{oral})]$$

Since the endpoints for the PODs were all based on cholinesterase inhibition, OEHHA considers the use of the total MOE equation appropriate. However, rationale should be provided as to why dietary exposure was not considered in the aggregate exposure for adult bystanders, when this human receptor is used to represent the general public.

Dietary exposure was also not included in the estimation of oncogenic risk for all human receptors. The aggregate oncogenic risk was calculated only for the handlers, and it was the sum of risks from dermal and inhalation exposures (pages 132-134). The draft RCD stated that dietary risk was not added because the risk of 2.9×10^{-6} already exceeds the acceptable level of 10^{-6} (page 134, Table IV-7a, footnote d).

OEHHA agrees with using the total cancer risk approach to calculate aggregate lifetime exposures. However, the decision of whether to include the dietary component for the lifetime aggregate risk should be based on the probability of lifetime exposure to carbaryl from multiple exposure pathways, rather than by the magnitude of the risk ($> 10^{-6}$) by the dietary route.

Margins of Exposure and Oncogenic Risk

For non-cancer hazard, the calculated MOEs for a single route and aggregate exposures were compared to a value of 100, which DPR considered to be protective of human health. This value reflected the default assumptions that (1) humans could be 10-fold more sensitive than animals and (2) that a 10-fold range of sensitivity exists within a human population (page 130).

OEHHA concurs with the use of a MOE of 100 as the threshold to evaluate non-cancer health risks, pending the additional consideration of the fetal mortality data in the dog study (discussed under Pre- and Post-natal Sensitivity in this document).

For cancer risk, DPR calculated lifetime exposures only for workers (dermal and inhalation routes) and for adult bystanders (inhalation route). DPR used a probability of one in a million for evaluating cancer risk. OEHHA concurs with the use of this level to evaluate cancer risk. However, OEHHA recommends that cancer risk should be calculated for the general population exposed to carbaryl in the ambient air. While ambient air concentration was not calculated in the EAD, the EAD indicated that the bystander exposure level could be used as an upper bound for an ambient air exposure level. OEHHA recommends that the lifetime exposure to carbaryl should include vulnerable periods such as third trimester in utero and early childhood. For this reason, OEHHA suggests the use of age-specific inhalation rates and ASFs (OEHHA, 2009b) in the calculation of lifetime inhalation exposure and cancer risk, respectively.

Editorial Comments

A list of tables would be helpful for the readers.

The information about the different exposure scenarios addressed in the draft RCD is not consistent with the actual scenarios evaluated in the document, and it needs to be revised. In various sections in the document, different scenarios were indicated, for example: 1) the title page indicates occupational and bystander exposures are addressed; 2) the Introduction section on page 119 listed occupational, bystander, and ambient scenarios; and 3) the second paragraph of the Risk Appraisal section (page 144) stated that the dietary exposure of both workers and the general public to carbaryl would be described.

Summary and Hazard Identification sections: It would be helpful to have a table summarizing the critical studies, PODs and endpoints used for the MOE and oncogenic risk calculations in this section instead of at the end of the document (Table VII-1, page 164) as part of the reference dose and concentration presentation.

Page 41, Section III, Toxicology Profile: The summary table for acute toxicity is missing. A summary table for subchronic toxicity and chronic toxicity study was provided on page 69.

Page 42, Paragraph 1: According to paragraph 1, "Subchronic NOELs and LOELs are summarized in Table III-8." These values are actually summarized in Table III-10.

Page 43: The data in Moser (2007) have been published in Toxicological Sciences 114 (1):113-123 (2010). The results, if necessary, and the citation in the RCD should be updated.

Pages 45 and 69: According to the text, the systemic NOEL for the subchronic dermal Austin (2002a) study is 20 mg/kg-day. However, Table III-10 lists the systemic NOEL as 100 mg/kg-day. This inconsistency should be addressed.

Page 48, Paragraph 1: According to paragraph 1, "Chronic NOELs and LOELs are summarized in Table III-9." These values are actually summarized in Table III-10.

Page 87, Paragraph 6: Space should be provided between the title for Table III-13, "NOEL and LOEL values in laboratory animal studies on the reproductive toxicity of carbaryl" and the statement above so that the title appears on the top of the table.

Page 95: In the table, superscripts 3 and 4 should be c and d.

Page 98, Paragraph 3, the paragraph below "**F1 adults.** There were no clear effects of carbaryl in the F_1 adults.", which begins with, "The LOEL determination for maternal effects....At 10 mg/kg, very clear body weight gain decrements, RBC and brain cholinesterase inhibition..." appears to be referring to F_0 adults instead of F_1 adults because there was "no clear effects of carbaryl in the F1 adult" as mentioned above. Therefore, there should be a space between the description of F1 adult and the following paragraph, which should be titled: Conclusion.

Pages 99-100 in Table III-16a: There seems to be an error in the incidence for "Signs" for the gd20 1.0 mg/kg group. It is lower (10/26) than that (11/26) for hypotonic gait alone. The incidence for "Signs" should be the same or higher since it represents the number of animals with one or more signs.

Page 100, footnote d: The incidences appeared to be for animals with one or more signs, not only those with "more than one sign" as indicated in this footnote.

Page 111, Paragraph 3, line 2: Hamada reference should be "Hamada 1987" instead of "Hamada 1997".

Page 116, the 2nd paragraph "...*Fourteen separate algorithms available in the USEPA version 1.3.2 benchmark dose...were compared as potential models for the male vascular tumor data...*": This paragraph was in the dietary RCD; it should be reviewed to see if it is still applicable for this RCD. As shown in Appendix III, the slope factor was calculated using the multistage cancer model from a more recent version (version 1.9) of the software. Multistage cancer model is the only appropriate model for the determination of potency. On this page and elsewhere, instead of "algorithm", the conventional term is "model" in reference to the statistical models in the BMD software.

Page 117: Paragraph 2, line 2: Typographical error, "adquate" should be "adequate".

Page 131, Paragraph 1, line 2: This line reads, ""hazard index" approach (footnote c, Table xxxg)." Please clarify which table is being referred to by Table xxxg. Also, location of Table xxxh on line 11 needs to be identified.

Pages 131-139: Mislabeling of the tables: xxxg, xxxh, xxxc.

Page 134, footnote c: The equation used to calculate the aggregate MOE is referred to as "Total MOE," not "Hazard Index" as written in the footnote.

Page 134, footnote d: Aggregate oncogenic risk was the "sum", not "product" as written in footnote.

Page 158, Paragraph 4, line 3: Which table is being referred to by "Table xxxl".

Page 162, Footnote 18: the units are wrong: "m^3/mg/hr" should be "m^3/kg/hr".

Page 179 (Appendix I): The description below the Probit Model stated, "Slight hypotonic gait data in males (top dose deleted)". This note appears to be a typographical error since the title of this Appendix I, "Benchmark dose extrapolation for induction of slight hypotonic gait in pregnant Sprague-Dawley rat" is for females (not male).

References: The list needs to be checked. Dange (1998) is not in the list. Some of the references listed were not cited in the RCD (for example: Bronzon and Jones, 1989).

REFERENCES

Austin, E.W. (2002a) (Covance Laboratories Inc.). 4 week repeated-dose dermal toxicity study with carbaryl technical in rats. Lab. Study #Covance 6224-268; DPR Vol. #169-413, Rec. #186206.

Brooks, W. and Broxup, B. (1995a) (Bio-Research Laboratories Ltd.). A time of peak effects study of a single orally administered dose of carbaryl, technical grade, in rats. Lab. project #97388. DPR Vol. #169-338, Rec. #142593.

Brooks, W. and Broxup, B. (1995b) (Bio-Research Laboratories Ltd.). An acute study of the time course of cholinesterase inhibition by orally administered carbaryl, technical grade, in rats. Lab. project #97392. DPR Vol. #169-340, Rec. #142600.

Brooks, W., Robinson, K. and Broxup, B. (1995) (Bio-Research Laboratories Ltd.). An acute study of the potential effects of a single orally administered dose of carbaryl, technical grade, on behavior and neuromorphology in rats. Lab. project #97389. DPR Vol. #169-341, Rec. #142602.

Chuzel, F. (1999) (Rhone-Poulenc Agro). Carbaryl: 6-month carcinogenicity study in p53 knockout mice by dietary administration. Study #SA 98155; DPR Vol. #169-398, Rec. #177755.

Collins, T.F., Hansen, W.H. and Keeler, H.V. (1971) The effect of carbaryl (Sevin) on reproduction of the rat and the gerbil. Toxicol Appl Pharmacol. 19(2), 202-216.

Cranmer, M.F. (1986) Carbaryl: a toxicological review and risk analysis. NeuroToxicology 7:247-332; DPR Vol. #169-148, Rec #46798.

DPR (2010) Carbaryl (1-naphthyl methyl carbamate): Dietary Risk Characterization Document (principal author: A.L. Rubin). Medical Toxicology Branch, Department of Pesticide Regulation, California Environmental Protection Agency.

Hamada, N.H. (1987) (Hazleton Laboratories America). One-year oral toxicity study in beagle dogs with carbaryl technical. Project ID #400-715; DPR Vol. #169-169, Rec. #056429.

Hamada, N.H. (1991) (Hazleton Laboratories America). Subchronic toxicity study in dogs with carbaryl technical. Project ID #656-152; DPR Vol. #169-239, Rec. #98146.

Hamada, N.H. (1993a) (Hazleton Laboratories America). Combined chronic toxicity and oncogenicity study with carbaryl technical in Sprague-Dawley rats. Project ID #656-139; DPR Vol. #169-271, Rec. #126241.
Hamada, N.H. (1993b) (Hazleton Laboratories America). Oncogenicity study with carbaryl technical in CD-1 mice. Project ID #656-138; DPR Vol. #169-267, Rec. #123769.

Immings, R.J., Shaffer, B. and Woodard, G.W.R.C. (1969) Sevin - Safety evaluation by feeding to female beagles from day one of gestation through weaning of the offspring. No project ID #; DPR Vol. #169-099.

Moser, V. (2007) Report on cholinesterase comparative sensitivity study of carbaryl. USEPA. MRID #47007001.

Murray, F.J., Staples, R.E. and Schwetz, B.A. (1979) Teratogenic potential of carbaryl given to rabbits and mice by gavage or by dietary inclusion. Toxicol Appl Pharmacol 51(1), 81-89.

OEHHA (2008) Air Toxics Hot Spots Risk Assessment Guidelines. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA (2009a) Comments on the Draft Carbaryl Dietary Risk Characterization Document. January 29, 2009. Memorandum from A.M. Fan to G.T. Patterson. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA (2009b) Air Toxics Hot Spots Risk Assessment Guidelines. Technical Support Document for Cancer Potency Factors: Methodologies for derivation, listing of available

values, and adjustments to allow for early life stage exposures. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Pant, N., Srivastava, S.C., Prasad, A.K., Shankar, R. and Srivastava, S.P. (1995) Effects of carbaryl on the rat's male reproductive system. Vet Hum Toxicol 37(5), 421-425.

Pant, N., Shankar, R. and Srivastava, S.P. (1996) Spermatotoxic effects of carbaryl in rats. Hum Exp Toxicol 15(9), 736-738.

Repetto-Larsay, M. (1998) (Rhone-Poulenc Agro) Carbaryl: developmental toxicology study in the rat by gavage. Study #SA 98070. DPR Vol. #169-383, Rec. #166125.

Robinson, K. and Broxup, B. (1997) (ClinTrials BioResearch Ltd.) A developmental neurotoxicity study of orally administered carbaryl, technical grade, in the rat. Lab. project #97391. DPR Vol. #169-384. Rec. #166126.

Savitz, D.A., Arbuckle, T., Kaczor, D. and Curtis, K.M. (1997) Male pesticide exposure and pregnancy outcome. American Journal of Epidemiology 146(12), 1025-1036.

Shtenberg, A.I. and Rybakova, M.N. (1968) Effect of carbaryl on the neuroendocrine system of rats. Food Cosmet Toxicol 6(4), 461-467.

Smalley, H.E., Curtis, J.M. and Earl, F.L. (1968) Teratogenic action of carbaryl in beagle dogs. Toxicol Appl Pharmacol. 13(3), 392-403.

Struble, C.S. (1994) (Hazleton Wisconsin, Inc.). Metabolism of 14C-carbaryl in rats (preliminary and definitive phases). Lab. Project #HWI 6224-184; PR-Ag study #EC-92-222. DPR Vol. #169-453, Rec. #209656.

Tyl, R.W., Marr, M.C. and Myers, C.B. (1999) (Research Triangle Institute). Developmental toxicity evaluation (with cholinesterase assessment) of carbaryl administered by gavage to New Zealand White rabbits. RTI ID #65C-7297-200/100. DPR Vol. #169-389, Rec. #170646.

Tyl, R.W., Myers, C.B. and Marr, M.C. (2001) (Research Triangle Institute). Two-generation reproductive toxicity evaluation of carbaryl (RPA007744) administered in the feed to CD (Sprague-Dawley) rats. RTI ID #65C-07407-400. DPR Vol. #169-410, Rec. #182115.

USEPA (2012) Benchmark Dose Technical Guidance. http://www.epa.gov/raf/publications/pdfs/benchmark_dose_guidance.pdf

USEPA (2007) Reregistration Eligibility Decision for Carbaryl. Case No. 0080. EPA-738R07- 018. September 2007.

Weinberg, J.T. (2008) (WIL Research Laboratories, LLC). An inhalation dose-response study of carbaryl-induced cholinesterase inhibition in albino rats. Study #WIL-21206. DPR Volume #169-490, Record #243620.

Wyrobek, A.J., Watchmaker, G., Gordon, L., Wong, K., Moore, D. II and Whorton, D. (1981) Sperm shape abnormalities in carbaryl-exposed employees. Environ Health Perspect 40, 255-265.

Xia, Y.K., Cheng, S.P., Bian, Q., Xu, L.C., Collins, M.D., Chang, H.C., Song, L., Liu, J.Y., Wang, S.L. and Wang, X.R. (2005) Genotoxic effects on spermatozoa of carbaryl-exposed workers. Toxicological Sciences 85(1), 615-623.