UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: October 4, 2022

SUBJECT: **Tetrachlorvinphos (TCVP):** Summary of the Joint Hazard and Science Policy Council (HASPOC) and Cancer Assessment Review Committee (CARC) Meeting on August 18th, 2022: Recommendations on the Need for Additional Mutagenicity Data

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HASPOC
Health Effects Division (7509T)

THROUGH: Brian VanDeusen *for* Joshua Godshall, Co-Chair
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CARC
Health Effects Division (7509T)

TO: Sarah Dobreniecki, Biologist
Michael Metzger, Branch Chief
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MEETING ATTENDEES:

HASPOC Members: Gregory Akerman, Moana Appleyard, Jeffery Dawson, Anwar Dunbar, Angela Gonzales, Kelly Lowe, Jacqueline Meadows, Elizabeth Mendez, Michael Metzger, Monique Perron, Hannah Pope-Varsalona, Brian VanDeusen, Cassi Walls, Krystle Yozzo, Joshua Godshall*, Sarah Dobreniecki

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CARC Members: Gregory Akerman, Jessica Kidwell, Anwar Dunbar, Hannah Pope-Varsalona*, John Patrick Rogers, Jeremy Leonard, Lori Brunsman, Linnea Hansen, Minerva Mercado, Abiy Mohammed, Sarah Dobreniecki

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Presenter: Sarah Dobreniecki

Other Attendees: Jesse Hale, Tom Moriarty, Ana Terman, Patricia Biggio, Harold Cooper, Matthew Zampariello, Emma Ford, Melissa Grable, Melantha Jackson, Yongqi Li, Rosanna Louie-Juzwiak, Megan Stallard, Nicholas Thomas, Heriberto Deleon, Alexandra Turley, Anna Senniger, Destiny Carter, Jorrell Fredericks, Dana Friedman, Adrian Britt, Jessie Wozniak#, Zachery Staley

Executive Secretary

I. PURPOSE OF MEETING

Currently, a linear low-dose approach for quantification of cancer risk using a Q_1* of 1.83 x 10^{-3} $(mg/kg/day)^{-1}$ is utilized for the tetrachlorvinphos (TCVP) cancer risk assessment (D456245, K. Lowe, 01/26/2022). This approach does not apply age dependent adjustment factors (ADAFs) for assessing susceptibility from early-life exposure to TCVP. The Agency's current supplemental guidance[1] provides guidance on applying the ADAFs when a carcinogen has been demonstrated to act through a mutagenic mode-of-action (MOA). To determine if TCVP is acting through a mutagenic MOA, and in turn, if the application of ADAFs are appropriate, additional mutagenicity studies were recommended for TCVP including a mouse micronucleus assay (OPPTS 870.5395) and an additional assay that examines possible genotoxic activity in the target organ (i.e., the liver) (D456245, K. Lowe, 01/26/2022; TXR 0057553, D437226, S. Dobreniecki, 05/01/2020). Since this time, an updated literature search has been conducted that identified one additional study that examined the mutagenic potential of TCVP. This study, in addition to all previously available mutagenicity data, were evaluated concurrently to determine the need for additional data to pursue a mutagenic MOA analysis. The HASPOC and CARC met on August 18th, 2022, to determine if additional mutagenicity data is still necessary to support the registrations for TCVP.

II. SUMMARY OF USE PROFILE, EXPOSURE, AND HAZARD CONSIDERATIONS

a. Use and Exposure Profile

TCVP is in the organophosphate (OP) class of pesticides that target the nervous system in insects and arachnids. It is used as a direct animal treatment to livestock (i.e., cattle, horses, poultry and swine) and their premises, in kennels, outdoors as a perimeter treatment, and as a flea treatment on cats and dogs. The TCVP livestock and perimeter treatment uses are formulated as dusts, liquids, feed-through (solid and liquid food additives), feed blocks, and wettable powders (WP). Some of the liquid, dust and WP formulations can be mixed with water

[1] Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. March 2005. https://www.epa.gov/risk/supplemental-guidance-assessing-susceptibility-early-life-exposure-carcinogens

to create a paint slurry used to treat livestock premises. The pet use formulations include collars and pump and trigger sprays. The personal protective equipment (PPE) required for occupational use of TCVP varies by formulation and application/equipment type. For feed-through (solid and liquid food additives) and feed blocks, occupational handlers are required to wear baseline clothing (i.e., long sleeved shirt, long pants, shoes, and socks) and chemical-resistant gloves. For all other end-use labels with livestock and outdoor perimeter uses, required PPE can vary dependent on the application/equipment type and range from baseline clothing and gloves to the addition of coveralls, or respiratory protection. None of the TCVP pet product labels require PPE as these are intended for residential sale or for occupational use.

Human exposure to TCVP in food may occur as a result of consuming residues in animal commodities (e.g., meat). Exposure may also occur from drinking water that may contain TCVP residues as a result of some outdoor use patterns. Residential exposures (handler and post-application) are anticipated from the use of TCVP pet products. Residential TCVP handler exposures (adults) are anticipated to be short-term (1 to 30 days), and post-application exposures (adults and children 1 to <2 years old) are anticipated to be short- (1 to 30 days), intermediate- (1 to 6 months – for pet collar scenarios only), and long-term (>6 months – for pet collar scenarios only) in duration.

Exposures *via* spray drift are not anticipated based on the registered use sites and application methods. Occupational handler exposures are anticipated from the use of TCVP in/on livestock, as an outdoor perimeter treatment, and on pets. Occupational post-application exposures are not anticipated for TCVP based on the manner in which it is applied and the use sites. Further, restricted entry intervals (REIs) are not included on TCVP product labeling, as the registered uses (i.e., livestock or other animals, or in or around animal premises) are not covered by the Worker Protection Standard (WPS).

b. Toxicity Profile

TCVP is a member of the OP class of pesticides. For TCVP, like other OPs, the initiating event in the adverse outcome pathway/mode of action (AOP/MOA) involves inhibition of the enzyme AChE *via* phosphorylation of the serine residue at the active site of the enzyme. This inhibition leads to accumulation of acetylcholine and ultimately to neurotoxicity in the central and/or peripheral nervous system. TCVP does not require metabolic activation to an oxon to inhibit AChE; i.e., the parent compound is the active form inhibiting AChE. While most OPs reach steady state within 2-3 weeks, other OPs, like TCVP, show no difference in response across duration. For TCVP, the steady state is reached after a single day of exposure. TCVP has cholinesterase data across multiple lifestages, durations, and routes for both red blood cell (RBC) and brain cholinesterase inhibition. RBC AChE inhibition is the most sensitive endpoint and is the endpoint from which the points-of-departure (PODs) for all TCVP exposure routes and durations were selected.

There is no evidence of increased quantitative or qualitative sensitivity in the developmental rat and rabbit studies or in the gestational (fetus) or juvenile components of the comparative cholinesterase assay (CCA) studies in rats. AChE data from the CCA studies suggest that the fetus is not more sensitive than the pregnant dam and that pregnant females are not more

sensitive than non-pregnant females with respect to cholinesterase inhibition. When comparing RBC BMD_{10} (benchmark dose) estimates from across the acute (single dose) CCA and repeat dose CCA studies, it is apparent that there are no age, duration, or sex-related differences. AChE data for the dermal and inhalation routes are also available and allow for route-specific evaluation. RBC AChE inhibition was observed in both sexes in the inhalation study (brain AChE was not assessed), while no inhibition of RBC or brain AChE was observed in the dermal study up to the limit dose.

TCVP is classified as a Group C possible human carcinogen (based on statistically significant increases in combined hepatocellular adenomas/carcinomas in female mice, suggestive evidence of thyroid c-cell adenomas, and adrenal pheochromocytomas in the rat, as well as mutagenicity concerns) with a linear low-dose approach for quantification of cancer risk using the oral slope factor (Q_1*) of 1.83×10^{-3} $(mg/kg/day)^{-1}$.

III. RECOMMENDATION ON THE NEED FOR ADDITIONAL STUDIES

a. Additional Mutagenicity Studies

A previous Agency review on the mutagenic potential of TCVP concluded that the data available at that time suggested a potential concern for mutagenicity (TXR 0057553, D437226, S. Dobreniecki, 05/01/2020). However, the uncertainty in the *in vivo* data precluded moving forward with a mutagenic MOA analysis, and as such, additional mutagenicity data was recommended including a mouse micronucleus assay (OPPTS 870.5395) and an additional assay that examines possible genotoxic activity in the target organ (i.e., the liver) (TXR 0057553, D437226, S. Dobreniecki, 05/01/2020; D456245, K. Lowe, 01/26/2022). Since that time an updated literature search was conducted by the Agency, in which one additional genotoxicity study was identified, and the previously available data were recently re-reviewed. These findings are used in a weight-of-the evidence (WOE) evaluation and presented below to determine the mutagenic potential of TCVP.

1. **Evidence of carcinogenicity in the TCVP database:** Evidence of carcinogenicity is presented within this memo to establish an understanding of rodent tumor formation following TCVP exposure. Treatment related tumors and the cancer classification will not be reevaluated at this time.

   The HED Carcinogenicity Peer Review Committee (CPRC) met in 1994 and classified TCVP as a Group C possible human carcinogen under the 1986 Carcinogen Risk Assessment Guidelines. The classification was based on a statistically significant increase in combined hepatocellular adenomas/carcinomas (predominantly carcinomas) in the female B6C3F1 mouse, suggestive evidence of thyroid C-cell adenomas and adrenal pheochromocytomas in the rat, mutagenicity concerns, and structure-activity relationship (SAR) support (TXR 0011438, B. Backus and E. Rinde, 03/06/1995). Data from the rat and mouse cancer bioassays are summarized below.

   Rat cancer bioassays: Two rat carcinogenicity studies were available during the 1994 peer review. The first study was conducted in 1978 in Osborne-Mendel rats at 0, 4250,

and 8500 ppm (mg/kg/day not reported) (MRID 00117443). Statistical significance was noted (trend and pairwise comparison) at 8500 ppm for thyroid C-cell adenomas and adrenal cortical adenomas in female rats. However, the incidence for both tumor types was below the historical control means and below or within the historical control ranges. The CPRC noted deficiencies in this study and determined the tumor findings were equivocal; however, the study deficiencies were not explicitly noted. An updated rat cancer bioassay was required and submitted. This study was conducted in 1993 in Sprague Dawley rats at 0, 100, 1000, or 2000 ppm (0/0, 4/6, 43/63, 89/125 mg/kg/day (male/female)) (MRIDs 42980901 and 43335101). A statistically significant trend for benign pheochromocytomas ($p<0.05$) was noted in male rats. Findings were not statistically significant by pairwise comparison, and historical control data was not available for this tumor type. In addition, there was a slight increase in the incidence of thyroid C-cell adenomas in males at 89 mg/kg/day; however, this finding was not statistically significant by trend or pairwise comparison but was slightly higher than the historical control range (29% vs. 4-23% historical control range). The first pheochromocytoma and thyroid C-cell adenoma were observed at week 76 and 74, respectively, after approximately 75% of the study was concluded. The CPRC noted that both thyroid and adrenal tumors were observed in the 1978 study and supportive of the results observed in the 1993 study. Thus the CPRC concluded that there was suggestive evidence that the thyroid and adrenal tumors were treatment related.

Mouse cancer bioassays: Two mouse carcinogenicity studies were also available for analysis during the 1994 CPRC. The later study was conducted in 1980 in B6C3F1 mice at 0, 17.5, 64, 320, 1600, 8000, or 16000 ppm (0, 2.6, 9.6, 48, 240, 1200, or 2400 mg/kg/day) for 103 weeks (MRID 00126039). Hepatocellular adenomas and/or carcinomas were treatment related in females at ≥1600 ppm (240 mg/kg/day) based on statistically significant increases in the incidences of adenomas at 16000 ppm ($p<0.05$), carcinomas at 8000 ($p<0.05$) and 16000 ppm ($p<0.05$), and combined adenomas and/or carcinomas at 1600 ($p<0.05$), 8000 ($p<0.01$) and 16000 ppm ($p<0.01$). It should be noted that combined hepatocellular adenomas and/or carcinomas were only slightly above the historical control (historical control data from the literature and not the conducting laboratory) mean (7-11% at ≥1600 ppm; historical control mean 8%) and were within the historical control range (0-20%) at all doses tested. The same pattern was observed for carcinomas and adenomas when evaluated separately. The highest dose tested (16000 ppm) was considered excessively toxic based on severe liver necrosis and was excluded from the estimation of the unit risk when calculating the Q_1*. The first carcinoma appeared at week 55 and the first adenoma appeared at week 79, with the first carcinoma appearing approximately 50% of the way through a 103-week study. An earlier study (MRID 00117443), in the same strain of mouse, tested doses of 8000 and 16000 ppm and identified a statistically significant increase by both trend and pairwise comparison ($p<0.001$) in hepatocellular carcinomas in males at both dose levels; however, deficiencies in the study design were identified. Despite the deficiencies, the data from this study were still considered supportive of the findings from the 1980 study. The CPRC concluded that the liver tumors were treatment related in female mice.

2. **Evidence of genotoxicity in the TCVP database:** TCVP has been investigated in a series of mutagenicity and clastogenicity studies submitted by the registrant or identified by the Agency in the open literature. The findings from these studies are summarized below and in Table A.3.

    a. **Gene mutations**: TCVP was not mutagenic in *Salmonella typhimurium* strains up to cytotoxic concentrations in both the presence and absence of S9 activation (MRID 41222508). Similar negative findings for gene mutations were reported by Moriya et al.[2] and Brooks et al.[3] in *S. typhimurium* and *Escherichia coli* WP2 and/or WP2uvrA. Brooks et al. also found that TCVP did not increase the mitotic gene conversion frequency in stationary-phase cultures of *Saccharomyces cerevisiae* D4.

    b. **Chromosome aberrations**:

    In vitro cytogenetics assays: In an *in vitro* cytogenetic assay (MRID 41312901) in Chinese Hamster Ovary (CHO) cells TCVP was tested at 29.9, 44.9, 59.9, 79.8, and 99.8 µg/mL. TCVP induced significant increases in structural chromosome aberrations (≥59.9 µg/mL; -S9 activation). This increase was observed at concentrations (79.8 and 99.8 µg/mL) that induced associated effects on the health of the cell monolayer and/or a reduction in visible mitotic cells. However, it should be noted that the data presented at 59.9 µg/mL does not mention the health status of the cell layer and only describes the slight reduction in visible mitotic cells. It's unclear if the reduction in visible mitotic cells is due to protective cell cycle arrest or irreparable DNA damage making a determination of cytotoxicity at this dose level difficult. A positive response was not seen in the presence of exogenous metabolic activation (+S9) or at the lowest noncytotoxic doses (29.9 and 44.9 µg/mL) in the absence of S9 activation.

    In a published study examining cytogenic effects of TCVP (MRID 51926101), primary cultures of mouse spleen cells were exposed to TCVP at concentrations of 0.25, 0.5, 1.0, and 2.0 µg/mL. Significant and concentration-related increases in chromosome aberrations (minus gaps) were observed at ≥0.5 µg/mL with chromatid and chromosome fragments being the major aberrations. Increases in chromosome aberrations were accompanied by marked increases in chromatid and chromosome gaps; however preliminary cytotoxicity testing indicated that cells were viable within the tested concentration range for the cytogenic assays. It is important to note that three separate experiments were conducted for each concentration with each experiment scoring 200 metaphases. The study authors noted that the differences between the percentage of chromosomal aberrations obtained from the three separate experiments conducted for each concentration were not statistically significant before the data was pooled (i.e., not statistically significant when 200 metaphases were analyzed). This deviates from the OPPTS 870.5375 guideline that recommends scoring ~200 metaphases per concentration

[2] Moriya, M., Ohta, T., Watanabe, K., Miyazzawa, T. (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat Res*. 116: 185-216.

[3] Brooks, T.M., Dean, B.J., Hutson, D.H., Potter, D. (1982). Microbial mutation studies with tetrachlorvinphos (Gardona®). *Mutat Res*. 105: 211-221.

and should be taken into consideration when interpreting the positive response observed by the study authors. In addition, the positive control (mitomycin C) produced an increase in the mean percentage of metaphases with chromosome aberrations; however, this increase was not statistically significant as would be expected for this type of positive control.

There was a statistically significant ($p<0.01$) and concentration-related increase in sister chromatid exchange (SCE) induction at ≥0.5 µg/mL; however, the slope of the response was shallow (8.22 ± 0.19, 8.96 ± 0.15, 9.90 ± 0.59, 10.60 ± 0.35, 11.92 ± 0.14 from solvent control to high dosed concentration, respectively). It should be noted that statistical analysis was not conducted on the response (i.e., trend analysis).

Micronuclei formation: In a published *in vivo* study examining the induction of micronuclei in mouse bone marrow by TCVP (MRID 51926102), three routes of administration were examined: intraperitoneal, oral and dermal.

Intraperitoneal: Mice were dosed at 50 and 100 mg/kg/body weight, and when repeated treatments were conducted, injections were given twice weekly. It should be noted that for multiple treatments it's unclear how far apart each administration was conducted; however, sufficient details were provided to determine the total number of applications over a known period of time. Following one, two, and three injections at 50 mg/kg/body weight, there were no statistically significant increases in the percentage of polychromatic erythrocytes (%PE) with micronuclei. After a total of four injections, with a 24-hour sacrifice, the %PE with micronuclei were statistically increased ($p<0.05$). However, data was not reported to determine if this finding occurred in the presence of significant bone marrow toxicity. When animals were sacrificed at 7 days, following four injections, the %PE with micronuclei was no longer statistically significant. The %PE after four injections of 50 mg/kg/body weight over a two-week period was also presented in graph form within the literature. The graph presented an increase in %PE as the number of injections increased; however, quantitative results were not presented, and it's unclear if these results were statistically significant. The study authors stated that the effect on the frequency of micronuclei was nearly the same as after double injection; however, there's no data to independently verify this conclusion.

Oral (dietary): oral administration of TCVP at 3000 and 6000 ppm over varying periods of time (24 hours, 7 days, 14 days, 3 weeks, 7 weeks, or 10 weeks) was tested. Following oral administration for 24 hours, there was a statistically significant increase ($p<0.01$) in the %PE when mice were dosed at 3000 and 6000 ppm. The %PE with micronuclei was only statistically significant at 6000 ppm ($p<0.05$). The same pattern of results were also observed in mice treated for 14 days. When mice were treated for 7 days, there was a statistically significant increase in both %PE and %PE with micronuclei at 6000 ppm. It's also noted that the results presented at 6000 ppm occurred at a dose level that was above the maximum tolerated dose (~4000 ppm) as was reported in the primary literature.

Mice were also exposed orally for 10 weeks at 3000 ppm with results presented in graph form within the literature. The graph showed an increase in %PE with micronuclei over

time; however, quantitative results were not presented. The study authors stated that this increase was statistically significant after oral treatments for 3, 7, and 10 weeks. They further concluded that no distinct relation could be traced between the time of oral treatment and the induced marrow toxicity indicated as a statistically significant increase in the %PE over that of the control. The later statement cannot be independently verified as the quantitative data for %PE was not presented within the literature.

The primary literature does not report the dose level in mg/kg/day. In order to estimate the dietary concentration in mg/kg/day, calculations were performed using generic inputs as study specific data was not available. The calculation[4] assumed mice weighed 19 grams[5] and consumed 4 grams of food per day. The results of the oral portion of this study were examined in the context of the tumorigenic dose level in mice. The Agency previously determined that liver tumors (adenomas and carcinomas combined) were treatment related at dose levels ≥ 240 mg/kg/day in mice. The results in this study observed at 3000 and 6000 ppm occurred at dose levels 2.6X and 5.3X higher, respectively, than where tumors were observed in a chronic *in vivo* cancer bioassay. In addition, the majority of the statistically significant responses in %PE with micronuclei occurred in the presence of significant bone marrow toxicity as noted by the statistically significant increase in the percentage of PE or at a dose level (i.e., 6000 ppm) above the maximum tolerated dose (~4000 ppm) as was reported in the primary literature.

Dermal: Dermal applications were conducted twice weekly during a two-week period at 1350 mg/kg/body weight. No quantitative data was available to evaluate following dermal application of TCVP. However, the study authors stated that after a total of four dermal treatments at 1350 mg/kg/body weight there was a statistically significant increase in the %PE, indicating bone marrow toxicity, but there was no effect on the %PE with micronuclei. In addition, it's noted that this dose level was above the maximum tolerated dose (900 mg/kg/body weight) as reported in the primary literature.

Cytokinesis-block micronucleus assay: An updated literature search[6] was conducted that returned one article of interest (MRID 51926104) that had not been previously reviewed by the Agency. Cytokinesis-block micronucleus and SCE assays were conducted using human peripheral blood lymphocytes. Micronucleus (MN) frequencies, SCE frequencies, and cytokinesis-block proliferation indices (CBPI) were evaluated after cells were administered 1, 5, 25, and 50 µg/mL TCVP. A chromosome instability (CIN) level was also calculated by combining the results of the micronucleus and sister chromatid exchange frequencies. After exposure to TCVP (1-50 µg/mL) there were no statistically significant responses in the percentage of MN or CBPI when compared to the negative control. The study authors reported a positive %MN association with concentration (via

[4] ((3000 ppm)(0.004 kg))/(0.019 kg) =630 mg/kg/day
((6000 ppm)(0.004 kg))/(0.019 kg) =1263 mg/kg/day

[5] Weight of SJL/J mouse; male aged 42 days – extracted from https://www.arc.wa.gov.au/?page_id=125

[6] Google scholar: tetrachlorvinphos, mutagenicity, genotoxicity, mutagen, genotoxic, clastogenicity, clastogenic
PubMED: ((tetrachlorvinphos)) AND (mutagenicity OR genotoxicity OR mutagen OR genotoxic OR clastogenicity OR clastogenic)

Spearman's rho test) but no statistically significant association with CBPI level and concentration.

TCVP at concentrations of ≥ 5 µg/mL induced statistically significant increases in the SCE frequency when compared to the negative control. However, the frequency of SCE was not statistically significantly associated with the concentration of TCVP (via Spearman's rho test). CIN was measured as the sum of MN and SCE frequencies. 50 µg/mL TCVP resulted in a significant increase in CIN level when compared to the negative control. The level of CIN was positively associated with the concentration of TCVP (via Spearman's rho test).

c. Other mutagenic mechanisms:

Sister Chromatid Exchange (SCE) induction: SCE induction in cultured mouse spleen cells (MRID 51926101) and human peripheral blood lymphocytes (MRID 51926104) has been discussed above. The SCE test is an assay that can measure the consequence of primary DNA damage. The mechanism(s) of action for chemical induction of SCE is unclear and thus, the consequences of increased frequencies of SCEs are unclear. As such, during the 2014-2015[7] update to the Genetic Toxicology Test Guidelines (TG) the Organization for Economic Co-operation and Development (OECD) no longer support the TG 479 (*in vitro* sister chromatid exchange test for mammalian cells). This should be taken into consideration when analyzing the positive response associated with TCVP exposure and should be given lower weight in the overall weight-of-the-evidence analysis of TCVP mutagenicity.

Unscheduled DNA synthesis (UDS): In an acceptable UDS study (MRID 42156401), cultures of primary rat hepatocytes were dosed with 5-40 µg/mL TCVP. Concentrations of 35 and 40 µg/mL were cytotoxic. Only the cells exposed to doses from 10 to 30 µg/mL were analyzed for evidence of UDS and the results were negative.

Alkylation of guanine: In a published study, the potential for TCVP to methylate guanine bases *in vivo* (i.e., formation of 7-methylguanine (7-MeGu)) was evaluated (MRID 51926103). Male Swiss mice were injected intraperitoneally with 25, 50, or 100 mg/kg/body weight ^{14}C-TCVP. DNA, RNA, and protein isolated from male mouse livers were found to contain ^{14}C-activity which increased with dose level and reached a maximum 24 hours post dosing. When calculated as a fraction of the total applied dose, formed 7-MeGu ranged from 6-11 X 10^{-5} and 36-41 X 10^{-5} for DNA and RNA, respectively, indicating that interaction with RNA may occur more preferentially as compared to DNA. ^{14}C-activity was found to be present in the adenine, guanine, and 7-MeGu fractions of the column effluent following ion exchange chromotography of the HCl hydrolysate of DNA and RNA. The ^{14}C-activity associated with 7-MeGu varied from 80-85% of the chromatographed radio-dose and contributed to ~12% and 16% of the radioactivity in DNA and RNA, respectively. Overall, there was evidence that TCVP has the potential to interact with DNA, RNA, and protein.

[7] https://www.oecd.org/chemicalsafety/testing/Genetic%20Toxicology%20Guidance%20Document%20Aug%2031%202015.pdf

3. **Evidence of mutagenicity from related chemicals:** A search was conducted using ChemIDplus for pesticides with >60% structural similarity to TCVP[8]. This search did not return any pesticides that would be considered an adequate analogue for TCVP. A search was also conducted using the Compendium of Pesticide Common Names[9]. TCVP is labeled as an organophosphate acaricide and organophosphate insecticide along with 17 other pesticides. Three of these pesticides (dichlorvos (DDVP), naled, and dicrotophos) are registered by the Agency and one has been canceled (mevinphos). Table A.4. summarizes the cancer classification, cancer quantification, treatment related tumors, mutagenic concerns, and chemical structures for each of these pesticides. A comparison of structural similarity between each of these pesticides and TCVP is also presented within Table A.4. These chemicals were considered acceptable analogs as their toxicological profiles were similar to TCVP (i.e., AChEI is the most sensitive toxicological effect, the parent compound is the active form inhibiting AChE, both positive and negative responses were observed in mutagenicity studies, and there was not a particularly robust tumor profile observed in long term cancer bioassays). A quantitative assessment of cancer risk was not required for any of the four chemicals. Mevinphos and naled did not produce treatment related tumors in either rat or mouse two-year cancer bioassays, while exposure to DDVP produced mononuclear cell leukemia in male rats and forestomach tumors in mice. Dicrotophos presented with treatment related thyroid tumors in mice. The mutagenicity results varied across chemicals with positive responses seen in *in vitro* bacterial systems (DDVP, mevinphos, and naled) and *in vitro* mammalian systems (DDVP, mevinphos, and dicrotophos). However, *in vivo* micronucleus tests were negative when available (DDVP, naled, and dicrotophos). While positive responses were seen across select mutagenicity studies, the negative *in vivo* micronucleus assays (DDVP, naled, and dicrotophos) and/or the lack of tumor response in the two-year cancer bioassays (mevinphos and naled), led to the Agency's conclusion that there was no concern for mutagenicity after exposure to the four SAR chemicals.

4. **Risk assessment considerations:** Currently, a linear low-dose approach for quantification of cancer risk using a Q_1* of 1.83×10^{-3} $(mg/kg/day)^{-1}$ is utilized for the TCVP cancer risk assessment (D456245, K. Lowe, 01/26/2022). This risk assessment approach does not apply age dependent adjustment factors (ADAFs) for assessing susceptibility from early-life exposure to TCVP. The Agency's current supplemental guidance[10] provides guidance on applying the ADAFs only after a carcinogen has been demonstrated to act through a mutagenic MOA. HED previously recommended that additional data (a mouse micronucleus assay (OPPTS 870.5395) and an additional assay that examines possible genotoxic activity in the target organ (i.e., the liver)) are needed to determine if TCVP is acting through a mutagenic MOA, and in turn, if the application of ADAFs are appropriate (D456245, K. Lowe, 01/26/2022). As the application of ADAFs have not been deemed appropriate at this time, the linear low-dose approach used to quantify adult exposures is presented below.

[8] https://chem nlm nih.gov/chemidplus/
[9] https://pesticidecompendium.bcpc.org/
[10] Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. March 2005. https://www.epa.gov/risk/supplemental-guidance-assessing-susceptibility-early-life-exposure-carcinogens

A summary of toxicological doses and endpoints for use in dietary, non-occupational, and occupational risk assessments are summarized in Tables A.1. and A.2. The refined cancer dietary (food and drinking water) assessment resulted in an estimated exposure to the highest exposed adult population subgroup (adults 20-49) to TCVP and its metabolites containing the 2,4,5-trichlorobenzene moiety (the residues of concern for cancer) of 0.000459 mg/kg/day. Applying the Q_1* of 1.83 x 10^{-3} (mg/kg/day)$^{-1}$ to the exposure value resulted in a cancer risk estimate of 8 x 10^{-7}. Drinking water was the major contributor to the cancer dietary risk estimate (see Table A.5.).

Residential cancer risks were assessed for both the registered pet collars and the pump/trigger spray products. Available chemical-specific transferable residue data for both types of products were used for the post-application assessment. For pet collars, there is uncertainty as to the ratio of liquid to dust in the formulated products. Therefore, residential exposures have been evaluated assuming TCVP is released from the collar at varying ratios (e.g., 1%/99%, 50%/50%, and 99%/1% liquid/dust). Residential handler cancer risks (combined dermal and inhalation) estimated for TCVP pet collars assuming a ratio of 1%/99% dust/liquid range from 10^{-8} to 10^{-9}; assuming a ratio of 50%/50% dust/liquid are all 10^{-8}; and when assuming a ratio of 99%/1% dust/liquid range from 10^{-7} to 10^{-8}. Residential handler cancer risk estimates for TCVP pump/trigger sprays range from 10^{-8} to 10^{-9}. Residential handler cancer risks are provided in Tables A.6 and A.7.

Residential post-application cancer risks estimated for TCVP pet collars assuming a ratio of 1%/99% dust/liquid are all 10^{-7}; assuming a ratio of 50%/50% dust/liquid from 10^{-5} to 10^{-6}; and when assuming a ratio of 99%/1% dust/liquid range from 10^{-5} to 10^{-6}. Residential post-application cancer risks estimated for TCVP pump/trigger sprays range from 10^{-7} to 10^{-8}. Residential post-application cancer risks are provided in Tables A.8 and A.9.

The cancer aggregate risk assessment combined residential and dietary (food and drinking water) expected lifetime exposures for adults. For TCVP, a cancer aggregate assessment was performed for adult post-application activities related to the residential pump/trigger spray uses since the registered pet collar uses resulted in non-cancer risk estimates of concern. The aggregate cancer risk estimate for adults was 1 x 10^{-6} (see Table A.10).

Occupational cancer risk estimates were estimated for both private/farmer handlers (assumed to be exposed for 10 days per year/35 working years/78 lifetime years) and contract/commercial handlers (assumed to be exposed for 30 days per year/35 working years/78 lifetime years). Cancer risk estimates ranged from 10^{-4} to 10^{-11} for private/farmer handlers and from 10^{-4} to 10^{-11} for contract/commercial handlers, depending on the scenario and PPE (see Table A.11).

IV. CARC CONCLUSIONS

(1) The tumor response observed in both the mouse and rat cancer bioassays was not particularly robust based on the following considerations:

Rat (MRIDs 42980901 and 43335101)

a. the pheochromocytomas were benign (malignant and combined benign/malignant were not treatment related), only treatment related in one sex (male), and only statistically significant *via* trend analysis (not pairwise comparison);
b. the thyroid C-cell adenomas were not statistically significant by trend or pairwise comparison, only treatment related in one sex (male), the findings did not occur in a dose-response manner, and carcinomas and combined adenomas/carcinomas were not treatment related;
c. the first pheochromocytoma and thyroid C-cell adenoma were observed late in the study; and

Mouse (MRID 00126039)

d. the combined hepatocellular adenomas and/or carcinomas were only slightly above the historical control mean and were within the historical control range at all doses tested. The same pattern was observed for carcinomas and adenomas when evaluated separately.

(2) There is low concern for mutagenicity based on the following considerations:

a. TCVP was not mutagenic in *in vitro* bacterial assays;
b. the increase in structural chromosome aberrations in the *in vitro* cytogenetic assay (MRID 41312901) only occurred at concentrations that induced associated effects on the health of the cell monolayer and/or a reduction in visible mitotic cells in the absence of exogenous metabolic activation;
c. there was evidence of an increase in %PE with micronuclei following intraperitoneal and oral administration of TCVP (MRID 51926102); however, it generally occurred in the presence of significant bone marrow toxicity, and in some cases, at a dose level that exceeded the maximum tolerated dose;
d. the positive results from the dietary portion of the micronuclei study (MRID 51926102) occurred at dose levels 2.6X-5.3X higher than where tumors were observed in the chronic mouse *in vivo* cancer bioassay and 225X-450X higher than the oral POD used in the TCVP risk assessment ($BMDL_{10}$ = 2.8 mg/kg/day based on AChE inhibition);
e. there was no evidence of an increase in %PE with micronuclei following dermal application;
f. the results from an acceptable UDS study (MRID 42156401) were negative;
g. there were no statistically significant responses in the percentage of micronucleus frequencies or cytokinesis-block proliferation indices in the newly identified cytokinesis-block micronucleus assay (MRID 51926104) which is considered a well-conducted, more up-to-date study as compared to the older TCVP literature studies and was conducted in human cells. This study examined the endpoint of concern from the originally recommended guideline mouse micronucleus assay;
h. SCE induction was observed in cultured mouse spleen cells (MRID 51926101) and human peripheral blood lymphocytes (MRID 51926104). However, as the

mechanism(s) of action for chemical induction of SCE is unclear and thus, the consequences of increased frequencies of SCEs are unclear, lower weight in the WOE approach should be given to these positive responses. In accordance with this recommendation, SCE assays are no longer supported under the OECD Genetic Toxicology Test Guidelines; and

i. the evidence that TCVP has the potential to interact with DNA, RNA, and protein in the *in vivo* methylation of guanine assay (MRID 51926103) should be given lower weight in the WOE approach as an interaction does not necessarily mean a mutation will occur within a cell or tissue, rather it only confirms exposure to the target tissue[11].

V. HASPOC CONCLUSIONS

Based on a WOE approach, the HASPOC recommends that additional mutagenicity data for TCVP including a mouse micronucleus assay (OPPTS 870.5395) and an additional assay that examines possible genotoxic activity in the target organ (i.e., the liver) are not needed at this time. This approach included the following considerations regarding tumor incidence, genotoxicity profile, and SAR findings: (1) the CARC determined that the tumor response observed in both the mouse and rat cancer bioassays was not particularly robust; (2) there is low concern for mutagenic potential following TCVP exposure; (3) the newly identified cytokinesis-block micronucleus assay (MRID 51926104) examined the endpoint of concern from the originally recommended guideline mouse micronucleus assay (MN frequency) and there were no statistically significant responses in %MN reported up to 50 µg/mL of TCVP; and (4) there was no concern for mutagenicity following exposure to chemicals within the same OP pesticidal MOA classification (DDVP, mevinphos, naled, and dicrotophos).

[11] Boysen, G., Pachkowski, B.F., Nakamura, J., Swenberg, J.A. (2009) The Formation and Biological Significance of N7-Guanine Adducts. Mutat Res. 678(2): 76-94.

VI. APPENDIX A

Table A.1. Summary of Toxicological Doses and Endpoints for TCVP for Use in Dietary and Non-Occupational Human Health Risk Assessments.				
Exposure/ Scenario	Point of Departure	Uncertainty Factors*	aPAD, cPAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (all populations, except adults 50-99)	$BMDL_{10}$ = 2.8 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF = 10x	Acute RfD = 0.028 mg/kg/day aPAD = 0.0028 mg/kg/day	Acute dose CCA study (MRID 48773401a) – Rat BMD_{10} = 3.2 mg/kg/day, based on PND 11 and 21 male and female RBC AChE inhibition
Acute Dietary (Adults 50-99)	$BMDL_{10}$ = 2.8 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF = 1x	Acute RfD = 0.028 mg/kg/day aPAD = 0.028 mg/kg/day	Acute dose CCA study (MRID 48773401a) - Rat BMD_{10} = 3.2 mg/kg/day, based on PND 11 and 21 male and female RBC AChE inhibition
Steady State Dietary (all populations, except adults 50-99)	$BMDL_{10}$ = 2.8 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF = 10x	Steady State RfD = 0.028 mg/kg/day ssPAD = 0.0028 mg/kg/day	Acute dose CCA study (MRID 48773401a) – Rat BMD_{10} = 3.2 mg/kg/day, based on PND 11 and 21 male and female RBC AChE inhibition
Steady State Dietary (Adults 50-99)	$BMDL_{10}$ = 2.8 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF = 1x	Steady State RfD = 0.028 mg/kg/day ssPAD = 0.028 mg/kg/day	Acute dose CCA study (MRID 48773401a) – Rat BMD_{10} = 3.2 mg/kg/day, based on PND 11 and 21 male and female RBC AChE inhibition
Incidental Oral (steady state)	$BMDL_{10}$ = 2.8 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF = 10x	Residential LOC for MOE = 1000	Acute dose CCA study (MRID 48773401a) - Rat BMD_{10} = 3.2 mg/kg/day, based on PND 11 and 21

Table A.1. Summary of Toxicological Doses and Endpoints for TCVP for Use in Dietary and Non-Occupational Human Health Risk Assessments.				
Exposure/ Scenario	Point of Departure	Uncertainty Factors*	aPAD, cPAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
				male and female RBC AChE inhibition
Dermal (steady state)	No potential hazard *via* the dermal route, based on the lack of treatment-related effects, including the lack of RBC and brain cholinesterase inhibition following repeat dermal exposure of rats at dose levels up to 1000 mg/kg/day and quantitative susceptibility was not observed.			
Inhalation (steady state)	$BMDL_{10}$=0.022 mg/L/day (males)	UF_A= 3x UF_H=10x FQPA SF = 10X	Residential LOC for MOE = 300	Subchronic Inhalation Toxicity Study (MRID 48803501) – Rat BMD_{10} = 0.12 mg/L/day, based on RBC AChE inhibition in both sexes

Table A.1. Summary of Toxicological Doses and Endpoints for TCVP for Use in Dietary and Non-Occupational Human Health Risk Assessments.				
Exposure/ Scenario	Point of Departure	Uncertainty Factors*	aPAD, cPAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Cancer (oral, dermal, inhalation)	Classification: A possible human (Group C) carcinogen. $Q_1^* = 1.83 \times 10^{-3}$ $(mg/kg/day)^{-1}$ (TXR 0012766, B. Fisher, 01/11/1995)			

[1] **Explanation of Abbreviations:** Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies); FQPA SF = Food Quality Protection Act Safety Factor; MOE = margin of exposure. LOC = level of concern. RBC = red blood cell. AChE = acetylcholinesterase. $BMDL_{10}$= benchmark dose lower limit for 10% response; CCA = comparative cholinesterase study; RfD = Reference Dose; PAD = population adjusted dose (a = acute and ss= steady state).

* The 10X FQPA SF is retained for all exposure scenarios with infants, children, youths, and women of childbearing age due to uncertainty in the human dose-response relationship for neurodevelopmental effects.

Table A.2. Summary of Toxicological Doses and Endpoints for TCVP for Use in Occupational Human Health Risk Assessments				
Exposure/ Scenario	Point of Departure	Uncertainty Factors*	Level of Concern for Risk Assessment	Study and Toxicological Effects
Dermal (steady state)	No potential hazard *via* the dermal route, based on the lack of treatment-related effects, including the lack of RBC and brain cholinesterase inhibition following repeat dermal exposure of rats at dose levels up to 1000 mg/kg/day and quantitative susceptibility was not observed.			
Inhalation (steady state)	$BMDL_{10}$=0.022 mg/L/day (males)	UF_A= 3x UF_H=10x UF_{DB}= 10x	Occupational LOC for MOE = 300	Subchronic Inhalation Toxicity Study (MRID 48803501) - Rat BMD_{10} = 0.12 mg/L/day, based on RBC AChE inhibition in both sexes
Cancer (oral, dermal, inhalation)	Classification: A possible human (Group C) carcinogen. $Q_1^* = 1.83 \times 10^{-3}$ $(mg/kg/day)^{-1}$ (TXR 0012766, B. Fisher, 01/11/1995)			

[1] **Explanation of Abbreviations:** Point of Departure (POD) = A data point or an estimated point that is derived from observed dose-response data and used to mark the beginning of extrapolation to determine risk associated with lower environmentally relevant human exposures. NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies); UF_{DB} = database uncertainty factor; MOE = margin of exposure. LOC = level

of concern. RBC = red blood cell. $BMDL_{10}$= benchmark dose lower limit for 10% response. AChE = acetylcholinesterase. SS = steady state

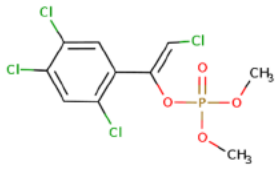
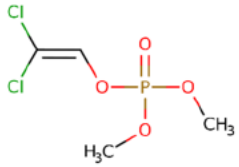
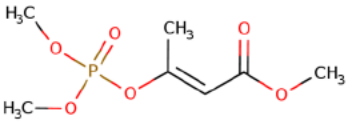
***** The 10X UF_{DB} is retained for infants, children, youths, and women of childbearing age for all exposure scenarios due to uncertainty in the human dose-response relationship for neurodevelopmental effects.

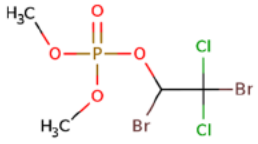
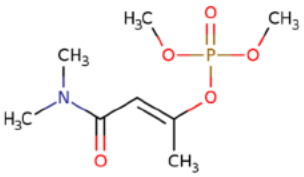
Table A.3.: Summary of Available TCVP Mutagenicity Data and Results

Guideline No./ Study Type	MRID No. (year)/ Classification/ Doses	Results
Gene Mutation 870.5100 *Salmonella/Escherichia* bacterial reverse mutation assay	41222508 (1989) Acceptable/Guideline 66.7, 100, 333, 667, 1000, or 3300 µg/plate in the presence of or 10, 33.3, 66.7, 100, 333, or 667 µg/plate absence of mammalian metabolic activation (S9 mix)	Strains TA98, TA100, TA1535, TA1537, and TA 1538 of *S. typhimurium* were exposed to TCVP from concentrations of 66.7 to 3300 µg/plate in the presence and 10-667 µg/plate absence of mammalian metabolic activation (S9-mix). There was no evidence of induced mutant colonies over background.
In vitro mammalian cytogenetics 870.5375 Chinese hamster ovary cells	41312901 (1989) Acceptable/Guideline 0, 29.9, 44.9, 59.9, 79.8, 99.8 µg/mL -S9 (20-hr cell harvest) 0, 12.5, 25, 37.6, 75.1 µg/mL +S9 (10-hr cell harvest)	-S9 activation: positive for inducing chromosomal aberrations at 59.9, 79.8 and 99.8 µg/mL in the presence of associated effects on the health of the cell monolayer and/or a reduction in visible mitotic cells. Data presented at 59.9 µg/mL does not mention the health status of the cell layer and only describes the slight reduction in visible mitotic cells. It's unclear if this is due to protective cell cycle arrest or irreparable DNA damage making a determination of cytotoxicity at this dose level difficult. Negative at 29.9 and 44.9 µg/mL. +S9 activation: negative for inducing chromosomal aberrations at 12.5, 25, 37.6, or 75.1 µg/mL.
Published literature Amer, S.M. and Aly, F.A.E. (1992) Cytogenetic effects of pesticides. IV. Cytogenetic effects of the insecticides Gardona and Dursban. ***Mutation Research***, 279: 165-170. *In vitro* mammalian cell clastogenicity assay in mouse spleen cells	51926101 (1992) Acceptable/Non-Guideline 0.25, 0.5, 1.0, 2.0 µg/mL (4-hr treatment)	There was evidence that exposure to TCVP induced chromosome aberrations and SCEs over background levels in primary mouse spleen cells.
Published literature Amer, S.M. and Fahmy, M.A. (1983) Cytogenetic effects of pesticides. II. Induction of micronuclei in mouse bone marrow by the insecticide gardona. ***Mutation Research***, 117: 329-336. Micronucleus assay in bone marrow cells of the mouse	51926102 (1983) Acceptable/Non-Guideline <u>Intraperitoneal</u>: 50 and 100 mg/kg/body weight (twice weekly for repeat treatments) <u>Oral (dietary):</u> 3000 and 6000 ppm (24 hours, 7 days, 14 days, 3 weeks, 7 weeks, or 10 weeks) <u>Dermal</u>: 1350 mg/kg/body weight (twice weekly during a two-week period)	There was evidence of an increase in %PE with micronuclei following intraperitoneal and oral administration of TCVP; however, it generally occurred in the presence of significant bone marrow toxicity, and in some cases, at a dose level that exceeded the maximum tolerated dose.
Unscheduled DNA Synthesis 870.5550 Mammalian cells in culture	42156401 (1992) Acceptable/Guideline Doses of 5, 7.5, 10, 15, 20, 23, 25, 27, 30, 35, or 40 µg/mL	Concentrations of 35 and 40 µg/mL were cytotoxic. Results at lower concentrations (≤30 µg/mL) were negative.

Guideline No./ Study Type	MRID No. (year)/ Classification/ Doses	Results
Published literature Zayed, S.M.A.D., Mostafa, I.Y., Adam, Y. and Hegazi, B. (1983) *In vivo* Methylation of Guanine by the Organophosphorus Insecticide Tetrachlorvinphos. *J. Environ. Sci. Health*, B18(6): 767-779. DNA adduct formation in Swiss male mice liver	51926103 (1983) Acceptable/Non-Guideline 25, 50 and 100 mg/kg intraperitoneal injection	There was evidence that TCVP has the potential to interact with DNA, RNA, and protein, causing the formation of the DNA alkylation lesion, 7-MeGu.
Published literature Cobanoglu, H. and Cayir, A. (2021) Assessment of the genotoxic potential of tetrachlorvinphos insecticide by cytokinesis-block micronucleus and sister chromatid exchange assays. *Human and Experimental Toxicology*, 40(12S): S158-S163 Cytokinesis-block micronucleus and sister chromatid exchange assays on human peripheral lymphocytes	51926104 (2021) Acceptable/Non-Guideline 1, 5, 25, and 50 µg/mL	TCVP did not induce an increase in %MN at the tested concentration range but did induce a statistically significant increase in SCEs and CIN. The reviewer assumes the increase in CIN is mostly driven by the increase in SCEs.

Table A.4. Summary of Carcinogenicity and Mutagenicity Data for Select Chemicals within the OP Pesticidal MOA Classification

Pesticide	Cancer Classification and Quantification	Treatment Related Tumors	Mutagenic Concerns	Chemical Structure	Chemical Similarity to TCVP[a]
TCVP	"*Group C Possible Human Carcinogen*" Q1* = 1.83 x 10^{-3} $(mg/kg/day)^{-1}$ based on female liver tumors	Combined hepatocellular adenomas /carcinomas (female mice), suggestive evidence of thyroid c-cell adenomas (male rat), and adrenal pheochromocytomas (male rat)	See Section III.a.2.		Not applicable
Dichlorvos (DDVP)	"*Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential*" No quantitative assessment of cancer risk is required	Mononuclear cell leukemia (male rats) and forestomach tumors (mice)	DDVP has been shown to be a direct acting mutagen *in vitro* bacterial and mammalian test systems. Clastogenic activity was identified in CHO cells *in vitro* with or without metabolic activation but was not identified in the *in vivo* micronucleus tests.		**AP** **Tanimoto:** 0.228261 **MCS** **Tanimoto:** 0.5000
Mevinphos	"*Not Likely to be Carcinogenic to Humans*" No quantitative assessment of cancer risk is required	No evidence of carcinogenicity	Weakly mutagenic in *S. typhimurium* (with or without activation), equivocally mutagenic in mammalian cell forward gene mutation assay, and significantly clastogenic in mammalian cells without activation.		**AP** **Tanimoto:** 0.207373 **MCS** **Tanimoto:** 0.4348

Pesticide	Cancer Classification and Quantification	Treatment Related Tumors	Mutagenic Concerns	Chemical Structure	Chemical Similarity to TCVP[a]
Naled	"*Group E Evidence of Non-Carcinogenicity for Humans*" No quantitative assessment of cancer risk is required	No evidence of carcinogenicity	Negative in an *in vivo* coat color mutation study in mice. *S. typhimurium* reverse mutation assay; mutagenic with metabolic activation and toxic in the absence of metabolic activation. Negative for DNA damage *in vitro*, negative for cytogenetic effects *in vivo*, and negative for clastogenic effects *in vivo*.		**AP** **Tanimoto:** 0.131818 **MCS** **Tanimoto:** 0.3913
Dicrotophos	*"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"* No quantitative assessment of cancer risk is required	Thyroid tumors (mice)	Negative in *Salmonella*/*E. coli* bacterial reverse mutation assay and micronucleus assay in *in vivo* mouse bone marrow cells. Positive gene mutation in the presence and absence of metabolic activation at non-toxic doses in a mammalian cell gene mutation assay in mouse lymphoma cells.		**AP** **Tanimoto:** 0.194805 **MCS** **Tanimoto:** 0.4167

[a] https://chemminetools.ucr.edu/; Similarity scores between compound pairs were computed with the Similarity Workbench. The interface calculates atom pair (AP) and maximum common substructure (MCS) similarities with the Tanimoto coefficient as the similarity measure (Chen & Reynolds, 2002; Cao et al. 2008). The MCS tool identifies the largest substructure two compounds have in common.

Table A.5. Summary of Cancer Dietary Exposure (Food and Drinking Water) and Risk for TCVP (and metabolites)

Population Subgroup	Exposure (mg/kg/day)	Risk
Adults 20-49 years old	0.000459	8×10^{-7}
Adults 50-99 years old	0.000445	8×10^{-7}
Females 13-49 years old	0.000450	8×10^{-7}

Table A.6. Residential Adult Handler Cancer Risk Estimates from Use of TCVP Pet Collars.												
EPA Reg. No.	Target Animal	Size of Animal	99% Dust / 1% Liquid			50% Dust / 50% Liquid			1% Dust / 99% Liquid			
			Dust LADD[2]	Liquid LADD[1]	Cancer Risk Estimate[3]	Liquid LADD[1]	Dust LADD[2]	Cancer Risk Estimate[3]	Liquid LADD[1]	Dust LADD[2]	Cancer Risk Estimate[3]	
2596-49	Cat	Medium / Large	4.0E-05	2.7E-08	7E-08	2.0E-05	1.3E-06	4E-08	4.0E-07	2.6E-06	6E-09	
2596-50, 62	Dog	Small	4.6E-05	3.1E-08	8E-08	2.3E-05	1.6E-06	5E-08	4.7E-07	3.1E-06	6E-09	
		Large	8.3E-05	5.6E-08	2E-07	4.2E-05	2.8E-06	8E-08	8.4E-07	5.5E-06	1E-08	
2596-83	Cat	Medium	3.4E-05	2.3E-08	6E-08	1.7E-05	1.1E-06	3E-08	3.4E-07	2.3E-06	5E-09	
		Large	4.6E-05	3.1E-08	8E-08	2.3E-05	1.6E-06	5E-08	4.7E-07	3.1E-06	6E-09	
2596-84	Dog	Small	4.6E-05	3.1E-08	8E-08	2.3E-05	1.6E-06	5E-08	4.7E-07	3.1E-06	7E-09	
		Large	8.3E-05	5.6E-08	2E-07	4.2E-05	2.8E-06	8E-08	8.4E-07	5.5E-06	1E-08	
2596-139	Cat	Medium	3.4E-05	2.3E-08	6E-08	1.7E-05	1.1E-06	3E-08	3.4E-07	2.3E-06	5E-09	
		Large	4.6E-05	3.1E-08	8E-08	2.3E-05	1.6E-06	5E-08	4.7E-07	3.1E-06	6E-09	
2596-139	Dog	Small	4.6E-05	3.1E-08	8E-08	2.3E-05	1.6E-06	5E-08	4.7E-07	3.1E-06	6E-09	
		Medium	6.5E-05	4.3E-08	1E-07	3.3E-05	2.2E-06	6E-08	6.5E-07	4.3E-06	9E-09	
		Large	8.3E-05	5.6E-08	2E-07	4.2E-05	2.8E-06	8E-08	8.4E-07	5.5E-06	1E-08	

1 Liquid LADD = [Inhalation + Dermal Dose (mg/kg/day)] × [Days per year of exposure (4 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy (78 yrs)]. Inhalation exposures considered negligible based on use of spot-on data for liquid pet collar formulation.

2 Dust LADD = [Inhalation + Dermal Dose (mg/kg/day)] × [Days per year of exposure (4 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy 78 (yrs)].

3 Cancer risk estimates = [(Liquid LADD * % TCVP released as liquid) + (Dust LADD * % TCVP released as DUST)] × Q_1^*, where $Q_1^* = 1.83 \times 10^{-3}$ $(mg/kg/day)^{-1}$

Table A.7. Residential Adult Handler Cancer Risk Estimates from Use of TCVP Pump/Trigger Spray Products.				
EPA Reg No.	Target Animal	Size of Animal	Total LADD[1]	Cancer Risk Estimate[2]
2596-126, -140 (Trigger)	Cat	Small	4.7E-06	8.6E-09
		Large	6.6E-06	1.2E-08
2596-140 (Pump)	Cat	Small	9.6E-07	1.8E-09
		Large	1.3E-06	2.5E-09
2596-125, -140 (Trigger)	Dog	Small	6.6E-06	1.2E-08
		Medium	7.5E-06	1.4E-08
		Large	1.3E-05	2.4E-08

1 Total LADD (mg/kg/day) = [Inhalation + Dermal Dose (mg/kg/day)] × [Days per year of exposure (6 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy (78 yrs)]..

2 Cancer risk estimates = Total LADD × Q_1^*, where $Q_1^* = 1.83 \times 10^{-3}$ $(mg/kg/day)^{-1}$

Table A.8. Residential Adult Post-Application Cancer Risk Estimates from Use of TCVP Pet Collars.												
EPA Reg. No.	Animal Type	Animal Size	Dust	Liquid	Dust	Liquid	Dust	Liquid	Dust	Liquid	Combined LADD (mg/kg/day)	Cancer Risk Estimate
			Transferable Residue (mg/cm^2)		Dermal Exposure $(mg/day)^2$		Dermal Dose (mg/kg/day)		$LADD^3$ (mg/kg/day)			
99% Dust / 1% Liquid												
2596-49	Cat	Medium	0.000571	0.0000058	61.55	0.023	0.027	0.000010	8.5E-03	3.2E-06	8.5E-03	2E-05
		Large	0.000357	0.0000036	38.47	0.014	0.017	0.000006	5.3E-03	2.0E-06	5.3E-03	1E-05
2596-50,62	Dog	Small	0.000410	0.0000041	44.15	0.017	0.019	0.000007	6.1E-03	2.3E-06	6.1E-03	1E-05
		Large	0.000244	0.0000025	26.25	0.010	0.011	0.000004	3.6E-03	1.4E-06	3.6E-03	7E-06
2596-83	Cat	Small	0.000815	0.0000049	87.88	0.020	0.038	0.000009	1.2E-02	2.7E-06	1.2E-02	2E-05
		Large	0.000417	0.0000042	44.94	0.017	0.020	0.000007	6.2E-03	2.3E-06	6.2E-03	1E-05
2596-84	Dog	Small	0.000410	0.0000041	44.15	0.017	0.019	0.000007	6.1E-03	2.3E-06	6.1E-03	1E-05
		Large	0.000244	0.0000025	26.25	0.010	0.011	0.000004	3.6E-03	1.4E-06	3.6E-03	7E-06
2596-139	Cat	Medium	0.000815	0.0000049	87.88	0.020	0.038	0.000009	1.2E-02	2.7E-06	1.2E-02	2E-05
		Large	0.000417	0.0000042	44.94	0.017	0.020	0.000007	6.2E-03	2.3E-06	6.2E-03	1E-05
2596-139	Dog	Small	0.000410	0.0000041	44.15	0.017	0.019	0.000007	6.1E-03	2.3E-06	6.1E-03	1E-05
		Medium	0.000280	0.0000028	30.23	0.011	0.013	0.000005	4.2E-03	1.6E-06	4.2E-03	8E-06
		Large	0.000244	0.0000025	26.25	0.010	0.011	0.000004	3.6E-03	1.4E-06	3.6E-03	7E-06
50% Dust / 50% Liquid												
2596-49	Cat	Medium	0.000288	0.000288	31.09	1.155	0.014	0.000502	4.3E-03	1.6E-04	4.4E-03	8E-06
		Large	0.000180	0.000180	19.43	0.722	0.008	0.000314	2.7E-03	9.9E-05	2.8E-03	5E-06
2596-50,62	Dog	Small	0.000207	0.000207	22.30	0.828	0.010	0.000360	3.1E-03	1.1E-04	3.2E-03	6E-06
		Large	0.000123	0.000123	13.26	0.492	0.006	0.000214	1.8E-03	6.8E-05	1.9E-03	3E-06
2596-83	Cat	Small	0.000247	0.000247	44.39	0.989	0.019	0.000430	6.1E-03	1.4E-04	6.2E-03	1E-05
		Large	0.000211	0.000211	22.70	0.843	0.010	0.000367	3.1E-03	1.2E-04	3.2E-03	6E-06
2596-84	Dog	Small	0.000207	0.000207	22.30	0.828	0.010	0.000360	3.1E-03	1.1E-04	3.2E-03	6E-06
		Large	0.000123	0.000123	13.26	0.492	0.006	0.000214	1.8E-03	6.8E-05	1.9E-03	3E-06
2596-139	Cat	Medium	0.000247	0.000247	44.39	0.989	0.019	0.000430	6.1E-03	1.4E-04	6.2E-03	1E-05
		Large	0.000211	0.000211	22.70	0.843	0.010	0.000367	3.1E-03	1.2E-04	3.2E-03	6E-06
2596-139	Dog	Small	0.000207	0.000207	22.30	0.828	0.010	0.000360	3.1E-03	1.1E-04	3.2E-03	6E-06
		Medium	0.000142	0.000142	15.27	0.567	0.007	0.000247	2.1E-03	7.8E-05	2.2E-03	4E-06
		Large	0.000123	0.000123	13.26	0.492	0.006	0.000214	1.8E-03	6.8E-05	1.9E-03	3E-06

Table A.8. Residential Adult Post-Application Cancer Risk Estimates from Use of TCVP Pet Collars.												
EPA Reg. No.	Animal Type	Animal Size	Dust	Liquid	Dust	Liquid	Dust	Liquid	Dust	Liquid	Combined LADD (mg/kg/day)	Cancer Risk Estimate
			Transferable Residue (mg/cm^2)		Dermal Exposure $(mg/day)^2$		Dermal Dose (mg/kg/day)		LADD[3] (mg/kg/day)			
1% Dust / 99% Liquid												
2596-49	Cat	Medium	0.0000058	0.000571	0.62	2.29	0.00027	0.00099	8.5E-05	3.1E-04	4.0E-04	7E-07
		Large	0.0000036	0.000357	0.39	1.43	0.00017	0.00062	5.3E-05	2.0E-04	2.5E-04	5E-07
2596-50,62	Dog	Small	0.0000041	0.000410	0.45	1.64	0.00019	0.00071	6.1E-05	2.3E-04	2.9E-04	5E-07
		Large	0.0000025	0.000244	0.27	0.98	0.00012	0.00042	3.6E-05	1.3E-04	1.7E-04	3E-07
2596-83	Cat	Small	0.0000082	0.000489	0.89	1.96	0.00039	0.00085	1.2E-04	2.7E-04	3.9E-04	7E-07
		Large	0.0000042	0.000417	0.45	1.67	0.00020	0.00073	6.2E-05	2.3E-04	2.9E-04	5E-07
2596-84	Dog	Small	0.0000041	0.000410	0.45	1.64	0.00019	0.00071	6.1E-05	2.3E-04	2.9E-04	5E-07
		Large	0.0000025	0.000244	0.27	0.98	0.00012	0.00042	3.6E-05	1.3E-04	1.7E-04	3E-07
2596-139	Cat	Medium	0.0000082	0.000489	0.89	1.96	0.00039	0.00085	1.2E-04	2.7E-04	3.9E-04	7E-07
		Large	0.0000042	0.000417	0.45	1.67	0.00020	0.00073	6.2E-05	2.3E-04	2.9E-04	5E-07
2596-139	Dog	Small	0.0000041	0.000410	0.45	1.64	0.00019	0.00071	6.1E-05	2.3E-04	2.9E-04	5E-07
		Medium	0.0000028	0.000280	0.31	1.12	0.00013	0.00049	4.2E-05	1.5E-04	2.0E-04	4E-07
		Large	0.0000025	0.000244	0.27	0.98	0.00012	0.00042	3.6E-05	1.3E-04	1.7E-04	3E-07

1 Transferable residue = [Application Rate (label defined) * Fraction Transferred (0.00092)] ÷ Surface Area of Cat/Dog (Cat: Small, 1,500; Medium, 2,500; Large, 4,000 cm^2 - Dog: Small, 3,000; Medium, 7,000; Large, 11,000 cm^2)

2 Dermal Exposure (mg/day) = [Transferable residue, mg/cm^2) x [Transfer Coefficient (1400 cm^2/hr for liquids and 38,000 cm^2/hr for dusts)] * x [Exposure Time (Adults, 0.77 hours/day; Children, 1.0 hours/day))

3 Dermal Dose (mg/kg/day) = Dermal exposure (mg/day) / Body weight (kg)

4 Liquid LADD = [Dermal Liquid Dose (mg/kg/day)] × [Days per year of exposure (180 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy (78 yrs)]

5 Dust LADD = [Dermal Dust Dose (mg/kg/day)] × [Days per year of exposure (180 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy (78 yrs)]

6 Combined LADD = (Liquid LADD * % TCVP released as liquid) + (Dust LADD * % TCVP released as dust).

7 Cancer risk estimates = Combined LADD × Q_1^*, where $Q_1^* = 1.83 \times 10^{-3}$ $(mg/kg/day)^{-1}$

Table A.9. Residential Adult Post-Application Cancer Estimates from Use of TCVP Pump/Trigger Spray Formulations.				
EPA Reg. No.	Animal Type	Animal Size	Total LADD[1]	Cancer Risk Estimate[2]
2596-126, 140 (Trigger)	Cat	Small	1.7E-04	3.0E-07
		Large	8.7E-05	1.6E-07
2596-140 (Pump)	Cat	Small	3.4E-05	6.2E-08
		Large	1.8E-05	3.2E-08
2596-125, -140 (Trigger)	Dog	Small	1.2E-04	2.1E-07
		Medium	5.7E-05	1.0E-07
		Large	6.3E-05	1.2E-07

1 Total LADD (mg/kg/day) = [Dermal + Inhalation Dose (mg/kg/day)] × [Days per year of exposure (180 days/yr) ÷ 365 days/year] × [Years per lifetime of exposure (50 yrs) ÷ Lifetime expectancy (78 yrs)]

2 Cancer risk estimates = Total LADD × Q1*, where Q1* = 1.83×10^{-3} $(mg/kg/day)^{-1}$

Table A.10. Cancer Aggregate Risk Calculations.					
Exposure Scenario	Lifestage	Food and Water Exposure (mg/kg/day)[1]	Residential LADD[2] (mg/kg/day)	Total Exposure (mg/kg/day)	Aggregate Cancer Risk
Post-application exposure to a small cat treated with a trigger pump spray	Adults	0.000459	0.00017	0.00063	1×10^{-6}

[1] See Table A.5.
[2] See Table A.9.
[4] Aggregate Cancer Risk = Total exposure (mg/kg/day) * 1.83E-03

Table A.11. Summary of Occupational Handler Cancer Risk Estimates for TCVP		
PPE combination	**Private/Farmer handlers**	**Contract/Commercial handlers**
SL/No G + No-R	10^{-4} to 10^{-10}	10^{-4} to 10^{-10}
SL/G + No-R	10^{-4} to 10^{-10}	10^{-4} to 10^{-10}
DL/G + No-R	10^{-4} to 10^{-10}	10^{-4} to 10^{-10}
SL/No G + PF10 R	10^{-4} to 10^{-10}	10^{-4} to 10^{-10}
SL/G + PF10 R	10^{-5} to 10^{-11}	10^{-5} to 10^{-10}
DL/G + PF10 R	10^{-5} to 10^{-11}	10^{-5} to 10^{-10}
SL/No G + PF50 R	10^{-4} to 10^{-11}	10^{-4} to 10^{-10}
SL/G + PF50 R	10^{-6} to 10^{-11}	10^{-5} to 10^{-10}
DL/G + PF50 R	10^{-6} to 10^{-11}	10^{-5} to 10^{-11}

All tables are based on the most up-to-date information available at the time of the joint HASPOC/CARC meeting and are subject to change