PESTICIDE REGISTRATION AND EVALUATION COMMITTEE (PREC)
Meeting Minutes – September 15, 2023

Committee Members/Alternates in Attendance:

Fabiola Estrada – U.S. Environmental Protection Agency (EPA), Region 9
Garrett Keating – Department of Industrial Relations (DIR)
Heather Williams – Department of Resources Recycling and Recovery (CalRecycle)
Krista Hoffmann – Department of Fish and Wildlife (CDFW)
Katherine Sutherland-Ashley – Office of Environmental Health Hazard Assessment (OEHHA)
Fatemeh Ganjisaffar – California Department of Food and Agriculture (CDFA)
Lisa McCann – State Water Resources Control Board (SWRCB)
Lynn Baker – Air Resources Board (ARB)
Mai Ngo – Department of Toxic Substances Control (DTSC)
Tulio Macedo – Department of Pesticide Regulation (DPR)

Visitors in Attendance:
Note: Only attendees who identified themselves using their full name are listed below

Ann Jonynas
Anne Katten - California Rural Legal Assistance Foundation
Christabelle Parnanthu
Griselda Arias
Jackie Ramsey - National Pest Management Association
James Nakashima - Office of Environmental Health Hazard Assessment (OEHHA)
Jing Tao – Office of Environmental Health Hazard Assessment (OEHHA)
John Bottorff
Lori Miyasato – Office of Environmental Health Hazard Assessment (OEHHA)
Kevi Mace – California Department of Food and Agriculture (CDFA)
Marcia Trostle
Nicholas Skoulis
Savannah Gosselin

DPR Staff in Attendance:

Aisha Iqbal - Pesticide Registration Branch
Alyssa Freeman - Pesticide Registration Branch
Andrew Turcotte - Pesticide Registration Branch
Brenna McNabb - Pesticide Registration Branch
Brittanie Clendenin - Pesticide Registration Branch
Daisy (Qiaoxiang) Dong - Human Health Assessment Branch
Dana Navarrete - Worker Health and Safety Branch
Elana Varner - Pesticide Registration Branch

Jolynn Mahomoudi-Haeri - Pesticide Registration Branch
Joy Dias - Groundwater Program
JT Teerlink - Pesticide Programs Division
Michel Oriel - Human Health Assessment Branch
Minh Pham - Environmental Monitoring Branch
Neelima Verma - Human Health Assessment Branch
Shelley DuTeaux - Human Health Assessment Branch
Svetlana Koshlukova - Human Health Assessment Branch
Taylor Whitehill - Pesticide Registration Branch

1. Introductions and Committee Business – Tulio Macedo, Chair, DPR

a. Approximately forty (40) people attended the meeting.

2. Summary of the Annual Well Sampling Report – Joy Dias, DPR

An overview of the Department of Pesticide Regulation's (DPR) 37th Annual Well Sampling Report, summarizing the groundwater sampling results for pesticide residues in California by DPR and other agencies. The Groundwater Protection Program produces the report each year as required by the Pesticide Contamination Prevention Act (PCPA).

DPR implements numerous steps to protect groundwater from the agricultural use of pesticides, some of which are described here. Identify potential groundwater contaminants and place them on the Groundwater Protection List (GWPL). Monitor groundwater for potential and known pesticide contaminants. Collect or maintain a database of pesticide monitoring in groundwater by DPR and other public agencies. Evaluate and respond to pesticide detections in groundwater by DPR and other public agencies. And prepare the annual Well Sampling Report to summarize monitoring results and actions taken in response to detections.

The 37th Annual Well Sampling Report includes well sampling data from January through December 2021. Data are compiled from DPR, State Water Resources Control Board (SWRCB), and United States Geological Survey (USGS). The data compiled by DPR, SWRCB, and USGS remains in line with previous years' data. In 2020 there was a slight dip in the number of wells sampled due to COVID, but in 2021 the number of wells sampled was back to previous years. The pesticides and wells tested by each agency varies due to each agency's different monitoring purposes. DPR monitors mostly shallow domestic wells in vulnerable areas with high pesticide use, while SWRCB data is collected from large and small water systems, and USGS generally monitors larger areas to determine overall groundwater basin quality.

Table 1 in the 37th Annual Well Sampling Report contains the summary of results for each pesticide analyzed. A total of 196 pesticides or degradates were sampled for by the three agencies, and 39 pesticides or degradates were reported detected. A bold row represents a detection in the samples analyzed, while a non-bold row indicates no detections of the pesticide or degradate. Sampling agency, reporting limit range, and detected concentration are also

summarized for each pesticide analyzed. Table 1 also differentiates whether a parent compound is registered or not for use in California.

DPR follows a set response process for all reported detections of pesticides. First DPR determines if the pesticide is currently registered for use in California. If the chemical is currently registered, then it is determined if the level detected is above the screening level. Then DPR evaluates these detections further. If, through the process outlined in the PCPA, DPR has formally reviewed the pesticide for potential risk to human health and the detection is below the level determined to pollute groundwater, then further assessment is not conducted at that site and monitoring of the pesticide will continue. If the detection is in a Groundwater Protection Area (GWPA) and the pesticide is already regulated under 3CCR section 6800(a) as a groundwater contaminant in those areas, then further assessment is not conducted at the site.

Table 2 of the report provides additional details about the 39 chemicals with reported detections and is divided into 4 tables to better display the data. Table 2A contains definitions of the human health drinking water standards included in the following tables. Table 2B gives a detailed summary of the responses to the GWPL 6800(a)-listed pesticides or degradates detected. This table includes 12 pesticides or degradates. These pesticides are identified as known groundwater contaminants and require mitigation measures if they are used in Groundwater Protection Areas. Table 2C contains a detailed summary of 6800(b)-listed pesticides or degradates detected. This table includes 10 pesticides or degradates. These pesticides are on the GWPL because they have been identified as having the potential to contaminate groundwater. Table 2D contains a detailed summary of pesticides or degradates detected but not currently included on the GWPL. This table includes 7 pesticides or degradates. Out of the 39 chemicals detected, 29 were of currently registered pesticides or their degradates. Tables 2 B-D include the name of the pesticide or degradate, number of wells with detections, detected concentration range, wells with detections at or above the screening level, State and Federal drinking water health and quality standards, and DPR's response to detection.

Table 2B 6800(a)-listed pesticides or degrades. These are pesticides that are restricted materials in GWPAs and require a permit and implementation of mitigation measures. All detections are below drinking water health and quality standards. Of the 12 pesticides, 7 do not require further action by DPR. For one of these, none of the detections exceeded the screening level. For the other 6, all detections were in GWPAs where they require a permit and management practices. There will be continued monitoring of the levels, but no additional sampling at those sites. Some detections of the five other pesticides or degradates detected will require further evaluation by DPR. There were 4 wells with detections of Atrazine, 1 well with a Bentazon detection, 1 well with Bromacil detection, 8 wells with detections of Simazine, and 3 wells with detections of DEA. Since the report was written, it has been determined that the well with a detection of Bentazon was resampled by the reporting agency and Bentazon was not detected in the samples.

Table 2C 6800(b)-listed pesticides or degradates which are on the GWPL. All detections of the 10 pesticides or degradates were below any drinking water health and quality standards. For 5 of the detections no further action is required by DPR. None of the detections of 1-Naphthol, 3,5-

Dichloroaniline and EPTC pesticides exceeded the screening level. The detections of Hexazinone and Imidacloprid were determined to not pollute at the levels detected. DPR continues to monitor to ensure that the levels stay low and determine if further action in the future will be needed. For the other 5 pesticides detected DPR will evaluate further. The well detection of Chlorantraniliprole and Fludioxonil were detected by DPR studies and are undergoing further evaluation. DPR has already initiated additional sampling for the 2 well detections of Metolachlor.

Table 2D lists pesticides or degradates not on the GWPL. All detections of the 7 pesticides were below any drinking water health and quality standards. For 3 of the pesticides there is no further action required by DPR. 2 of the pesticides did not have any detections that exceeded the screening level. And all detections of the other pesticide detected were at levels that were determined not to pollute groundwater. DPR will evaluate the detections of 4 of the pesticides diquat dibromide, flutriafol, methoxyfenozide, and P-DCB.

Table 2E- Detailed summary of compounds detected not currently registered for use as a pesticide. Reported detections of the 10 compounds with legacy pesticide use or non-pesticidal uses. DPR includes these compounds in the annual report and WIDB but does not conduct further evaluation. These compounds include 1-2 DCP, carbon disulfide, DBCP, ethylene dibromide, ethylene dichloride, formaldehyde, methoxychlor, molinate, ortho-dichlorobenzene, and tefluthrin. Table shows limited information, as DPR does not test for pesticides. The information included is the pesticide name, number of wells with detections, detected concentration range, state and federal drinking water health and quality standards, and registration status.

For questions or more information see Groundwater Protection Program at <cdpr.ca.gov/docs/emon/grndwtr/index.htm>. And the Annual Well Sampling Reports at <cdpr.ca.gov/docs/emon/grndwtr/wellinv/wirmain.htm>

Committee Comment

No comments to report from the committee.

Public Comment

Anne Katten asked what pesticide degrades into DEA? Joy Dias replied that Atrazine degrades into DEA. Atrazine is currently not widely used in many areas of California, so it is likely that these detections may be from legacy use.

Anne followed up in the Q&A box, Will DCPA screening level be reevaluated in light of new data on toxicity made available recently? Joy replied need further discussion with DPR's Human Health Assessment branch and will look at as a department to see if need to reanalyze.

3. **Proposal to Add Chitosan to List of Active Ingredients Allowed in Minimum Risk Pesticides – Jolynn Mahmoudi-Haeri and Neelima Verma, DPR**

Minimum risk pesticides are defined as pesticides that pose little to no risk to human health or the environment. U.S. Environmental Protection Agency (EPA) created the exemption for minimum risk pesticides to eliminate the need for the EPA to expend significant resources to regulate products that were deemed to be of minimum risk to human health and the environment. The exemption is found under federal law in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) section 25(b) and federal regulation: 40CFR section 152.25(f). If the product meets the criteria, then it does not require to be registered with U.S. EPA. During this presentation, minimum risk pesticides will be referred to as 25(b)s.

U.S. EPA has 6 conditions a product must meet to qualify under the exemption from registration. Federal regulation 40CFR 152.25(f)(1) and (2) list the active and inert ingredients allowed the exemption. Inert ingredients include commonly consumed food commodities, animal feed items, and edible fats and oils described in 40CFR 180.950(a), (b), and (c); and certain chemical substances listed under 40CFR 180.950(e). The remaining conditions have to do with product labeling. All ingredients (both active and inert) must be listed on the label. The active ingredients must be listed by label display name and percentage by weight. Each inert ingredient must be listed by label display name. The product must not bear claims to either control or mitigate organisms that pose a threat to human health, or insects or rodents carrying specific diseases. The label cannot include any false or misleading statements. The name of the producer or the company for whom the product was produced, and the company's contact information must be displayed prominently on the product label. More information on the federal exemption can be found U.S. EPA 25(b) website < epa.gov/minimum-risk-pesticides>.

For a product to qualify for exemption from registration in California, it must first meet the federal exemption requirements. Second, it must meet California exemption requirements according to California law and regulation. The California exemption is different than the federal exemption. For certain active ingredients, a CAUTION signal word and precautionary statements are required. And if a product contains more than 1% of citronella or citronella oil and is topically applied to human skin, the product requires registration. These are typically mosquito repellents. If a product does not qualify for exemption in California, it must be registered. For more information on California's exemption, see Department of Pesticide Regulation's website <cdpr.ca.gov/docs/registration/sec25/sect25intro.htm>

Chitosan is one of the most common compounds found in nature and a naturally occurring chain of glucose molecules that is structurally related to cellulose. Chitosan is commonly derived from lobster, crab and shrimp shells, fish scales, and many types of insects and fungi by deacetylation of chitin. Chitosan has several biomedical applications, including as a carrier molecule for pharmaceuticals. The chemical name is Poly-D-Glucosamine. Commercial extraction of chitosan is mostly from shellfish like crab shells. Chitin isolation from shellfish requires the removal of the two major constituents of the shell, proteins by deproteinization and inorganic calcium carbonate by demineralization. Next, Chitin is treated with a hot concentrated solution of sodium

hydroxide for a few hours, and this produces chitosan as an insoluble residue. The manufacturing process should remove and denature any proteins or contaminants of allergenic or other concern. To be named chitosan the deacetylated chitin must contain 60% or more D-Glucosamine residues. Based on the extraction method the molecular weight may vary and the deacetylation may vary anywhere from 60 to 100%. The insoluble residue formed is called "dry" chitosan. Soluble salts of chitosan are formed by reaction of chitosan to various acids and are called "wet" chitosan.

U.S EPA's review criteria for adding Chitosan and Chitosan Salts to the 25(b) list, including seven factors which are described in 61 FR 8876 on March 6, 1996. The criteria are used when considering substance to be added to the list of substances exempted from FIFRA requirements as pesticides. The first factor is if the pesticidal substance is widely available to the general public. Chitosan and its salts have many agricultural, biochemical, cosmetic, food additive and other applications. The second factor is if it is common food or a constituent of a common food. While chitosan is not a food, it has numerous food related uses. It is a fat blocker, dietary supplement and used as an incipient in medicine. Chitosan salts are used for making films for packaging food. The third factor is if it has a nontoxic mode of action. No known adverse effects have been reported for chitosan or its salts. The fourth factor is if it is recognized by the Food and Drug Administration as safe; also known as "Generally Recognized as Safe" (GRAS). Chitosan and its salts do not have the FDA status. The fifth factor is if there is no data showing that it causes significant adverse effects. Human toxicity data show that chitosan and its salts show low acute toxicity. The sixth factor is if its use pattern will result in significant exposure. Nonagricultural exposure to chitosan and its salts through diverse avenues of exposure and use in applications such as cosmetics and textiles is widespread and continuous, far exceeds any exposure through pesticidal use of chitosan. And the final factor is if it is likely to be persistent in the environment. Chitosan applied as a minimum risk pesticide would be unlikely to persist in the environment due to chitosan degrading microorganisms.

DPR review of Chitosan Acute Toxicity Profile. Eight chitosan-related products have been evaluated by DPR's Human Health Assessment (HHA) Branch to date. Product uses include fungicide, plant growth regulator, antimicrobial agent, and adjuvant. Chitosan concentrations ranged from 0.95% to 30%. DPR's evaluation of the products showed that two products have U.S. EPA toxicity category III eye irritation hazard, which is mild eye irritants. However, irritation was overall so mild that if the Global Harmonized System (GHS) classification was used, these products would be "Not classified" for eye irritation hazards. Overall, all products showed low acute toxicity.

DPR also reviewed the acute toxicity of chitosan salts. The standard method of solubilization utilizes acetic acid. There is some data available on the toxicity of chitosan acetate, chitosan lactate, and chitosan hydrochloride in open scientific literature. Although limited, the data suggests these salts show low human toxicity. The scientific literature does not contain enough information on the potential toxicity of all the chitosan salts. There is the possibility that the U.S. EPA could add more acids to the 25(b) list in the future which could lead to other types of salts. Again, the limited data that is available shows that the chitosan salts have low acute toxicity.

Chitosan and chitosan salts are used in many biomedical products such as wound dressing, hair and skin care products and dietary supplements. The results of several human studies evaluating the effect of chitosan in reducing fat absorption suggest chitosan is well tolerated in humans. Chitosan salts are also being evaluated for various drug delivery applications such as ocular and nasal delivery.

DPR also reviewed chitosan related incidents. In open science literature there was one case of a chitosan induced, immediate-type allergy was observed in an adult female after eating chitosan from an unknown source. The same manuscript reported cases of contact dermatitis induced by chitosan-containing cream was observed in France and Portugal. There was also a review of DPR data bases and found that DPR did not receive any Adverse Effects Reports for chitosan or its salts or any reports to the Pesticide Illness Surveillance Program.

The conclusion is that DPR concurs with the U.S. EPA's decision to add chitosan and its salts to minimum risk pesticide list. Although limited, the available evidence suggests chitosan and its salts show low human toxicity. However, many factors, such as molecular weight, degree of deacetylation, salt form, source, and purity could influence the toxicity of chitosan. DPR recommends that the current data supports adding chitosan and chitosan salts to the 25(b) list in Title 3 CCR 6147(5)(A). Products containing chitosan should at a minimum bear the phrase "Keep Out of Reach of Children" on the label.

U.S EPA determined that there are low risk concerns for human health or the environment if chitosan is intended for use as a minimum risk pesticide. The conclusion is supported by information in the U.S.EPA's reviews of registered pesticide products containing chitosan as an active ingredient. At the beginning of this year, U.S. EPA added chitosan to the list of active ingredients eligible for use in 25(b) products exempt from registration and other requirements of FIFRA. In doing so, U.S. EPA is specifying that the listing also include those chitosan salts that can be formed when chitosan is mixed with the acids that are listed as active or inert ingredients eligible for use in 25(b) products. For more information see the U.S. EPA final rule <regulations.gov/document/EPA-HQ-OPP-2019-0701-0026>.

DPR proposes to align with U.S. EPA's decision by revising Section 6147 to add chitosan to the list of active ingredients permitted in pesticide products. Currently DPR is drafting rulemaking proposal documents that will be reviewed by California Environmental Protection Agency. This winter, a Notice of Proposed Action along with a Public Comment Period will follow. By spring of next year, the rulemaking package will be finalized and will be reviewed by the Office of Administrative Law. DPR anticipates regulation to be adopted in the summer of 2024. Proposed rulemaking documents <cdpr.ca.gov/docs/legbills/rulepkgs.htm> will be available on DPR's website. And updates on Regulatory Notices <cdpr.ca.gov.docs/dept/listserv/listdesc.htm> can be received by subscribing.

Committee Comment

No committee member comments to report.

Public Comment

Ricki Schneider with Tidal Grow Agriscience asked in the Q&A box: "It was mentioned that chitosan is not GRAS. What about the GRAS notification for the fungal-derived chitosan that is active?" Neelima Verma responded that DPR is looking for whether the GRAS status has been made or not. Currently the FDA has not yet made the determination for the fungal-derived chitosan.

Ricki S. thanked the presenters for a thorough presentation and followed up with a live question. Ricki S. is aware of a dry chitosan which is soluble and does not require an organic acid to go into solution, and wanted to know if it was on DPR's radar. Neelima clarified that the dry chitosan she was referring to, was the end product of an industry-standard manufacturing process. Ricki clarified that depending on the manufacturing process, chitosan can be produced that has a low enough molecular weight that it will be soluble in water without an acid.

4. **Mechanistic Studies of Chloropicrin: Current Results and Future Research – Dr. Daisy (Qiaoxiang) Dong, DPR**

The chemical name of chloropicrin is trichloronitromethane (CCI_3NO_2). Chloropicrin was first patented for use as an insecticide in 1908, and is a broad-spectrum fumigant with insecticidal, fungicidal, nematocidal and herbicidal properties. Chloropicrin is highly reactive and has a low odor threshold. With the low odor threshold, chloropicrin is used as a warning agent for other odorless fumigants like methyl bromide, methyl iodide, 1,3 Dichloropropene (1,3 D), and sulfuryl fluoride.

Chloropicrin formally entered reevaluated in 2001. Air monitoring data showed exceedances of National Institute of Occupational Safety and Health (NIOSH) recommended exposure limits (REL) 100 parts per billion. The adverse effects of chloropicrin were evaluated under the California Birth Defects Prevention Act of 1984 (BDPA). In 2010 there was a Toxic Air Contaminant Assessment for inhalation exposure to bystanders. A risk management directive for mitigation was drafted in 2010, and a comprehensive risk assessment began. The 2012 risk characterization document extended inhalation exposure to workers and bystanders.

Chloropicrin remains in re-evaluation because of concerns related to its long-term effect or carcinogenicity in humans. The U.S. Environmental Protection Agency (EPA) does not list it as a carcinogen through inhalation exposure. The Office of Environmental Health Hazard Assessment (OEHHA) does not list it on Proposition 65, and it is not on the International Agency for Research on Cancer (IARC) list. DPR's risk assessment concluded that chloropicrin is a carcinogen based on tumors in two species by two routes. The two routes of carcinogenicity include lung tumors in female mice through inhalation route, and mammary fibroadenomas in

female rats through oral route. DPR's assessment was peer reviewed by scientific review panel (SRP) and OEHHA, both concur with DPR's conclusion that chloropicrin in a carcinogen.

The main discrepancy between EPA and DPR on the carcinogenicity issue lies on the critical study by Burleigh-Flayer et al., 1995. This study was a registrant submitted guideline study, where a significant increase in combined lung adenoma and carcinoma were seen at the high dose group in the female mice after 18-19 months of whole-body exposure to chloropicrin vapors. The issue centered at the female dataset, in contrast to DPR's conclusion, EPA agreed with a significant trend of increase but considered high dose effect marginal. There was no significant increase in male mice. The reasons for less pronounced effect could be high tumor incidence in concurrent controls, and the length of study as incidence might have been higher in a longer study. From a health protective standpoint, DPR's weight of evidence analysis concluded that chloropicrin is a carcinogen, thus this study was used to conduct a quantitative assessment of carcinogenicity.

For quantitative assessment of carcinogenicity, DPR follows the EPA's cancer guidance. The Threshold Approach assumes a non-mutagenic mode of action (MOA), there are "safe" dosages, and Point of Departure (POD) is based on upstream effects. Some examples of the threshold approach are allyl isothiocyanate (AITC), propanil and fipronil. The non-threshold approach assumes a mutagenic MOA or evidence for direct DNA damage by a mutagen. There is no "safe" dose, meaning any dose can lead to cancer. It is based on cancer effects and some examples are 1,3-D, chlorothalonil, and carbaryl. The non-threshold approach is DPR's default approach when we don't know the MOA.

In 2010 and 2012 risk assessments, DPR used a non-threshold approach (linear extrapolation) to calculate cancer potency that assumes no safe dose. A non-threshold approach was used due to the MOA for tumor development being unknown. Mixed results in in vitro genotoxicity tests, and negative findings in in vivo genotoxicity tests, so evidence for direct DNA damage was inconclusive. Although the linear extrapolation approach assumes no safe dose, it is still possible to calculate an exposure level that corresponding a negligible risk level (DPR uses one in a million excess cancer case). The calculated Reference Concentration (RfC) is 0.00024 ppb, which is the exposure level that can lead to one in a million excess cancer cases, or a single increased incidence of cancer among a population of one million individuals exposed in a similar manner. This level is much smaller than the NIOSH REL of 100 ppb.

So why require a mechanistic study? In December 2014, the mode of action of lung tumor was unclear and data needed to close reevaluation (mitigation). In January 2015 DPR proposed another two-year mouse study to resolve the carcinogenicity issue. DPR also proposed to use a different mouse strain in the study that has a lower incidence of lung tumors in the control mice. In July 2015 Chloropicrin Manufacturers Task Force (CMTF) instead proposed a mechanistic study. This type of mechanistic study was supposed to fill the data gap, also evaluate possible non-mutagenic MOA, and would be faster and less costly. The study was contracted to Dr. Laura S. Van Winkle from UC Davis. In July 2016 DPR approved a three-phase mechanistic study. Phase 1 of the study would identify the target cells in the respiratory tract. Phase 2 would

characterize proliferative responses and investigate if there is a possible non-mutagenic MOA. Phase 3 would determine whether chloropicrin forms DNA adducts and if any such adducts are stable, which will tell if chloropicrin is a mutagen, that to say causing direct damage to DNA.

There are two studies in Phase I. For Phase I-study 1, the goal was to identify the target area in the lung and the target cell type. The study used the same strain of mice from the two-year study, and only female mice were used. The only method of exposure was by nose. The dose was within the range of the 1995 study and included a slightly lower dose as well. The duration was acute (a single six-hour dose) and repeated (six hours a day for five days). Sample size included six mice per dose and per time point. The tissue area examined included extrapulmonary (trachea/lobar bronchi) and intrapulmonary airways (lobar bronchi, midlevel bronchioles, terminal bronchioles, and alveoli). The main result found for toxicity is the effect on vacuoles of epithelial cells in conducting airways. The target issues found were severe effects in intrapulmonary midlevel and terminal bronchioles and mild to moderate effects in traches and lobar bronchus. There was no effect in the alveoli. The target cell: club cells that protect the bronchioles, detoxify harmful inhaled substances. Human lungs contain very few club cells as compared with rats and mice.

The Phase 1 Study 2 goal is to examine the sex effect or why there were significantly more lung tumors in the earlier study female mice; and also, to see if oxidative stress is a mechanism. The study principal investigator wanted to examine oxidative stress to see if oxidative stress plays a role in chloropicrin induced acute cytotoxicity (cell vacuolization). The study used both males and females of the same strain of mice as in earlier studies. Exposure was by nose only and dose given was 0 versus 0.5 ppm. The duration of exposure was acute or one single six-hour inhalation. The sample size was six mice, similar to the earlier studies as well. There was no evidence of oxidative stress.

In Phase 1, DPR requested that nasal tissue be examined as well. The goal was to identify the target area in the nasal tissue. All the nasal tissue from Phase I study 1 females and the male/female mice from Phase I study 2 were sent to a contract lab for histopathology. Five sections from the nasal tissue T2 cross section were examined which included parts of the olfactory, respiratory, and transitional epithelium. Histopathology was completed by the Experimental Pathology Laboratories (EPL). The effects were eosinophilic globules, atrophy, and vacuolation with olfactory epithelium appears to be the most sensitive site in the nasal cavity.

A summary of Phase 1 findings – The nasal tissue only showed minimal or mild grade while the lung showed moderate and severe grade in histopathology. The target site was found to be the intrapulmonary airways. In the nose the target cell is the olfactory epithelium, and in the lung is the club cells. The sex difference is equivocal, and oxidative stress has no effect.

Earlier this year DPR regrouped and reevaluated the mechanistic study. Phase 1 results did not answer the central question whether chloropicrin induces lung tumors in mice via mutagenic or

non-mutagenic mode of action and since the initiation of the mechanistic study back in 2016, there are significant advances in the field of cancer risk assessment.

A new framework was proposed by Cohen and colleagues in 2020. This framework provides a stepwise evaluation of the relevance of lung tumors in mice to human cancer risk. If lung tumors are formed in mice, the first question is whether the chemical is DNA reactive; meaning whether the chemical is mutagenic or non-mutagenic. If mutagenic then move on to answer if the chemical's metabolism in mice is similar to humans, in other words, whether the chemical induced lung tumors is through its metabolites, and same metabolites will be produced in humans, thus answer the question whether the lung tumors in mice is relevant in humans, if yes, then it is likely human carcinogen and if no, it is unlikely to be human carcinogen. If the chemical is not DNA reactive, then move on to examine whether it is cytotoxic, if yes, then followed by the analysis of regenerative proliferation. If not, then followed by examining whether it is a direct mitogen. Same for the cytotoxicity branch, we need to look into the metabolism relevance to humans. Phase I study showed that chloropicrin has cytotoxicity -the cell vacuoles. To reflect all the new science and answer the central question of DNA reactivity, DPR worked together with CMTF earlier this year and revised the next phase studies to fill the required data gaps. For the central question of DNA reactivity, DPR/CMTF agreed on two studies to address whether chloropicrin is mutagenic or not. One study would be in vivo mutagenesis following a 28-day inhalation exposure in mice using error-corrected next generation sequencing (ecNGS). And the second study would be ex vivo DNA adduct quantification using lung slices ex vivo culture and radiolabeled chloropicrin. During initial discussion, CMTF communicated to DPR about possible difficulty in obtaining radiolabeled chloropicrin, thus an alternative test was proposed. If the radiolabeled chloropicrin cannot be procured within three months after the starting of the study, then CMTF should proceed with in vitro DNA adduct formation using HepaCometChip assay. These two studies will help answer the central question whether chloropicrin is DNA reactive. Study 2 will examine the alternative mode of action cell proliferation to see if chloropicrin cancer risk can be evaluated by the threshold approach. Studies 4 and 5 will test the human relevance to see if chloropicrin induced lung tumors in mice is relevant to humans. Study 4 first test if chloropicrin caused cytotoxicity is mediated by CYP enzymes; if yes, then proceed with study 5 where CYP2F2 knockout or humanized (CYP2F1) mice will be used to evaluate human relevance. CYP2F2 knockout mice refer to mice without this particular CYP enzyme, thus if chloropicrin can induce cytotoxicity in these knockout mice, indicating the cytotoxicity is not mediated by this enzyme, otherwise suggesting CYP2F2 mediated mechanism. For humanized mice, the mouse CYP2F2 enzyme was replaced with the human isoform CYP2F1, which is used to see if chloropicrin can be metabolized by this CYP enzyme in humans and thus cause cytotoxicity.

These next phase studies will answer the central question: whether chloropicrin is mutagenic or non-mutagenic. As well as answer the question about the human relevance of lung tumors in mice. The outcome will direct DPR to use the appropriate method for cancer risk assessment for chloropicrin. And all studies are expected to be completed within one year.

Committee Comment

Garret Keating asked about study 4 on slide 24. How does the error-corrected next generation sequencing (ecNGS) inform about DNA adducts? And is the HepaCometChip an assay of measuring DNA adducts? Daisy (Qiaoxiang) Dong responded that the error-corrected next generation sequencing (ecNGS) is also called duplex sequencing. By sequencing both strands of DNA, it provides high resolution to detect DNA mutations. The contract lab has been able to validate known carcinogens using this assay. It detects stable mutation spectrum in various tissues after chemical exposure. It thus can be used to inform chemical's DNA reactivity. The HepaCometChip assay is a modified comet assay. This assay uses DNA synthesis inhibitors to block the nucleotide excision repair, which is the main repair pathway to remove bulky lesions such as DNA adducts. As we know, the regular comet assay can only detect single strand breaks, by blocking the nucleotide excision repair, this modified assay converts comet-undetectable bulky lesions into comet-detectable single strand breaks.

Garret followed up with a question on whether chloropicrin gas is reactive? Daisy answered that it was reactive.

Katie Sutherland asked about the enzymes tested in Study 4 whether it is any specific CYP enzyme. Daisy responded that it is a pan-CYP inhibitor, which inhibits all CYP enzymes, not specific to any particular CYP enzyme to see if chloropicrin induced cytotoxicity is mediated through CYP enzymes. The focus is on enzymes found in mice rather than rats due to the past study results of lung tumors in mice.

Lynn Baker thanked for the update.

Public Comment

James Nakashima asked, "The testing for CYP-mediated cytotoxicity (study 4) – is that an Ames-type in vitro test?" Daisy answered that it is not, and the study is trying to identify the mechanism, which can help answer the question whether lung tumors found in mice is relevant to humans.

1. **Agenda Items for Next Meeting**

The next meeting is scheduled for January 19, 2024, at 10:00 a.m. This meeting will be held virtually on the Zoom platform and broadcast live on the CalEPA webcast page. <video.calepa.ca.gov/>

2. **Adjourn**