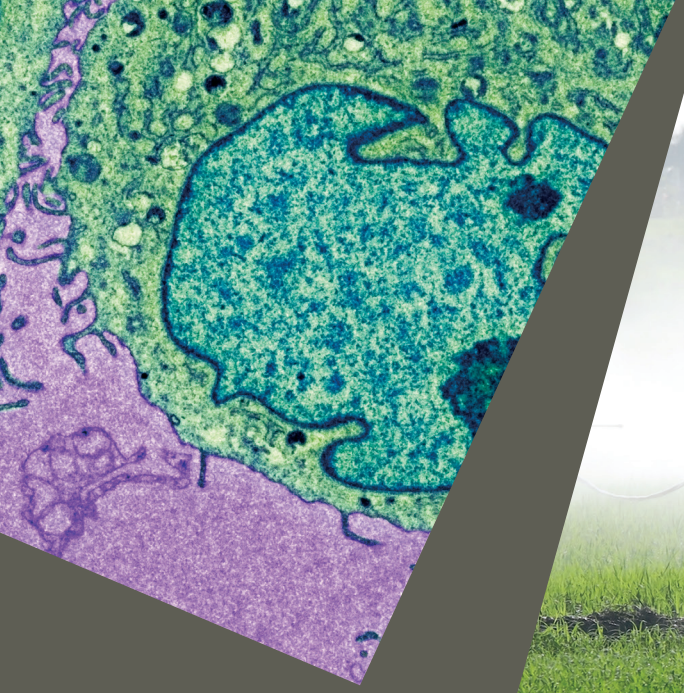

EXPOSURE AND INTERACTION

The Potential Health Impacts of Using Multiple Pesticides

stpp
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The Sustainable Technology & Policy Program, a joint undertaking of the UCLA School of Law and the Fielding School of Public Health, is an interdisciplinary research and education program. Its mission is to advance public health and environmental protection by integrating science, law, and engineering to promote prevention in public and private decision making.

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Appendices and Supplementary Materials

All appendices and supplementary materials mentioned in this report are available at: http://www.stpp.ucla.edu/node/586

KEY TERMS AND DEFINITIONS

Aggregate exposure	Combined exposure to a single stressor (e.g. a pesticide) across multiple routes and multiple pathways.
Cumulative exposure	Combined exposure to multiple stressors that affect a single biological target. This report focuses on the cumulative exposure to three commonly used fumigants, with a focus on their effect on cancer risk.
Cumulative risk	Combined risk from aggregate exposures to multiple stressors. There are different ways to consider cumulative risk.
Additive effects	When the effect of two or more stressors is equal to the sum of each of the agents when used alone. Often called dose addition, in this case the stressors do not interact in a direct way. Common Mechanism Groups (CMG) are an example of additive effects.
Interactive effects	When two or more stressors interact with each other to either amplify or reduce a toxic effect. One form of interactive effects is synergism, which is the focus of this report.
Synergistic effects	When different stressors interact in a way that makes their impact on toxicity greater than additive (e.g. multiplicative). This includes potentiation, when one substance that normally does not have a toxic effect accentuates the toxicity of a second chemical. Synergistic effects are the focus of this report, though the effects discussed are often referred to by the more general term interactive effects.
Common Mechanism Group (CMG)	When stressors act on the same target, and the mechanism by which they act is known, they can be classified as a CMG. CMG classifications can be used in CRA, but to date they have only been used to determine additive effects, not interactive effects.
Cumulative Risk Assessment (CRA)	An analysis, characterization, and possible quantification of the combined risks to health and the environment from multiple agents or stressors. The level of consideration of cumulative risk can vary. Most CRAs only consider additive effects.
Scientifically reasonable hypothesis	A hypothesis, in this case of potential interactive effects, based on scientific knowledge and judgment, and available toxicity data. If there is a scientifically reasonable hypothesis of interactive effects, additional testing and analysis should be completed to assess whether the hypothesis is accurate. This report adopts a purposely broad definition for scientifically reasonable hypothesis.

CONTENTS

I. INTRODUCTION

People are exposed throughout their lifetimes to mixtures of chemicals and other agents; this may result from numerous exposure circumstances, including contaminated drinking water, polluted air, intake of pharmaceuticals, use of cosmetics and consumer products, occupational exposures, proximity to industrial facilities, and pesticide exposures.

Conventional agriculture relies heavily on pesticides, often applied as mixtures of products. Each chemical in the mixture targets different soil pests, and co-application saves farmers time and fuel. With a few exceptions for known interactions between pesticides that alter their pesticidal activity, there are no label restrictions on combining pesticides. Exposure to multiple pesticides is thus widespread, from the most heavily exposed farm workers, to neighbors adjacent to or downstream from pesticide application sites such as agricultural fields or structural fumigations.

Increasingly, research shows that cumulative exposures can have larger than anticipated impacts on public health.[1] While the potential for interactive effects is recognized in both scientific and regulatory communities, pesticide testing requirements by the California Department of Pesticide Regulation (DPR) and the U.S. Environmental Protection Agency (EPA)—the state and federal agencies responsible for pesticide regulation—are currently focused primarily on the effects of a single chemical. While there are some efforts underway to assess pesticides with common mechanisms of action,[14] pesticides are still assessed and regulated assuming exposure to only a single chemical. Assessing the risks of multiple exposures is challenging for already resource-stressed regulatory agencies, but is essential for fully understanding the potential for adverse health effects.

This report evaluates the possible cumulative health effects of three pesticides commonly used in California, and makes policy suggestions for implementing a framework for cumulative risk assessment that accounts for potential interactive effects, not just those occurring by the same mechanism of action.

A. FUMIGANT USE IN CALIFORNIA

Fumigant pesticides are highly toxic chemicals that exist as gases or liquids that vaporize readily at ambient temperatures. Fumigants (or their breakdown products) are applied at high rates—typically 50–400 pounds per acre—characteristics that make it difficult to control off-target drift to neighboring properties. Because of their toxicity and ability to permeate soil and other materials, fumigants are effective pesticides. They allow the planting of the same crop in the same field year after year, reducing the need for crop rotation to control soil pests. However, the very same characteristics that make these chemicals effective as pest control agents—their toxicity and volatility—have also led to numerous acute poisonings of workers and neighbors, and pose risks for birth defects and chronic health effects such as neurological damage, respiratory disease, and cancer.

California has the highest use of fumigants in the U.S., primarily because of the large acreage of high-value specialty crops. They are intended for use to control insects, plant parasitic nematodes (worms), soil-borne pathogens, and weed seeds for field grown strawberries, peppers, tomatoes, stone fruits, tree nuts, grape vines, ornamental plants and turf, as well as nursery plants. They are also used in commodity fumigation to kill insect pests in stored grains and nuts and for quarantine treatments of produce and timber transported internationally. In California, approximately 30 million pounds of soil fumigants are used every year (see Figure 1), accounting for approximately 20 percent of total reported pesticide use in the state in most years. Although the mix of fumigants has changed over the years, the total annual use has been relatively

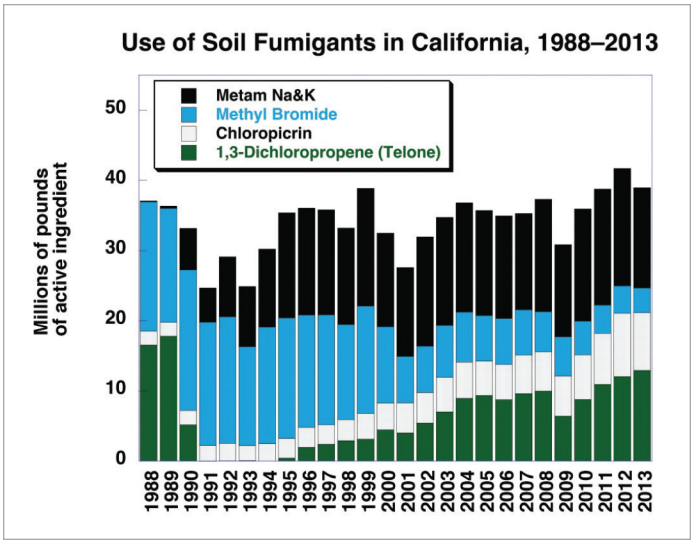


Figure 1: Fumigant use in California fluctuates between 30 and 40 million pounds per year.

constant over time. Within California, the regions of highest chloropicrin and Telone use are the strawberry-growing areas of the Central Coast (Monterey and Santa Cruz counties primarily) and South Coast (Ventura, Santa Barbara and San Luis Obispo counties primarily), with additional use in the San Joaquin Valley for orchard and vineyard replants, carrots, peppers and tomatoes. Metam sodium use is the highest in Fresno and Kern counties.

B. FUMIGANTS AS A CASE STUDY

Chloropicrin, metam sodium, and 1,3-dichloropropene (Telone®) are key fumigant pesticides used in California. While the EPA and/or California Department of Pesticide Regulation (DPR) have conducted risk assessments of these three agents on a chemical-by-chemical basis, neither agency has addressed the potential for interactive effects among these fumigants in exposed populations. This shortcoming presents a potentially serious health concern, because toxicological responses to mixtures of chemicals can be quite different compared to responses to each chemical separately.

This report focuses on pesticide mixtures, using Telone, chloropicrin, and metam salts as a case study,* and seeks to demonstrate three key points:

1. **It is possible these pesticides interact to increase human health hazard.** The report investigates the potential for interactive effects among these agents. The report first introduces the concept of cumulative exposure and risk assessment, and summarizes the current state of the science and policy on this issue. Then, the report assesses the health risks from simultaneous or sequential exposures to Telone, metam sodium, and chloropicrin, identifies health effects reported for each of these chemicals, and assesses the mechanistic bases for potential interactive effects. This section of the report identifies critical data gaps that impede full evaluations of the cumulative health risks from exposure to these three fumigant pesticides, and recommends expanding the range of studies and computational techniques to better understand and characterize interactive effects that might occur at the cellular and molecular levels and result in enhanced adverse health outcomes.
2. **Some residents of California are exposed to these pesticides simultaneously and/or sequentially.** The report investigates use of these three fumigants, and concludes application of multiple pesticides is common, and surrounding communities are likely exposed.
3. **DPR is required to assess the risk of cumulative exposure and protect public health, but is not currently doing so.** This section reviews the adequacy of existing risk assessment approaches and regulatory policies toward cumulative exposures. It suggests actions regulators in California could take to develop science-based risk assessments of chemical mixtures that would guide regulatory agencies in setting exposure limits that protect human health from cumulative exposures.

*Methyl bromide was not included in this study despite its widespread use because of its anticipated phase out. However, the authors acknowledge its continued use is significant and is likely to contribute to interactive effects.

II. CUMULATIVE EXPOSURE AND RISK ASSESSMENT

A. TYPES OF INTERACTIONS: NOT ALL MIXTURES ARE CREATED EQUAL

Chemical exposures do not happen in isolation; everyone is exposed to a range of chemicals on a daily basis. This includes aggregate exposures to a single chemical, as well as cumulative exposure to chemical mixtures. The combined toxicological effects of these cumulative exposures usually take one of two forms:[2]

- ***Additive effects:*** The effect of two or more chemicals is equal to the sum of each of the agents when used alone. Often called dose addition, in this case the compounds do not interact in a direct way. Mixture constituents acting via dose addition generally belong to a Common Mechanism Group (CMG), meaning the detailed biological steps leading to particular disease or toxic effect are mechanistically identical. Alternatively, two or more chemicals could have a similar effect on a particular target organ. Therefore, even when individual compounds are present at concentrations below their respective No Observed Adverse Effect Levels (NOAELs), combined exposures could result in measurable effects due to the combined doses of individual components in the mixture.
- ***Interactive effects:*** Two or more chemicals can interact with each other to either amplify or reduce a toxic effect. This is particularly true when a scientifically reasonable hypothesis exists for enhancing or reducing a particular effect by metabolic inhibition or induction of enzymes responsible for detoxification. For example, activation of cytochrome P450 by organophosphates can decrease an organism's ability to detoxify pyrethroids, so greater-than-additive toxicity may be observed during periods of simultaneous exposure.[3] Interactive effects can lead to responses that are greater than or less than those predicted using simple dose-addition models:
    - **Synergistic—greater than additive.** This type of effect includes potentiation, when one substance that normally does not have a toxic effect accentuates the toxicity of a second chemical. It also includes synergistic effects, when the combined effect of two chemicals is greater than the sum of each agent individually.
    - **Less than additive.** This type of effect is called antagonism, when the toxicity of one chemical is reduced as a result of the presence of the other chemical. This type of effect can occur when one chemical induces the production of enzymes responsible for the degradation and clearance of the other chemical, thereby reducing its effect.

This report focuses on **synergistic effects, and uses the term interactive effects interchangeably**. The largest collection of knowledge regarding interactive effects relates to drug-drug interactions. Indeed, drug-drug interactions are so common and have such potentially severe effects that there are multiple references to aid physicians, pharmacists and patients in avoiding undesirable combinations of drugs.[4,5,6] For environmental exposures, epidemiological data and a meta-review of common chemicals also provide evidence of greater than additive interactions between compounds. For example, greater than additive interactions have been shown between cigarette smoke and arsenic[7,8] or asbestos exposure[9,10,11] in increasing the risk of lung cancer. These examples arose from epidemiological data, and raise the concern that there may be many more examples of interactive effects among different environmental toxicants. This is particularly true for fumigants, which are often applied together or in quick succession, and which routinely drift away from the application site, resulting in widespread exposures.

Published meta-reviews and drug interaction studies also indicate that greater than additive effects are prevalent. Recently 174 cancer biologists and environmental health scientists from 28 countries examined the possibility that low levels of chemical mixtures in the environment may be combining to contribute to environmental carcinogenesis.[1] Based on their analyses of dose-response relationships for 85 chemicals on 11 phenotypic hallmarks of cancer, the

authors suggested that the cumulative effects of individual chemicals, even those that have not been identified as human carcinogens, may act on different cancer pathways to synergistically produce carcinogenic effects at low exposure levels.[1] There are no epidemiological data or meta-review to use in assessing potential interactions between the three fumigants in this case study, so instead this report assesses potential mechanisms of interaction. We review four possible sources of interactive effects—direct chemical reactions among the fumigants, decreased cellular capacity to detoxify, inhibition of DNA repair, and inhibition of enzymes that regulate gene expression.

Increasing awareness of the potential health impacts of cumulative exposure has led regulatory agencies to develop approaches to cumulative risk assessment. The next section summarizes existing approaches.

III. CURRENT STATE OF THE SCIENCE ON INTERACTIVE EFFECTS

Regulatory agencies and the international health community are increasingly recognizing the potential risks associated with cumulative exposures to chemical residues. This includes both aggregate exposures to a single chemical as well as exposure to chemical mixtures. Indeed, the Food Quality Protection Act of 1996 (FQPA) mandated the inclusion of exposure through multiple pathways and routes—such as food, drinking water, and occupational activities—in the risk assessment of pesticides.[12] FQPA further directed U.S. EPA to include a focus on the cumulative effects of pesticides that have a common mechanism of toxicity, considering dietary and non-occupational pathways of exposure in its assessments of pesticide safety.[13] However, there are no statutory requirements for the evaluation of the random chemical mixtures to which humans are frequently exposed, despite evidence from the scientific literature indicating interactive effects may be possible for chemicals acting through different modes of action.[2]

The situation with pesticides stands in contrast to the work done on pharmaceutical compounds. Drug interactions are common,[14] and systems and safeguards are in place to ensure that physicians and pharmacists do not inadvertently prescribe drugs that can produce serious or life-threatening interactions.[15,16,17] Every prescription drug approved by the U.S. Food and Drug Administration (FDA) for human use comes with FDA-approved labeling. Product labels for both prescription and over-the-counter drugs must indicate known interactions with foods and other drug products.[18] For example, the drug labeling for statins includes warning statements regarding the potential for adverse interactions with chemical components of grapefruit juice.

A. CURRENT APPROACHES FOR ASSESSING CUMULATIVE TOXICITY

A consortium of 16 laboratories sponsored by the European Commission recommended "precautionary actions on the assessment of chemical mixtures even in cases where individual toxicants are present at seemingly harmless concentrations" based on the results of a series of mixture studies.[19] The National Academy of Sciences recommends "exploration into interactions of exposures to chemicals that have similar or different Modes of Action (MOAs) but affect the same toxicologic process."[20] The 1996 amendments to the Safe Drinking Water Act required consideration of chemical mixtures in drinking water and recommended that EPA conduct studies "to determine the prospects for synergistic or antagonistic interactions that may affect the shape of the dose-response relationship of the individual chemicals." Synergistic effects are not considered in EPA's cancer risk assessments; these assessments assume independence of action of individual substances.[21] There is some reason for this as interactions with background processes tend to approach linearity at the limit of low doses.[22] On the other hand, some greater than additive interactions are possible when chemicals with interacting mechanisms make appreciable incremental contributions to cancer risks at relatively large doses.

There are several approaches for assessing interactive effects currently in use or in development by regulatory agencies. To date, only one of these approaches—the Common Mechanism Group—is applied to pesticides in the U.S.

Common Mechanism Groups: Compounds acting at the same molecular target belong to the same common mechanism group (CMG) and may be identified as such for toxicological purposes. Examples of CMGs include organophosphate pesticides known to act on the enzyme acetylcholinesterase and dioxin-like compounds, which bind the aryl hydrocarbon receptor. A substantial amount of information is required to determine membership of a CMG, leading to a relatively small number of established CMGs for the purposes of cumulative risk assessment. Based on EPA methods, preliminary identification of a CMG should be based on one or more of the following criteria: similarities in chemical structures, mechanism of pesticidal action, general mode/mechanism of mammalian toxicity, or common specific toxic effect(s). The general approach for assessing risk from exposure to multiple members of a CMG is to assign Relative Potency Factors (RPF) to each chemical in the group compared to an index chemical and convert each chemical exposure into the equivalent exposure level of the index chemical. Addition of all exposures then provides the total dose in units of the index chemical.[13] The EPA cumulative risk assessment for organophosphorus insecticides provides a current example of this approach.[23]

Cumulative Assessment Groups: The European Food Safety Authority (EFSA) recently developed criteria for inclusion of compounds in a cumulative assessment group (CAG).[24] The classification of pesticides into CAGs is based on identifying compounds that exhibit similar toxicological properties in a specific organ or system. EFSA's Panel on Plant Protection Products and their Residues (PPR) has taken the first step of applying this methodology to define groups of pesticides exhibiting toxicity to the thyroid and central nervous systems. In addition, the PPR Panel has carried out a significant amount of preliminary work for the development of groups for effects on other organs/organ systems, such as the reproductive system, liver, eye and adrenals. Future work in this area will involve the gradual implementation of cumulative risk assessment for pesticide CAGs.

Assessment of Cumulative Exposures: California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) has developed guidance for evaluating differential exposures to pollutants based on race, socioeconomic status, age, health status, proximity to point source and other key factors.[25] Since this resource is not designed to assess molecular-level interactive effects, it is not discussed further here.

None of these approaches is being employed by DPR. To date, none of the methods used to assess cumulative effects by regulatory agencies accounts for interactive effects that could result in amplification of adverse effects, or antagonism that could reduce toxicity. Thus, the picture of interactive effects is incomplete, leaving potentially large gaps in the understanding of the potential impacts of pesticide chemicals. For further analysis of existing efforts at conducting CRA, please see Appendix A.

B. LABORATORY EVALUATION OF COMPLEX CHEMICAL MIXTURES

There are two possible experimental research tracks that could support cumulative risk assessment: one focused on strengthening individual chemical data for use in a Relative Potency Factor (RPF) approach for a Common Mechanism Group, or alternatively, experimental evaluation of whole mixtures could begin with the complex mixture and proceed to a determination of whether the mixture is associated with adverse effects.[26] The National Toxicology Program (NTP) of the National Institutes of Environmental Health Sciences has engaged in component-based mixture assessments of dose additive interactions as well as the experimental evaluation of complex mixtures. Comparison of these two approaches has shown that the CMG approach may not accurately predict the potential for genotoxicity and immunotoxicity.

These shortcomings have been attributed to interactive effects that produce larger effects than would additive interactions between components.[27]

High-throughput *in vitro* screening (HTS) assays are now being developed to complement the resource intensive and time consuming nature of *in vivo* toxicity testing using laboratory animals. The current animal-based toxicity-testing paradigm is too resource intensive to effectively address the data needs for understanding the potential risks associated with exposure to chemical mixtures.[27] In contrast, existing HTS technologies can evaluate hundreds of thousands of chemicals per week per assay. Therefore, applications of the HTS approach to mixtures has promise in resolving the resource and time restrictions that limit the fields of mixtures toxicity research and risk assessment. The NTP, EPA, the National Institutes of Health Chemical Genomics Center, and FDA have established a collaborative research program termed Tox21, which uses robotics technology to screen thousands of chemicals for potential toxicity.[28] In addition, EPA has initiated the *in vitro* and *in silico* screening of environmental chemicals for targeted testing and prioritization under the ToxCast Program.[29,30] HTS approaches such as those used in the Tox21 and ToxCast projects could potentially lead to the rapid determination of hazards associated with exposure to individual chemicals and complex mixtures, and provide information on the mechanisms by which interactive effects occur. However, these technologies have not yet been extensively applied to the detection of interactive effects. Additionally these methods are most straightforwardly adaptable to detection of interactions that occur within individual cells. Interactions that involve system-wide pharmacokinetic or pharmacodynamic mechanisms will not normally be detected by any means other than *in vivo* testing in whole animals.

C. CAUSAL MODELING OF INTERACTIVE EFFECTS

Data limitations have made it difficult to conduct reliable quantitative risk assessments for potential health effects associated with exposures to chemical mixtures. However, in situations where mixture components act through a common mode of action but no significant modulating interaction occurs, the toxicologically relevant dose for the mixture is considered to be equivalent to the sum of individual constituent doses (i.e., dose addition). This approach, which has been developed by the U.S. EPA and the WHO International Programme on Chemical Safety, is used for toxic or carcinogenic chemicals that produced their effects by the same mode of action. The use of response addition to assess risks of mixtures is based on the assumption that the constituent agents act independently (i.e., by different modes of action) to cause the same disease outcome. These approaches do not address chemical mixtures that can produce synergistic carcinogenic effects due to the various constituents acting on different processes involved in carcinogenesis.

Mode of Action (MOA): Pesticides have a common mode of action when they "cause a common toxic effect to human health by the same, or essentially the same, sequence of major biochemical events."[121] It is used interchangeably with Mechanism of Toxicity.

Simultaneous or sequential exposure to a mixture of chemicals may cause interactions in the pharmacokinetics (time course for absorption, distribution, metabolism, and elimination) and pharmacodynamics (effects on dose/response, target organ toxicity) of the individual chemicals. Physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models can be employed to describe the mechanisms of action and tissue responses for individual chemicals and simple (e.g., binary) chemical mixtures.[31] Researchers have developed PBPK/PD models for a number of individual pesticide active ingredients, such as the carbamate insecticide, carbaryl,[32] and the organophosphate insecticide, chlorpyrifos.[33] Knowledge of PK or PD interactions can be quantitatively integrated into the PBPK/PD model for the chemical mixture. Although progress has been made in this area, relatively few examples of PBPK/PD models for pesticide mixtures have been validated and published in the peer-reviewed literature.[143,144]

This report now turns from a review of existing efforts at cumulative risk assessment to focusing on the three pesticides that form the case study.

IV. HEALTH EFFECTS OF FUMIGANTS

The three fumigants have known individual health effects. This section reviews what is known about individual fumigant toxicity, and highlights data gaps for key endpoints and measures of toxicity.

A. OBSERVED HUMAN HEALTH EFFECTS

Chloropicrin: Chloropicrin is a strong electrophilic agent that reacts with glutathione or protein thiol groups including those of hemoglobin. Its metabolites can also react with glutathione, and a possible intermediate of chloropicrin biotransformation is a highly toxic and reactive compound. Chloropicrin itself is a sensory and respiratory irritant.[69] Multiple episodes of community illnesses in California have been associated with the use of chloropicrin for agricultural pest control.[34] Respiratory effects of chloropicrin may pose greater health risks in persons with a pre-existing respiratory disease. Because of its irritant and corrosive properties, chloropicrin was used as a warfare agent during World War I.

Metam Sodium: Both metam sodium and its degradation products and metabolites are toxic irritants. In soil after the first day of application, metam sodium is rapidly converted to the degradation product, methyl isothiocyanate (MITC). Subsequently, MITC volatilizes, resulting in potential inhalation exposure for workers and the general public. Metam sodium and MITC are reactive chemicals that can be converted to other toxic chemicals, including methyl isocyanate (MIC), carbon disulfide (CS_2), and hydrogen sulfide (H_2S). Thus, use of metam as a fumigant can result in exposures of farm workers and neighboring residents to multiple reactive chemicals.

1,3-Dichloropropene (Telone): The EPA classifies Telone as a Group B2, probable human carcinogen.[35] Epidemiological studies show a correlation between increased incidence of pancreatic cancer and long-term residence in areas with the highest use of four pesticides, including Telone.[36] Telone also demonstrates acute toxicity to humans when inhaled.[35]

B. TOXICOLOGICAL EFFECTS OBSERVED IN LABORATORY ANIMALS

Each chemical demonstrates individual toxicity across a range of endpoints. Table 1 on the following page provides a summary of the toxicological effects of the three fumigants reported in laboratory animal studies. A more detailed list of available toxicology studies, including dosage and test animal information, is available in Appendix B.

C. ENDPOINTS OF SIGNIFICANT CONCERN AND HEALTH EFFECTS DATA GAPS

Table 1 summarizes known toxicity information for the three compounds, but some additional endpoints have incomplete information. This section raises two specific concerns about these compounds, and the lack of available data for certain relevant endpoints and calculations: 1) neurotoxicity; and 2) carcinogenicity, genotoxicity, and immunotoxicity.

Neurotoxicity

The paucity of available data on the neurotoxicity of the three compounds is concerning. The EPA requires 90-day neurotoxicity studies for conventional pesticide products and developmental neurotoxicity studies if the pesticide causes neurological effects in adult animals or humans.[64] Although there is no available information on the neurotoxicity of chloropicrin and Telone, metam sodium, and its degradation products show signs of neurotoxicity,[35,36,58,59,60,61] the EPA requirement for thorough evaluations of neurological effects of the three fumigants has not been satisfied. None of the three fumigants are included on an EPA-maintained list of developmental neurotoxicants (i.e., chemicals that damage the fetal and infant brain), probably due to inadequate data.[65] However, numerous pesticides and several chemicals with similar structures

Table 1: Summaries of the Toxicity of Three Individual Fumigants Across Key Endpoints

	Chloropicrin	Metam Sodium (and degradation products)	Telone
Clinical and Histopathology	*Inhalation:* Inflammation and epithelial hyperplasia of the nasal cavity and lungs in rats and mice.[37] *Oral:* Hematological changes and histopathological changes (inflammation, necrosis, hyperplasia, and ulceration) in the forestomach of rats.[38]	*Inhalation:* Erosive gastritis, pulmonary histiocytosis, and inflammation and epithelial hyperplasia of the nasal mucosal membrane in rats.[39] *Oral:* Stomach ulcerations and disorganization of the nasal cavity olfactory epithelium in rats, hepatocyte degeneration and necrosis and bile duct proliferation in dogs;[40] inflammation and bladder mucosal hyperplasia in mice.[41] *MITC:* Ocular and respiratory irritation. *MIC:* Tissue damage in respiratory, circulatory, and gastrointestinal systems.[41]	*Inhalation:* Increased incidences of degeneration and hyperplasia of the nasal epithelium in rats and mice, and epithelial hyperplasia of the urinary bladder and forestomach in mice.[42,43] *Oral:* Regenerative hypochromic microcytic anemia in dogs;[44] increased incidences of basal cell hyperplasia of the forestomach in rats and mice, nephropathy in rats, and epithelial hyperplasia of the urinary bladder in mice.[45]
Reproductive Toxicity	One inhalation study showed decreased litter size in rats at 2.0ppm.[46] Another rat study showed no effects on reproduction at 1.5ppm and below.[47]	*Metam Sodium:* One study showed a decrease in ovulation in rats,[48] while another two generation drinking water study showed no significant reproductive effects. *MIC:* Decreased female fertility.[49,50] *CS_2:* Reproductive toxin.	No evidence of reproductive effects in a two generation inhalation study in rats.[51]
Developmental Toxicity	*Inhalation:* Exposure of pregnant animals to chloropicrin induced skeletal variations in the developing fetus in rats,[46] and led to pre- and post-implantation losses, reduction in fetal body weights, and induction of visceral and skeletal variations in rabbits.[52]	*Oral:* Administration to pregnant rats caused an increase in resorptions due to early post implantation loss, suppression of fetal weights, and skeletal developmental delays.[53,54] Gestational exposure of rabbits induced early absorptions,[55] skeletal variants, and fetal malformations.[56] *MIC:* Spontaneous abortions, resorptions, suppresses fetal skeletal growth. *CS_2:* Developmental toxin.	*Inhalation*: No developmental effects observed after inhalation in rats or rabbits.[57]
Neurotoxicity	No available information.	*Oral*: Rats displayed altered behavior and decreased ambulatory and total motor activities.[58] *MIC*: Tissue damage to the central nervous system.[41] *CS_2:* Central nervous system toxin.[40] Neurological dysfunction has been demonstrated in experimental animals.	Clinical signs of possible neurological effects (lethargy, salivation, lacrimation, and labored respiration) were reported in rats, and ataxia of the hind limbs and loss of the righting reflex was reported in rabbits.[59,60,61] Other inhalation studies did not show clinical signs of neurotoxicity in rats, guinea pigs, rabbits, or dogs, although sensitive neurological tests were not included in these studies.[62,63]

are listed,* further underscoring that the EPA requirement for neurotoxicity testing of these three pesticides needs to be fulfilled, and developmental neurotoxicity studies of metam sodium and MITC as well as of fumigant mixtures should be required.

Carcinogenicity, Genotoxicity, and Immunotoxicity

The three fumigants in question display multiple hallmarks of cancer, including positive carcinogenicity tests in rodents and evidence of genotoxicity and immunotoxicity. Genotoxic chemicals

*Compounds on the EPA list that are similar to the three pesticides at issue here include S-ethyl dipropylthiocarbamate, aldicarb, CS2, trichloroethylene, 1,1,1-trichloroethane, dichloromethane, tetrachloroethylene, and other halogenated hydrocarbons.

damage genetic information within cells causing mutations, DNA strand breaks, and/or chromosome aberrations.

All three fumigants show signs of **carcinogenicity**. An epidemiological study noted an elevated risk for pancreatic cancer mortality among residents of areas with high use of Telone.[66] No adequate epidemiological studies have been published on the cancer risk of chloropicrin or metam sodium in humans. Table 2 provides a listing of the sites of tumor induction by the three fumigants in rats and mice and the cancer potency estimates derived by DPR (chloropicrin and metam sodium) or EPA (Telone). A more detailed list of available carcinogenicity studies, including dosage and test animal information, is available in Appendix B. The EPA has concluded that Telone is clearly a rodent carcinogen, and is "likely to be carcinogenic to humans."[67]

EPA and DPR cancer potency estimates for chloropicrin, metam sodium, and Telone may underestimate cancer risk. No adjustments were made for variability in human susceptibility, for children who generally have greater sensitivity due to their rapid growth and development, or for naturally occurring genetic differences in metabolizing genes or DNA repair genes that influence the ability to repair DNA damage.

All three fumigants also raise questions about **immunotoxicity**. Inhibition of immune surveillance may play a role in fostering the growth of tumors once the initial generation of malignant cells has occurred, and therefore may contribute to cancer.[69] Though exposure to chloropicrin resulted in signs of decreased immune function, no studies have been reported on the effect of chloropicrin on immune system function.[38,69] Similarly, no studies have been reported on the effect of Telone on immune system function.[63,67] The immunotoxicity of metam sodium in mice

Table 2: Summary of Evidence of Carcinogenicity and Hallmarks of Cancer.
Studies in blue are from rats, studies in green are from mice.

	Chloropicrin	Metam Sodium	Telone
Carcinogenicity	Mammary gland	Mammary gland (MITC)[a] Blood vessels—angiosarcoma Cutaneous fibrosarcoma (MITC)[a]	Forestomach Liver Adrenal gland Urinary bladder Forestomach Lung Liver
Genotoxicity	Positive Ames test; evidence of clastogenicity in hamster and human cells	Negative Ames test; evidence of clastogenicity in hamster and human cells	Positive Ames test; evidence of clastogenicity in mouse, rat, hamster and human cells
Immunotoxicity	Signs of decreased immune function[38]	Unknown	Unknown
Cancer potency (mg/kg/day)$^{-1}$	2.2 based on lung tumors in female mice[68]	0.19 based on angiosarcomas in male mice[40]	0.1 based on bladder tumors in mice; inhalation unit risk is 4E-6 ($\mu g/m^3$)$^{-1}$ based on lung tumors in mice[67]
Deficiencies in Available Data	Despite findings of toxicity to developing fetuses, chloropicrin has not been evaluated for its carcinogenic potential based on neonatal exposures.	Potency estimates are likely underestimated because of degradation of the chemical in the route of exposure used in the study. The lack of chronic/carcinogenicity inhalation studies on MITC is also a serious deficiency. Despite findings of fetal toxicity for metam, MIC, and CS_2, has not been evaluated for its carcinogenic potential based on neonatal exposures.	Cancer results were questioned because formulations in some studies contained a carcinogenic stabilizer, however, the concentration was not high enough to account for the multiple-organ tumor response of this fumigant.

is due to MITC and/or the parent compound and other decomposition products.[70] Thus, there is a critical need to fully evaluate effects of the three fumigants individually and in combination on immune function.

All three chemicals also show evidence of **genotoxicity**. Chloropicrin is a mutagenic and clastogenic chemical—a chemical that induces disruption or breakages in chromosomes. The parent compound or its metabolites induced mutations in *Salmonella*, chromosomal aberrations in rodent cells, sister chromatid exchanges in human lymphocytes, and DNA strand breaks in TK6 cells. These results are consistent with the electrophilic nature of this fumigant. Though conjugation with glutathione detoxifies chloropicrin, it also results in activation of this compound to a mutagenic intermediate.[71] Metam sodium was negative in gene mutation studies, but positive for clastogenicity in *in vivo* and *in vitro* studies. In Chinese hamsters and in cultured human lymphocytes (in the absence or presence of metabolic activation), metam sodium produced increases in the frequency of chromosome aberrations.[72] Telone is mutagenic in the Ames Salmonella test with or without metabolic activation, and is also a clastogen; it induced DNA damage in multiple organs of mice, among other genotoxic results.[67,73]

Results from toxicity testing of all three chemicals lead to concern that each fumigant independently may be carcinogenic in humans, and that cumulative exposure may increase cancer risk in an additive or greater than additive manner.

Summary of Deficiencies in Available Data

The review of toxicological data for the three fumigants reveals gaps in available information about these three chemicals when considered individually that need to be addressed to adequately assess potential health risks in exposed human populations. These gaps are described in Table 3 below.

Table 3: Data Gaps for Fumigant Pesticides

	Chloropicrin	Metam Sodium	Telone
1) Lack of studies on the toxicity and cancer risk from neonatal exposures	⚠	⚠	⚠
2) Not fully evaluated for carcinogenic potential in mice	⚠		
3) No chronic or carcinogenicity inhalation studies on primary degradation product		⚠	
4) Not adequately evaluated for immunotoxicity as required by EPA	⚠	⚠	⚠
5) Not adequately evaluated for neurotoxicity as required by EPA	⚠	⚠	⚠
6) Lack of studies on the impact of degradation products		⚠	

The metabolism and known health effects of fumigants also suggest the possibility of interactive effects, which the next section of this report explores.

V. POTENTIAL INTERACTIVE EFFECTS OF THE THREE FUMIGANTS

Until this point, this report has focused on the effects and data gaps associated with individual fumigants. It now turns to possible interactive effects between the fumigants. Although there are no studies on health effects of combined exposures to chloropicrin, metam sodium, and Telone, there are a number of scientifically reasonable hypotheses for interactive effects.

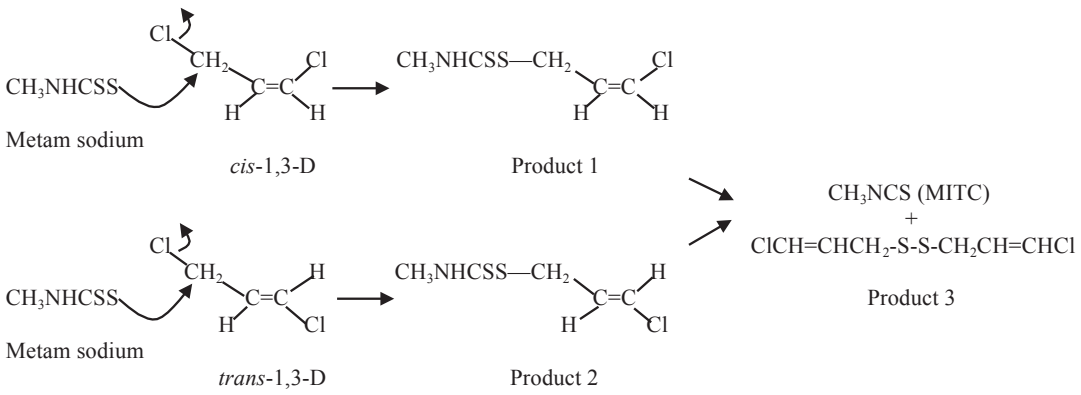
Based on existing knowledge of chemical and drug interactions, along with additional mechanistic information on the biological effects and potential interactions of these agents, this report attempts to identify and characterize potential interactive effects resulting from exposure to these toxic fumigants and to devise approaches to assess human health risks that might not be identified in assessments of the individual fumigants. This report focuses on (a) chemical reactions between the fumigants; (b) "primary level" interactive effects such as the depletion of the same detoxification enzyme cofactor or disabling of DNA repair enzymes that have sulfhydryl or amine groups in their active sites; and (c) the way these primary level interactive effects could combine to create secondary level interactive effects, such as the impact of combined genotoxicity and inhibition of DNA repair. These possible interactive effects are likely to impact the carcinogenic risk associated with cumulative risk, however, there may be interactive effects that impact other endpoints, such as reproductive, developmental, or neurotoxicity.

A. DIRECT CHEMICAL REACTIONS AMONG THE FUMIGANTS

If the fumigants are applied together or in close geographic or temporal proximity, the active ingredients may react with each other and form products that have toxic health effects. The halogenated fumigants chloropicrin and Telone have long been known to react with metam sodium. Because of this, some agricultural researchers have considered these fumigants to be "incompatible" even though they are occasionally used together by growers.[74]

At ambient application temperatures, all chloropicrin is converted to reaction products within an hour of application, and conversion of the Telone isomers to reaction products will likely be appreciable (see Appendix C). Particularly for the more rapidly-reacting chloropicrin, this raises a safety concern about what kinds of health effects the reaction products cause.

Reactions between the fumigants themselves are also possible. Figure 2 below illustrates one likely reaction between metam sodium and both isomers of Telone.



(Adapted from Zheng et al, 2004)

Figure 2: Reaction of Metam Sodium with Telone

The two reaction products both raise human health concerns. One product is MITC. The human health concerns of that substance are detailed above in the health effects section. The second product retains the double bonds from the original Telone which, like vinyl chloride, are likely to be converted to DNA-reactive epoxides. Whether the resultant molecule (and the intermediate addition product) will have greater or lesser carcinogenic potency than the Telone isomers is not yet known. This would be a natural issue for evaluation with future experimental work.

B. PRIMARY LEVEL INTERACTIVE EFFECTS

There are a number of ways the three pesticides might interact to affect the same cellular processes. This section details three opportunities for interactive effects: consumption of glutathione as part of pesticide metabolism, increased genotoxicity, and inhibition of DNA repair enzymes and DNA methyltransferases.

Consumption of Glutathione as Part of Pesticide Metabolism in Cells

Interactive effects may occur when the metabolism of two or more chemicals consume the same reagent. This could result in depletion of enzymes or cofactors responsible for chemical metabolism, leaving the organism more vulnerable to chemical effects. Alternatively, enzyme production may be up-regulated, which may alter metabolism of other chemical substances. As an example, we consider glutathione, one of the primary means of detoxifying electrophilic chemicals in mammalian systems.

Glutathione (GSH—a tripeptide composed of glutamate, cysteine and glycine) plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events, including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, and immune response, among others. Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and many diseases including Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes.[75] This section first explains how the metabolism of each of the three fumigants uses glutathione. It then assesses two possible scenarios for glutathione depletion—one based on glutathione synthesis and depletion throughout the body, and the other focused on localized glutathione levels in the lungs.

Glutathione detoxifies a number of compounds from the human body, including:

- **Fumigants**
- **Other environmental agents (*e.g.* air pollution)**
- **Reactive oxygen species (*e.g.* peroxides)**

If any one compound depletes GSH, it will make it harder for the body to metabolize other compounds that are detoxified by binding with glutathione.

Fumigant Exposure Depletes Glutathione

Glutathione conjugation is frequently a step in the elimination of toxicants via the kidney. As can be seen in Figure 3, glutathione is a cofactor for the metabolism of all of the fumigants—with chloropicrin consuming two to four moles per mole of fumigant, and the other fumigants consuming one mole of glutathione per mole of fumigant.

Chloropicrin is a strong electrophilic agent that reacts with glutathione or protein thiol groups including those of hemoglobin. The initial reaction of chloropicrin with glutathione forms a metabolite; this metabolite can react further with glutathione to form dichloronitromethane or monochloronitromethane or react with cysteine. Thiophosgene, a highly toxic and reactive compound, may also be an intermediate of chloropicrin biotransformation. Metabolism of the metam degradate MITC involves conjugation with glutathione forming mercapturic acid conjugates that are excreted in the urine. The major metabolic pathway for Telone involves conjugation with glutathione; the resulting mercapturic acid metabolite is excreted in the urine. A second metabolic pathway involves cytochrome P450-catalyzed epoxidation to 1,3-dichloropropene oxide, which can be detoxified by GST-catalyzed conjugation with glutathione. However, this epoxide intermediate can also undergo an internal rearrangement to 2,3-dichloropropanal (2,3-DCPA), which spontaneously eliminates HCl and forms the mutagenic carcinogen 2-chloroacrolein.[76]

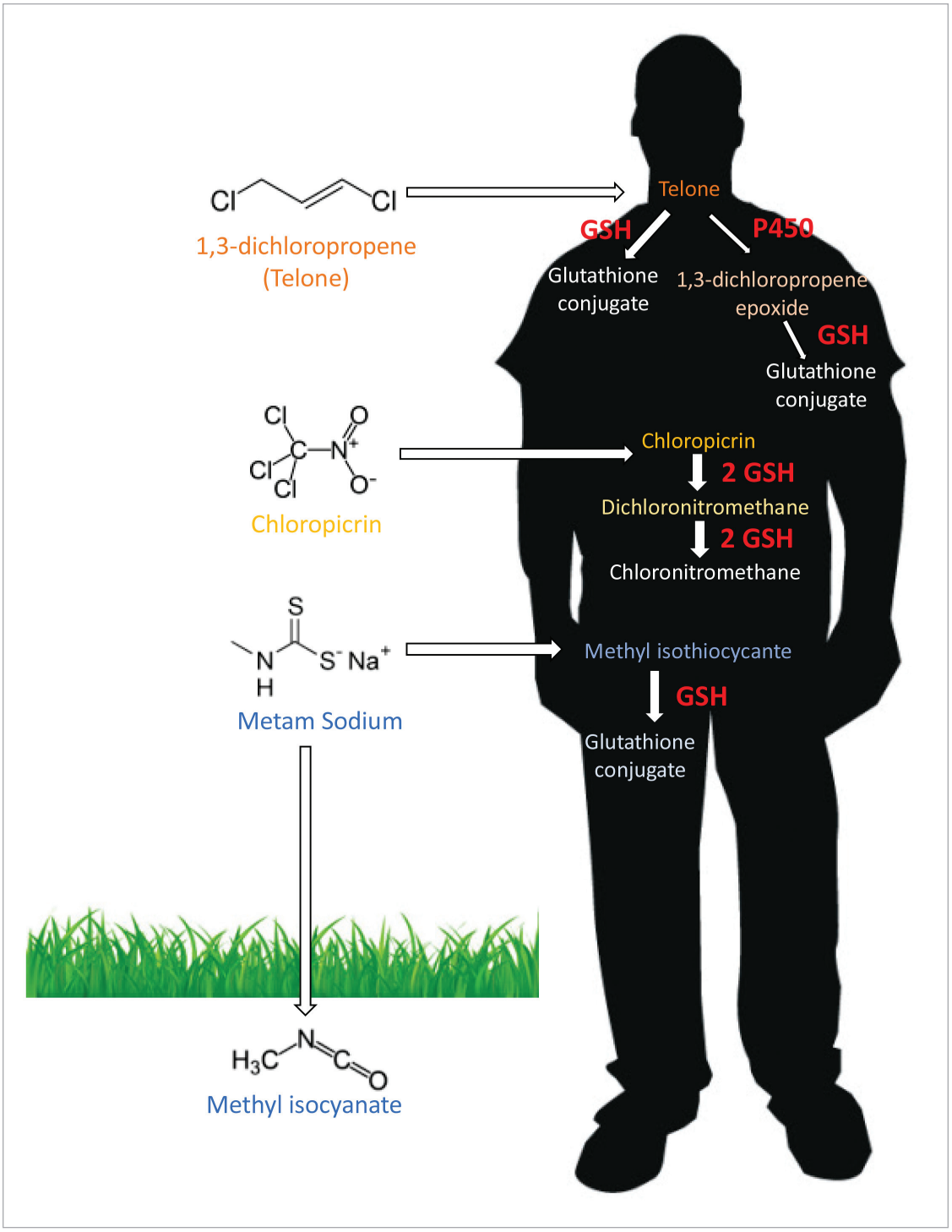


Figure 3: Metabolism of Three Fumigants in Human Body. Shows metabolic pathways for three fumigants. Metabolism is not tissue or organ specific, therefore, all three pesticides would use, and therefore deplete, the same pools of glutathione.

The human body naturally replenishes glutathione, but there is evidence that exposure to fumigants leads to decreased blood glutathione levels. In one study Dutch fumigators' blood glutathione levels were significantly decreased during the flower bulb culture season when they were exposed to Telone at time weighted average concentrations of 0.4-4.2 ppm.[77] Furthermore, natural genetic variation in genes coding for enzymes involved in glutathione synthesis have not been characterized, making it possible that certain individuals replenish glutathione less quickly, and are therefore more susceptible to glutathione depletion. If glutathione were to be appreciably depleted by reaction with one fumigant, this would lead to slower metabolic elimination of the other fumigants, thus enhancing their opportunity to react with cellular molecules and alter cellular functions.

Whole-Body Glutathione Depletion During Typical Fumigant Exposures

To determine if glutathione depletion is an issue of concern with fumigant exposure, it is instructive to calculate the rate of production of glutathione and compare it to the rate at which glutathione is used up by reaction with fumigants at typical concentrations in air, i.e. the flux of glutathione. Calculations (see Appendix C), suggest that for particularly vulnerable populations, exposure to just one of the fumigants (chloropicrin) at exposure levels predicted in DPR exposure assessments could result in the depletion of 10 percent of available glutathione (see Table 4). It would be challenging for these individuals to process simultaneous or possibly sequential exposures to other chemicals that also consume glutathione—including Telone and metam sodium. This may increase the amount of time that the fumigants, or damaging metabolites thereof, are in tissues and capable of causing other damage.

Table 4: Concentrations of the individual fumigants needed to deplete 10 percent of glutathione in the body of select demographic groups

	Chloropicrin needed (assuming 2 moles)	Chloropicrin needed (assuming 4 moles)	Telone or metam sodium needed (assuming 1 mole)
Average adult, light activity	0.63 ppm	0.32 ppm	1.26 ppm
Average adult, moderate activity	0.29 ppm	0.15 ppm	0.58 ppm
Adult, 95th %ile breathing rate, moderate activity	0.22 ppm	0.11 ppm	0.44 ppm
Elementary school children	0.34 ppm	0.17 ppm	0.68 ppm
Average infant, light activity	0.14 ppm	0.07 ppm	0.28 ppm
Infant, 90th %ile breathing rate, light activity	0.09 ppm	0.05 ppm	0.18 ppm

Estimation of Glutathione Depletion in the Liver and Lung Due to Fumigant Exposures

The previous section assessed the likely depletion of glutathione in the entire body. This section builds off that analysis to focus on glutathione depletion in the lung—the organ that first absorbs inhaled fumigants. There is insufficient available data to develop a full model of the expected depletion of glutathione in different tissues following exposure to the fumigants.

Because the exact rate of reaction with glutathione and other biomolecules containing sulfhydryl groups in lung tissue is not known, this report addresses that uncertainty with two polar cases:

- Case 1—assume the local reactions of the fumigants in the lung are so fast that nearly all of the fumigant molecules are destroyed before reaching the general circulation. This represents the “worst case” for depletion of glutathione in the lung.
- Case 2—assume that very little of the fumigants react locally in the lung—implying that glutathione stores in the lung and elsewhere in the body have a roughly equal chance to react with the fumigants, leading to relatively uniform percentage loss of glutathione in different tissues.

The calculations are summarized here; full calculations are available in Appendix C.

Table 5 below shows the expectations for case 1 (essentially complete reaction in the lung) for the expected percentage reduction in lung glutathione for various concentrations of chloropicrin exposure, assuming 4:1 consumption of glutathione. For metam and Telone the figures would be a quarter of those for chloropicrin.

Table 5: Expected Percentage Reduction in Lung Glutathione Under the Case 1

Time after start of exposure (hrs)	ppm chloropicrin exposure			
	0.5	1	2	4
	% reduction in lung GSH			
1	8%	15%	30%	60%
2	10%	19%	39%	77%
4	10%	21%	42%	84%
8	11%	21%	42%	85%

It must be stressed that these are in most respects the results of "worst case" calculations. Although sulfhydryl groups, such as those on glutathione, have the greatest reactivity with the fumigants, other groups such as amino and hydroxyl groups would be expected to consume some of the active metabolites made from chloropicrin and other fumigants. Moreover, it is quite likely that some, perhaps large, proportion of the inhaled fumigants would escape the lung to the general systemic circulation. On the other hand, the scenario represented in this table is for constant concentrations of fumigant over extended periods. In fact it is highly likely that actual exposures averaging the values heading the columns would have considerable hour-to-hour and even minute-to-minute fluctuations. Figuring likely true maximal GSH reduction levels which would potentially give rise to increased concentrations of Reactive Oxygen Species and prolonged internal half-lives of other fumigants would need to take into account the temporal variability likely in actual exposure scenarios and the competing reactions with biomolecules *in vivo*, based on new experimental observations *in vivo* in animals and in human and animal lung tissue explants *in vitro*.

Nevertheless, it should be noted that under these assumptions, exposures of several ppm would be expected to lead to appreciable reductions in lung glutathione, and corresponding increases in the expected reaction of other fumigants with cellular macromolecules—leading to enhanced toxicity and likely mutations that increase risks of cancer. A recent risk characterization document from DPR suggests that workers and to a lesser extent bystanders may be exposed to levels of Telone similar to those analyzed in Table 4, suggesting that if localized glutathione depletion is the predominant model, then exposed populations may have significantly reduced lung glutathione levels.[78]

Considering the estimates in Table 4, it is of interest to anticipate here the results of the exposure modeling detailed in Appendix D. Air dispersion modeling was done for a peak period of fumigant use in strawberry fields near Rio Vista High School in the Oxnard area of Ventura County. In all, the modeling yielded 7,384 predicted 7-day average exposures for different locations based on 2013 emissions and meteorological conditions. Of these, the largest value for chloropicrin was 0.11 ppm and the resulting glutathione depletion was not appreciably increased by the amounts of Telone and methyl bromide expected to be in the air based on modeling.

This result could be increased appreciably if the whole of the 7-day estimated exposures were delivered over a shorter time. It is likely that fumigants are applied and re-released into the air during daylight hours. If the cumulative 7-day exposures were released in a single 8-hour period, that would increase the expected air concentrations by about 168/8 = 21 fold. Applying this factor to the sum of the chloropicrin-equivalents for all three fumigants would yield a glutathione depletion potential equivalent to about 2.3 ppm of chloropicrin. Thus under these worst case assumptions, the estimates in Table 4 could yield glutathione depression approaching 40 percent—sufficient to raise concerns for some prolongation of the presence of reactive fumigant metabolites *in vivo* and likely some increased generation of reactive oxygen species.

There is also some indication that Case 2 may be a more likely scenario for at least some of the fumigants. A recent draft report by DPR on Telone referring to pharmacokinetic studies in both humans and rats indicates considerable persistence of Telone in people following a six-hour inhalation exposure.[78] Further follow-up of the cited pharmacokinetic studies will help refine estimates of the rate of reaction of at least this fumigant with glutathione in the lung and elsewhere in the body.

Genotoxicity

The three fumigants under consideration are genotoxic in a variety of *in vitro* and/or *in vivo* systems. Depending on the mechanism of action, the effects of these pesticides on genotoxicity may be more than additive. Few studies have examined the formation of DNA adducts by these fumigants. Such studies are needed to characterize the DNA reactive forms of each fumigant and the type of DNA alteration that they induce. *In vivo* and *in vitro* studies of mixtures of the three fumigants are needed to determine if genotoxic effects of these chemicals are additive or synergistic. If one of these fumigants affects physiological or biochemical processes that influence the genotoxicity of the other agents in the mixture (*e.g.*, alters their metabolism, their rates of clearance, or critical processes such as DNA repair or cell division rates), then such an effect could increase the genotoxicity of the mixture in a greater than additive manner. Studies demonstrating synergistic genotoxicity of other chemical mixtures have been reported.[79,80,81]

Studies demonstrate that chemical mixtures can have synergistic effects on genotoxicity.[79,80,81] Genome instability caused by genotoxicity underlies one of the hallmarks of cancer.[69] The results of the cancer studies available for the three fumigants indicate that a fumigant mixture containing chloropicrin, metam sodium, and Telone represents a multiple-organ carcinogenic risk to exposed populations. The potential magnitude of these possible interactive effects should not be underestimated, particularly in light of a recent assessment of chemical mixtures that has shown that even low dose exposure to chemicals not known to be carcinogens can lead to cancer.[1] If such synergism of low dose exposures to chemicals not labeled carcinogens can lead to hallmarks of cancer, it is entirely possible that real world exposures to these three pesticides could lead to similar increases in cancer risk, particularly given their genotoxicity.

Inhibition of Critical Enzymes Through Binding to Protein Sulfhydryl and Amine Groups

All three pesticides are electrophilic—i.e. they seek out negatively charged electrons. Electrophilic chemicals can attack both sulfhydryl and amine groups in proteins and can attack and mutate the nucleic acids that make up DNA. Some environmental agents may contribute to cancer development by binding to sulfhydryl groups in the active site of DNA repair enzymes and inhibiting their functional activities.[82] Also, electrophilic chemicals can induce abnormal DNA methylation and alter gene expression by inhibiting the activity of DNA methyltransferases by binding to sulfhydryl groups in the active sites of these enzymes.[83] The electrophilic pesticides can also be expected to attack amine groups thereby inactivating DNA repair enzymes with amine groups in critical locations. While there are no studies showing this experimentally, there are DNA repair enzymes that are known to have a lysine residue in their active sites, and electrophilic chemicals could be expected to attack the amine group of these critical lysines and inhibit the DNA repair function of the enzymes.[84,85,86]

DNA methylation is a process by which methyl groups (CH_3) are added to DNA. Methylation typically suppresses gene expression, therefore, any compounds that modify DNA methylation may alter when and how genes are expressed.

Inhibition of DNA repair and altered DNA methylation caused by chemical exposure can contribute to genomic instability by allowing cells with damaged or hypo-methylated DNA to produce abnormal daughter cells upon cell division. The Halifax project demonstrated that low-dose exposures to environmental chemicals may contribute to genomic instability by a variety of mechanisms including inhibition of DNA repair.[87]

The impact of electrophilic fumigants on DNA repair has not been fully studied. Because the three fumigants are electrophiles and can bind covalently with protein sulfhydryl and amine groups or other tissue nucleophiles, they may alter biological functions. Such reactions may account for the major toxicological effects of these agents. There is direct evidence of this for chloropicrin and the degradation products of metam sodium.[88,89]

Consequently, there is a critical need to characterize underlying mechanisms by which exposures to low levels of chemical mixtures, especially those containing electrophilic chemicals, can induce genomic instability and promote carcinogenesis.

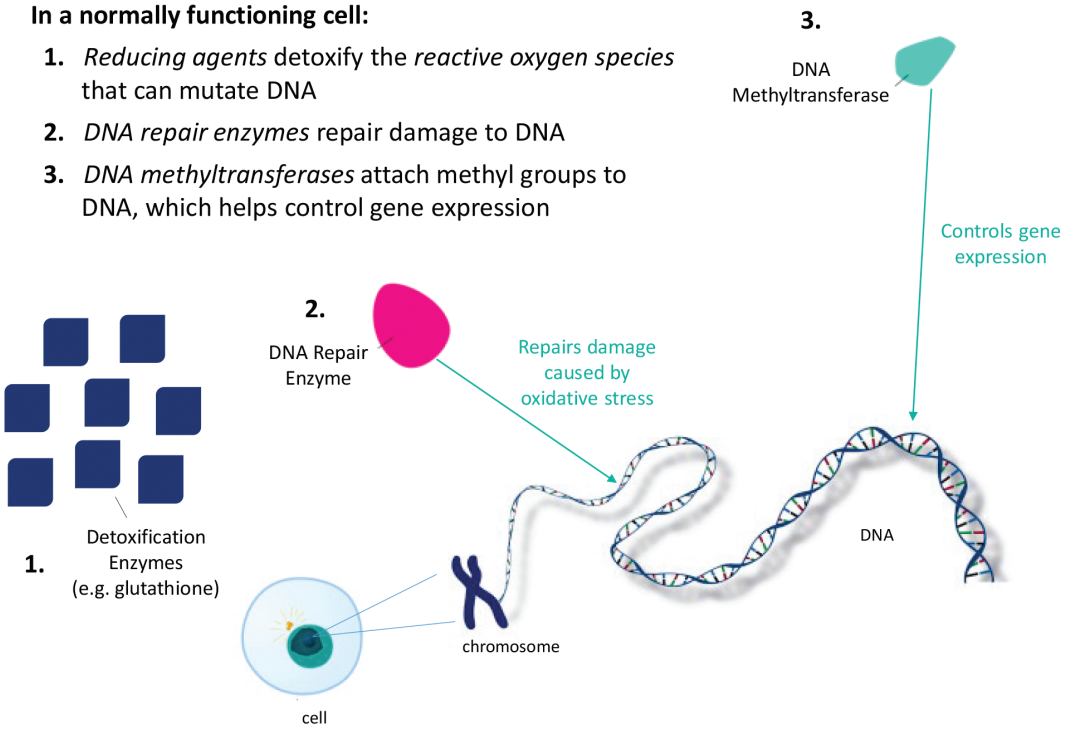
Table 6 below summarizes potential interactive effects between the chemicals and suggests testing that could be employed to investigate the risk associated with each effect.

Table 6: Possible Effects and Recommended Additional Testing of Three Fumigant Pesticides

Possible Interactive Effect	Assessment of Concern	Suggested Next Steps
Elimination rates of metam sodium and Telone are affected by chloropicrin exposures	Chloropicrin's consumption of 2–4 moles of GSH means it may deplete GSH in tissues and slow the detoxification of Telone and metam sodium. There are no available studies assessing the health effects of chloropicrin-induced GSH depletion or providing dose response data for assessing human risk.	Pharmacokinetic studies that provide blood time-course data on MITC, Telone, and Telone degradation products in rats exposed to chloropicrin
Enhanced oxidative stress due to decreased glutathione	Oxidative DNA damage, enhanced neurodegenerative diseases and immunotoxicity	Mixture study of the three fumigants, with measurements of reactive oxygen species and 8-hydroxy-deoxyguanosine in lung, liver and urine, and evaluations of neurotoxicity and immunotoxicity
Enhanced bladder carcinogenicity of Telone by metam sodium	Bladder hyperplasia induced by metam sodium might affect the dose-response for bladder tumor induction by Telone.	Evaluate bladder tumor response in mice exposed to both Telone and metam
Reaction of the electrophilic parent compounds or their metabolites and degradation products with protein sulfhydryl or amine groups	Electrophilic chemicals have been suggested to contribute to cancer development by binding to sulfhydryl groups and inactivating DNA repair enzymes or inducing abnormal DNA methylation and altering gene expression by binding to the cysteine thiol group at the active site of DNA methyltransferase.[85,86,90]	*In vitro* enzyme kinetic studies need to be performed to determine whether chloropicrin, metam sodium, and Telone or their metabolites inhibit the DNA repair enzyme O^6-alkylguanine DNA alkyltransferase or DNA methyltransferase, and other enzymes that have thiol groups at their active sites. *In vivo* mixture studies are needed to evaluate potential synergistic effects of these fumigants on tumor induction due to inhibition of DNA repair or altered DNA alkylation. mRNA analyses should also be performed to determine if the individual fumigants affect DNA methylation and gene expression in target organs and whether the patterns of altered gene expression are different for the individual fumigants compared to fumigant mixture.

In a normally functioning cell:

1. *Reducing agents* detoxify the *reactive oxygen species* that can mutate DNA
2. *DNA repair enzymes* repair damage to DNA
3. *DNA methyltransferases* attach methyl groups to DNA, which helps control gene expression



How the pesticides might interact to disrupt normal cellular function:

1. All three pesticides consume detox resources as part of their metabolism.
   - **1a.** This results in DNA-damaging compounds being present in the cell for a longer time.
2. The pesticides, their metabolites, and the compounds generated in 1a damage DNA.
3. All three pesticides may attack:
   - **3a.** Amino and sulfhydryl groups of DNA repair enzymes, impairing their ability to repair DNA damage.
   - **3b.** The sulfhydryl groups of DNA methyltransferases, impairing their ability to control gene expression.

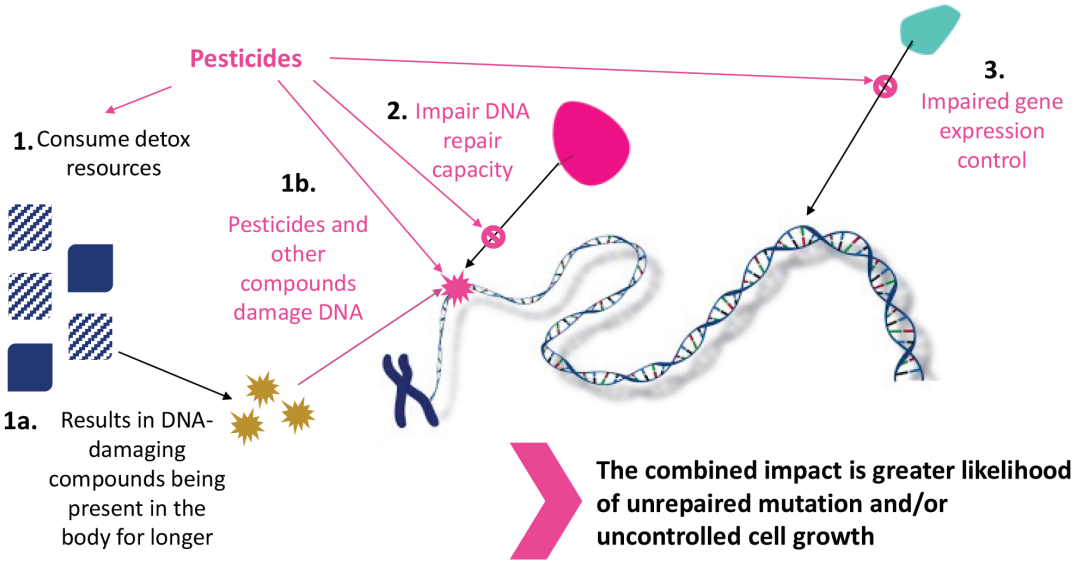


Figure 4: DNA Damage and Repair in Two Cells

C. PRIMARY INTERACTIVE EFFECTS MAY COMBINE TO CREATE SECONDARY INTERACTIVE EFFECTS

The previous section outlined the ways the three pesticides could have an impact on an individual cellular function like detoxification and DNA repair. The combination of these individual impacts may also cause greater than additive effects. For instance, many of the primary interactive effects impact the damage and repair of DNA. Thus, they may combine to create additional human health impacts, as was suggested recently by the Halifax project.[1] The greater than additive enhancement of liver cancer risk from exposures to both aflatoxin and hepatitis B virus (HBV) is an example of a potential carcinogenic synergistic interaction.[90,91] The mechanistic basis for this interactive effect has been attributed to a protein produced by HBV that inhibits excision repair of bulky DNA adducts, such as those produced by aflatoxin. Reduction in exposure to aflatoxin or HBV immunization has been effective in reducing liver cancer incidence.[92,93]

Figure 4 on the previous page demonstrates how these pesticides might demonstrate secondary interactive effects.

There is a need to identify and characterize mechanistic effects of these fumigants in targeted *in vitro* studies where the presence of multiple fumigants might affect the dose-response of the other agents in the mixture and to evaluate in animal models the effects of mixed exposures on the actual dose received of each fumigant and the combined health risks of the fumigant mixture. Relevant alternative testing strategies—including *in vitro* and *in vivo* studies—could also assist in determining what interactive effects might occur from exposures to two or more chemicals. Such work is vital given that residents of California are being exposed to these pesticides simultaneously.

VI. FUMIGANT EXPOSURES IN CALIFORNIA

The previous section detailed how interactive effects could occur between the pesticides and increase the risk of cancer or other human health harms resulting in greater than additive risk associated with exposure to multiple pesticides. This section provides information on typical exposures from fumigant use in California and demonstrates that co-application of fumigants is common, and thus co-exposures pose a real threat to Californians living in areas where fumigants are used.

A. SIMULTANEOUS APPLICATION OF MULTIPLE FUMIGANTS IS COMMON

The three fumigants studied here are often applied simultaneously or within a few days in California. An evaluation of the California Pesticide Use Reporting data[94] allows determination of the frequency of simultaneous applications of different fumigants, providing insights into the potential for multiple exposures and interactive effects. In 2013 over the entire state of California, there were 9,108 soil fumigant applications of chloropicrin, metam salts, dazomet, Telone, or methyl bromide. Twenty-six percent of these applications, accounting for 12.11 million pounds of fumigants applied, involved use of a product containing multiple fumigant active ingredients. Thirty-five percent (2.64 million lbs) of fumigants were applied on the same day to the same field (there is some overlap between the first group and the second). Only 42.2 percent of applications involved a single fumigant on a particular day. The most common mixtures used are chloropicrin/methyl bromide and chloropicrin/Telone.

Because of the seasonal use of fumigants, exposure is not evenly distributed over the course of a year (see Figure 5), and in high-use areas during peak application season, the potential for exposure to multiple fumigants is high.

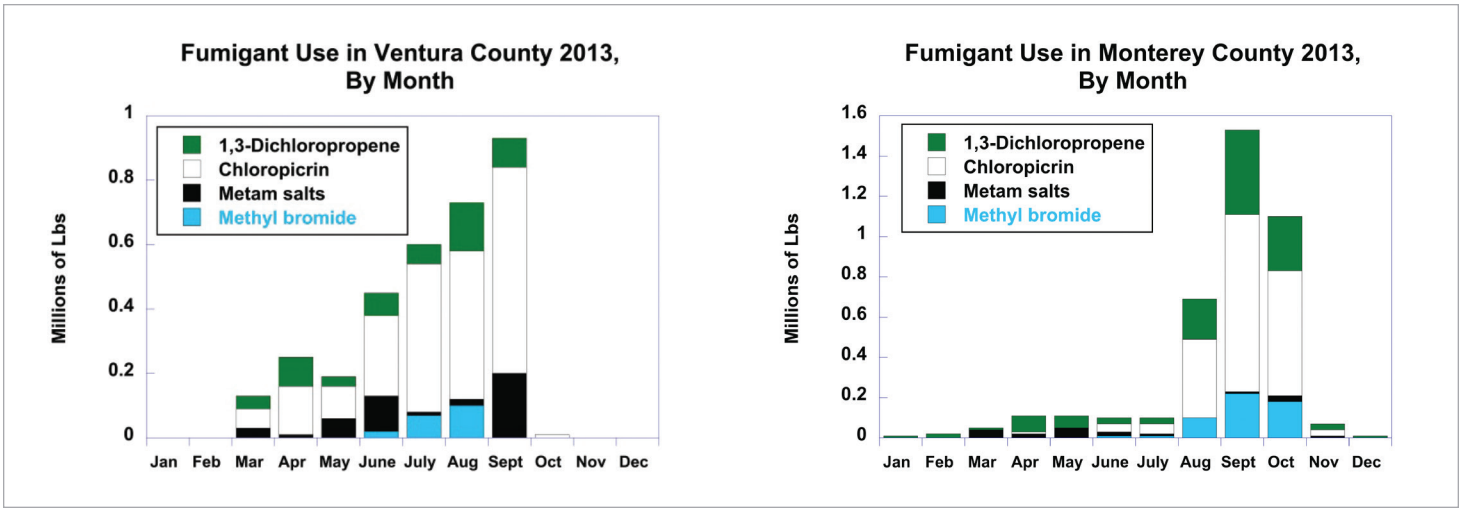


Figure 5: A mixture of fumigants is used in Ventura and Monterrey counties over the course of the year. Peak use occurs in the Central and South Coast regions in the fall with the replanting of the strawberry crop. Chloropicrin and Telone are often co-applied.

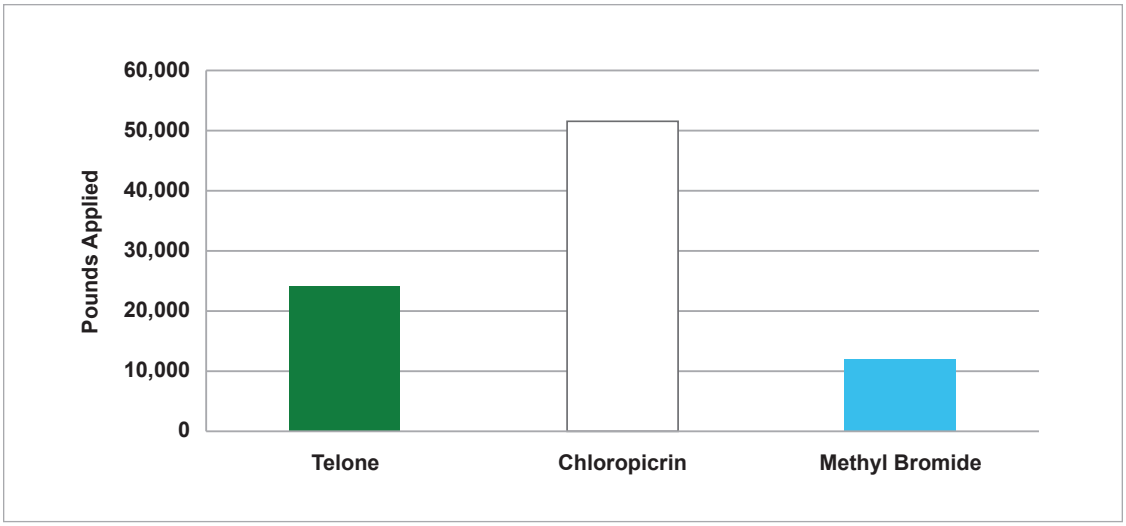


Figure 6: Fumigants Applied from July 26, 2013 through August 3, 2013 Near Rio Mesa High School

As a case study, this report focuses on fumigant use in a high-use area of Ventura County near Rio Mesa High School during a high use period in summer 2013. During the week of July 27, 2013, nearly 85,000 lbs of the fumigants Telone, chloropicrin, and methyl bromide were applied in the area surrounding Rio Mesa High School (see Figure 6). On every day of that week, multiple pesticides were applied, indicating a risk of co-exposure to multiple fumigants.

While fumigant use around Rio Mesa High School is high, this level of use is not uncommon for agricultural areas in the state of California. An interactive map showing the pesticide application rates by township* for weeks of fumigant use for a variety of locations in California is available at: https://www.pesticideresearch.com/site/?project=exposure-to-multiple-fumigants.

*A township is a six square mile block that is the foundational geographic unit of much of DPR's pesticide use management and monitoring.

B. FUMIGANT DRIFT IS COMMON, EXPOSING NEARBY WORKERS AND RESIDENTS

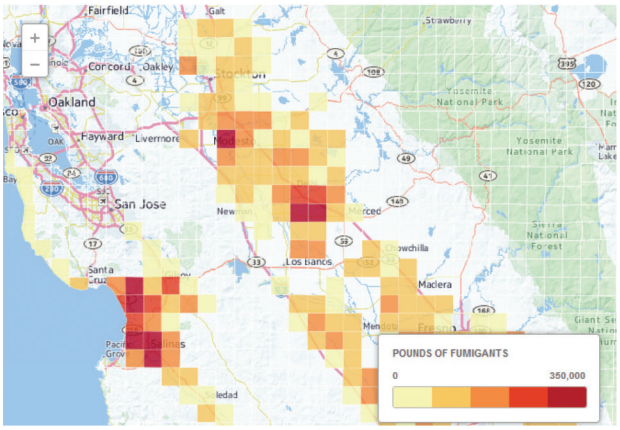


Figure 7: Snapshot of Pesticide Application by Township. See Interactive Map here.

The propensity of fumigants to volatilize and drift away from the application site results in exposure, both for workers involved in the application and for people living or working near the application site. A number of studies have been conducted under the mandate of the California Toxic Air Contaminant Act by DPR and the CA Air Resources Board (ARB) to determine typical concentrations of fumigants in air near application sites, as well as ambient concentrations in areas and seasons of high use to estimate longer-term exposures.[95] Fumigant concentrations in air have also been measured as part of DPR's Air Monitoring Network, a program started in 2011 to sample ambient air for multiple pesticides in three communities on a regular schedule.[96]

The occurrence of fumigants in air is a seasonal event that correlates strongly with crops grown in the area, fumigants used on those particular crops, and their replant schedules. For tree and vine crops, fumigation only occurs prior to new plantings. For annual crops like strawberries or carrots, fumigation typically is carried out annually. A typical exposure pattern for a fumigation is shown in Figure 8 below, with concentrations peaking during the first 24–48 hours after the application, and remaining measureable in the air for three to seven days afterwards. Peak concentrations are a function of application method, with untarped fumigations producing generally higher concentrations than those that are tarped.[97]

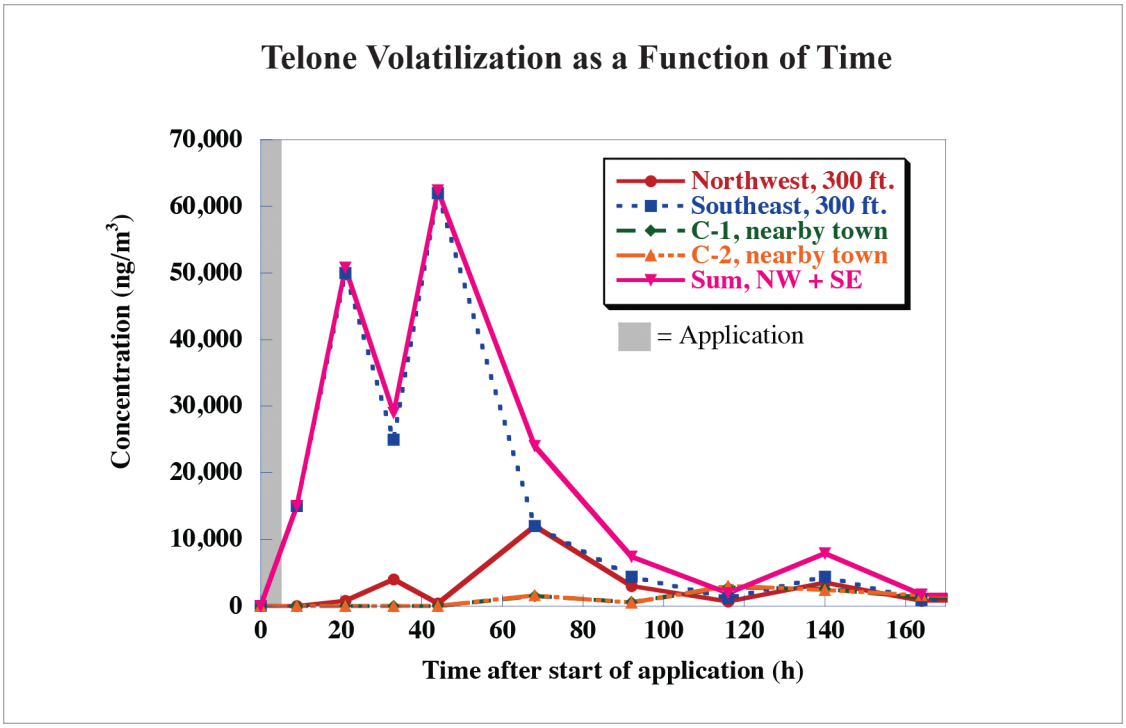


Figure 8: Typical exposure profile from an untarped fumigation utilizing Telone. The concentration peaks during the first day or two after the application, but remains measureable for a week. *Data source:* Reference 98

Exposure Profile: Chloropicrin

Chloropicrin use has increased substantially over the last twenty years (see Figure 1), as methyl bromide use has been phased out. The top crop for chloropicrin use is strawberries, accounting for 5.9 million pounds or 72 percent of the total used in 2013. High use areas include the Central Coast and South Coast regions of California. ARB has published three application site monitoring studies of chloropicrin and two ambient air monitoring studies.[99] Chloropicrin has also been documented in the Air Monitoring Network studies,[100] and by Drift Catcher Studies conducted by non-governmental organizations.[101]

Exposure Profile: Metam Salts Producing Methyl Isothiocyanate (MITC)

Fumigation using metam salts has remained relatively constant over the last twenty years (see Figure 1). Carrots, tomatoes and potatoes accounted for 4.8, 2.2, and 1.3 million pounds, respectively, in 2013, for 80 percent of the total use. ARB has published 10 application site monitoring studies and six ambient air monitoring studies of methyl isothiocyanate (MITC) derived from metam salts or dazomet.[102] MITC has also been documented in DPR's Air Monitoring Network studies.[100]

Exposure Profile: Telone

Fumigation using Telone has increased substantially over the last twenty years (see Figure 1). Soil, strawberries, and almonds accounted for 3.8, 2.4, and 1.2 million pounds, respectively, in 2013, for 57 percent of the total use. After DPR completely suspended use in 1990 due to concerns about excessive cancer risk, new application methods were introduced and use resumed on a limited basis in 1995, with a maximum cap of 90,250 pounds per year per township (a 6 mi x 6 mi area).[103] Over time, DPR relaxed the strict requirement of township caps, but air monitoring conducted in 2011 and 2012 indicated that measured levels of Telone in air exceeded DPR's regulatory target level. In February 2014, DPR ended the practice of approving applications above the township caps until the Telone risk assessment has been completed. ARB has published one application site monitoring study of Telone and 12 ambient air monitoring studies in regions and seasons of high use.[104] Telone has also been documented in the Air Monitoring Network studies,[100] and by Drift Catcher Studies conducted by non-governmental organizations.[101]

Rio Mesa High School Case Study

The case study of the area near Rio Mesa High School illustrates how these chemicals volatilize and spread. A simulation was generated using a standard EPA model of air dispersion, and the following location and time specific input data:

- The type, amount, location, and timing of pesticide use in this area for the period from July 26, 2013 through August 3, 2013. This information was gathered from Pesticide Use Reporting (PUR) and Notice of Intent (NOI) documents filed with the Ventura County Agricultural Commissioner's office.
- DPR established emissions ratings for each of the fumigants considered. DPR has established emission ratings for field fumigants. An emission rating is described as: "the emissions of fumigant to the air under field conditions, expressed as a proportion (percentage) of applied fumigant, and is fumigant- as well as application method-specific."[105] An emission rating can be thought of as the total amount of fumigant expected to volatilize during and after the application. For methyl bromide, and the fumigation method used by the applicable strawberry growers, the emission rating is 48 percent; for chloropicrin, the emission rating is from 12 to 44 percent (depending on the field fumigation method); and for Telone, the emission rating is 19 percent.[97]
- Rural dispersion coefficients.

- Local terrain maps and meteorological data from local weather stations during the week in question.
- A list of local "receptors," i.e. sensitive locations like schools, day cares, and parks.

The model employed was USEPA's AERMOD air dispersion model, version 15181, obtained from the Support Center for Regulatory Atmospheric Modeling (SCRAM) website. Version 15181 is the latest version of the AERMOD model, which was publicly released on June 30, 2015. Additional information about the methodology for exposure modeling is available in Appendix D.

The results of the modeling for the period are shown in Figure 9. The results demonstrate exposure to multiple pesticides occurs at a number of locations in the area, including sensitive sites like schools and day cares. The exposure is shown in total micrograms (µg) over a nine-day period assuming a standard breathing rate of 20 cubic meters per day. The purpose of the map is to demonstrate that significant co-exposure is happening. Assessing the concentrations residents were exposed to at specific times would require further disaggregating the modeling data into smaller periods of time and is beyond the scope of this report.

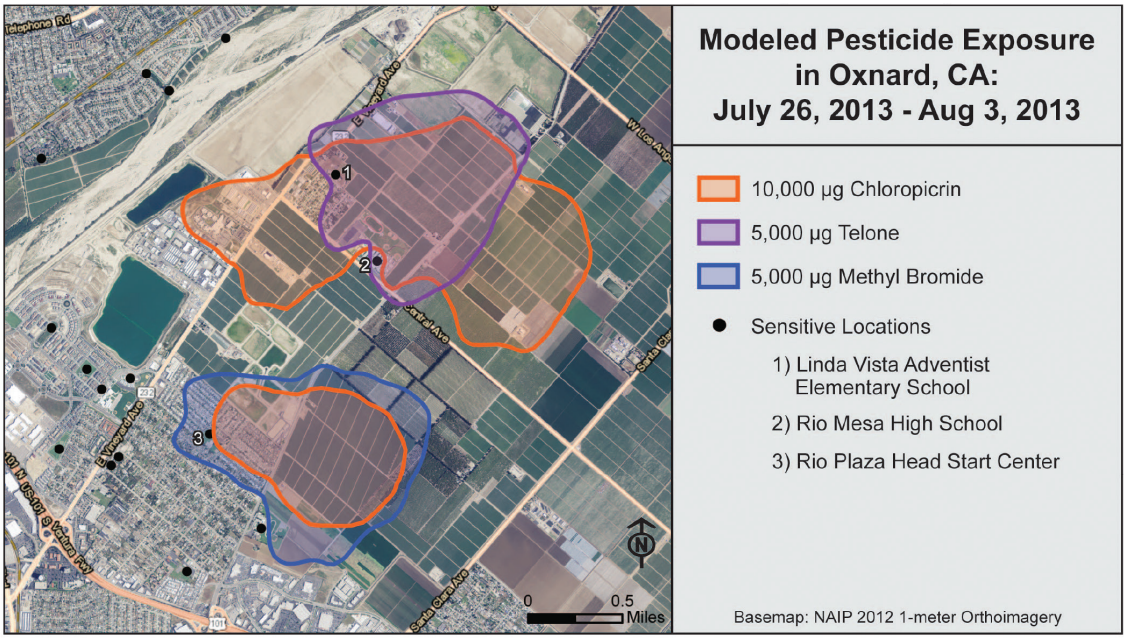


Figure 9: Exposure Isopleths Around Rio Mesa High School for the Period July 26, 2013 – August 3, 2013

This map and the statewide pesticide use data presented demonstrates that co-exposure is likely to occur in a number of communities across California. Human health risks from simultaneous or sequential exposures to Telone, metam sodium, and chloropicrin may be significantly greater than the added risks of the individual components. Because these fumigants are often applied in combination, the cumulative health risks of this mixed exposure need to be carefully assessed. This report now turns to the legal and policy guidance on how and when to assess these cumulative risks.

VII. RECOMMENDATIONS FOR EVALUATION AND REGULATION OF PESTICIDE MIXTURES

Based on the health effects of the fumigant pesticides highlighted above, it is clear that these reactive agents are toxic and carcinogenic in multiple organs. In addition, there are scientifically reasonable hypotheses for interactive effects that would not be detected in studies of the individual agents. The exposure assessment section demonstrates that co-application of these fumigants is common in California communities. Possible interactions include the depletion of glutathione by multiple pesticides or the combined effect of genotoxicity and possible inhibition of DNA repair enzymes. Recent publications from the Halifax Project stressed the plausibility of non-carcinogenic chemical mixtures in the environment producing synergistic carcinogenic effects at low exposure levels by acting on different cancer pathways.[1] While regulatory agencies recognize that exposures to multiple chemicals can pose a greater health risk than exposures to single chemicals and that there is a need to address potential risks associated with cumulative exposures, most risk assessments are still conducted on a chemical-by-chemical basis. This is because other than applying dose addition methodologies for agents in a mixture that act at the same site or by a common mode of action, there is no generally agreed approach for how to evaluate health risks from multiple chemical exposures when interactions with different pathways leading to toxicity may produce synergistic effects.

Simultaneous or sequential exposures to multiple fumigant pesticides may produce interactive effects. Regulatory agencies lack sufficient methods for assessing the impact of interactive effects of chemical mixtures on human health risks—CMGs cover only some potential common mechanisms of action, and there is no method for assessment of interactive effects that do not fit the narrow existing definition of a common mechanism. For example, this report identified examples of potential, but plausible, interactive effects among these fumigants. While a common mechanism has not been identified, it is likely that due to their electrophilic nature. The parent compounds and/or their metabolites or degradation products bind to tissue nucleophiles (e.g., glutathione and/or protein sulfhydryl groups, and nitrogen and oxygen groups on DNA bases) and disrupt normal cellular functions.

This section reviews possible policy approaches to decrease human health risk from interactive effects of pesticides, including pesticides formulated with two or more chemicals, mixtures prepared in the field, and incidental exposures of separately applied pesticides. It first considers two aspects of conventional risk assessment relevant to individual chemicals as well as mixtures. It next summarizes how interactive effects could be considered in the scoping phase of risk assessment. The concluding two sections review necessary changes to the risk assessment and risk management, respectively. As appropriate, it discusses the extent to which current law requires, authorizes or prohibits each recommendation.

A. RECOMMENDATIONS REGARDING CONVENTIONAL RISK ASSESSMENT

This report illustrates that pesticide mixtures present scientific and policy challenges beyond those created by individual chemicals. However, that fact does not diminish the importance of addressing deficiencies in the assessment and management of individual chemicals. In considering changes needed to confront the challenges created by mixtures, one should not ignore problems in conventional practice. Two such problems are particularly relevant here: inadequate testing and failure to consider aggregate population risk in risk assessment.

As noted in Section V.C. above, there are substantial data gaps for each of the three fumigant pesticides. These include studies of toxicity/cancer for neonatal exposures, immunotoxicity and neurotoxicity for all three pesticides; testing of carcinogenic potential in mice of chloropicrin; and chronic toxicity and carcinogenicity inhalation studies on the primary degradation product of metam sodium. EPA's regulations for toxicity testing of conventional pesticides expressly mandate testing for carcinogenicity (including neonatal where appropriate), neurotoxicity and

immunotoxicity.*[106,107] Two-year cancer bioassays in rats and mice are required where, among other things, the active ingredient, metabolite or degradate is structurally similar to a recognized carcinogen, is mutagenic, or produces a morphologic effect in any organ.[106] While the default neurotoxicity and immunotoxicity testing requirements do not mandate testing of degradates, EPA has the authority (and obligation) to require such testing on a case-by-case basis where appropriate.[108,109] The California statute and regulations acknowledge that EPA test requirements are relevant to the state registration, and thus in most cases require submission of such testing results.[110,111] Moreover, the DPR has independent authority to require testing of the type missing in this case.[111,112]

A variety of factors explain the reticence on the part of regulators to require submission of comprehensive data despite their clear authority under the relevant statutes and regulations. Testing can be both time-consuming and expensive for the registrants, who have ample financial and market incentives to minimize testing that may reveal health and environmental concerns.[113] Reviewing agencies often have limited resources, which are furthered impaired when faced with the need to justify testing requirements and evaluate results. Data gaps in the evaluation of pesticide registration applications are a long standing issue. For example, although the Birth Defect Prevention Act of 1984 mandated submission of toxicity studies for a variety of endpoints, gaps continue to exist.[114] Almost thirty years later, these requirements were still not fully implemented; a study of the registration of methyl iodide found serious deficiencies in scope of required testing.[74,145]

Failure to consider aggregate population risk is another shortcoming in DPR's current risk assessment practice. When looking at the way DPR conducts its risk assessments, the agency generates worst-case exposure estimates for bystanders and then estimates what the maximum individual person's cancer risk (MIR) would be, as well as non-cancer risks.[115] If the levels of risk exceed acceptable levels, DPR typically requires mitigation measures. On the downside, DPR does not make an attempt to evaluate an "aggregate population risk," that is, the agency does not attempt to calculate the total number of persons who would be exposed to the pesticide at high exposure levels. Focusing just on the single person who is exposed to the highest level of pesticide may miss the fact that a very large population could be exposed.[116] When considering hazardous air pollutants, U.S. EPA estimates the number of persons expected to develop cancer as an important measure of health risk.[116] Appendix E illustrates that long term transport of fumigants may result in large numbers of persons potentially suffering a cancer risk, when looking at the population exposed as a whole, even when the maximum individual risk is within levels acceptable to the agency.

The Health and Safety Code supports consideration of aggregate population risk. Section 12825 authorizes DPR to reject any pesticide that "has demonstrated serious uncontrollable adverse effects either within or outside the agricultural environment."[117] This broad language, specifically addressing impacts "outside the agricultural environment," provides the agency with ample authority to address population risks created beyond the location of the maximally exposed individual. Existing exposure modeling and risk assessment methods are sufficient to estimate aggregate population risk. EPA has engaged in such analysis under the residual risk provisions of the Clean Air Act Amendments for over 15 years. Due primarily to the limits of conventional air dispersion models, EPA's analyses are limited to populations within 50 kilometers of the regulated facility.[118] Appendix E discusses the type of analyses required to consider national impacts beyond 50 kilometers.

*Note some of these requirements may be affected by application of EPA's 2013 Part 158 Toxicology Data Requirements: Guidance for Neurotoxicity Battery, Subchronic Inhalation, Subchronic Dermal and Immunotoxicity Studies. Analysis of the applicability of this guidance is beyond the scope of this report.

B. RISK ASSESSMENT SCOPING: DETERMINE WHETHER TESTING FOR INTERACTIVE EFFECTS SHOULD BE REQUIRED

Addressing potential interactive effects in the scoping phase of risk assessment is the first step in assessing and regulating cumulative exposures that might exhibit adverse interactive effects.[20] During the scoping or problem formulation phase of risk assessment the environmental problem at issue is defined, potential options are summarized, and the risk and technical assessments needed to evaluate the issue are identified.[20] This necessarily requires balancing the possibility of interactive effects and their possible impact on human health with the need to delineate a scope and level of complexity that are appropriate to the problem.[20] This report recommends that the agency consider interactive effects when determining the scope of the risk assessment; in this case risk assessment is synonymous with the pesticide registration or reevaluation process. This report suggests concepts DPR could use to better incorporate interactive effects that might not act through a common mechanism, building in part on EPA's growing guidance on cumulative risk assessment.

The EPA's existing approach to CRA, which relies on chemicals that act through a common mechanism, is a valuable effort to address cumulative effects, but has two main flaws. First, the process of identifying and validating CMGs is time consuming and expensive. The EPA recognizes this, and is in the process of drafting and enacting guidance to incorporate cumulative hazard and exposure issues in risk assessments involving pesticides that may have common mechanisms, but have not formally been established as CMGs.[120]

Second, because the approach is based on evidence of common mechanistic effects, it will not capture pesticides that may have interactive effects that act through different mechanisms or for which the mechanism is unknown. Mixture components that act on different pathways leading to toxicity may increase health risks by targeting additional organ sites and/or by increasing the potency of observed effects to levels that are greater than that of the individual agents. For example, simultaneous or sequential exposures to the three fumigants presented in this case study may cause the following interactive effects, none of which would be captured using the common mechanism approach:

- Synergistic genotoxicity by inducing multiple gene mutations and chromosome damage at different stages and inhibition of DNA repair, which are part of a multi-stage process leading to cancer;
- Depletion of glutathione, which could increase oxidative stress and reduce the metabolic elimination of the fumigants, other toxins, or naturally generated reactive oxygen species;
- Inhibition of DNA methyltransferases through binding of these electrophilic pesticides or their metabolites to sulfhydryl groups or amines in the active sites of these enzymes that could result in uncontrolled growth of mutated cells.

DPR does not have a method in place for considering interactive effects when registering pesticides. While there is no silver bullet approach for assessing the likelihood of interactive effects at the problem formulation phase, this report offers the following recommendations to guide DPR when considering whether interactive effects should be included in the risk assessment:

For Products Sold as a Mixture, Require Testing

Testing for potential interactive effects should be mandatory for all pesticides that are sold as part of a mixture. DPR clearly has the authority to require testing of mixtures. The relevant regulations identify a range of testing required for pesticide products undergoing registration or reevaluation.[111,112,121] Additionally, Section 6192 of the California Food and Agriculture Code sets out omnibus authority to require any other data needed to carry out the registration evaluation called for by Section 12824 of the Food and Agricultural Code.[124] These broad authorities relate to testing of "pesticides" and "pesticide products," terms which *explicitly* include mixtures of two or more active ingredients or other substances.[122,123]

Indeed a careful reading of the statute demonstrates that DPR has the obligation to require testing of mixtures that may produce interactive effects that could lead to enhanced adverse outcomes. Section 12824 directs DPR to engage in a "thorough and timely evaluation" of a pesticide so as to eliminate any pesticide that endangers the agricultural or nonagricultural environment.[124] As we discuss in more detail below, given the scientific literature regarding interactive effects generally, a thorough evaluation of a pesticide mixture must include assessment of potential interactive effects of the component substances. Accordingly testing of the mixture itself is needed to generate data to support that assessment.

For Products Used in Combination or Sequentially With Other Pesticides, Require Testing (or Default Protective Management Measures) When Supported by a Scientifically Reasonable Hypothesis of Interactive Effects

As the exposure section of this report clearly established, pesticides that are not sold as a mixture are still often used simultaneously or sequentially in ways that lead to exposures to multiple pesticides. Pesticides that are commonly applied to the same plants or at the same time of year are more likely to lead to exposure to mixtures. This section summarizes a two-step approach to use when considering interactive effects from co-applied pesticides: (1) determine whether there is reason to believe there will be interactive effects, and (2) either perform testing or adopt stringent restrictions to avoid the likelihood of health impacts. In some cases, such mixing, sequential, and adjacent use is reasonably foreseeable during the registration process and can be addressed by DPR during its registration evaluation. In others, the uses may not become apparent until restricted material permit requests are submitted to the local County Agricultural Commission (CAC). Such cases will require coordination between DPR and the relevant CACs, as described below.

Step 1: Determine Whether Reasonable, Scientifically-Based Hypotheses of Interactive Effects Exist

As a first step the agency should determine if there is (1) a reasonable, scientifically-based hypothesis for potential interactive effects among multiple agents to which humans are exposed, and (2) the hypothesized health risks due to these interactive effects would be greater than the added risk of the individual components of this mixed exposure.

A scientifically reasonable hypothesis would be formed based on available evidence such as mechanistic studies and scientific judgment. For instance, there is evidence that the three electrophilic chemicals considered in this case study induce DNA damage and that each of the chemicals may inhibit DNA repair enzymes by reacting with sulfhydryl or amine groups in their active sites. While there is no publicly available direct evidence that these three chemicals inhibit DNA repair, there is a plausible hypothesis for this mechanism, and they are carcinogenic electrophiles. Based on this, it is reasonable to hypothesize that exposure to more than one of these fumigants may lead to a greater than additive increase in cancer risk. This does not preclude other sources of evidence for a scientifically-reasonable hypothesis of interactive effects. For instance, if epidemiological data suggests a potential interactive effect, that would also justify additional investigation. This report does not attempt to develop a specific definition of the term or standards for its implementation. The concept is not simply a scientific or legal issue; rather it implicates important social values as well. DPR should establish a task force composed of scientists and experts from pertinent disciplines to address the issue with input from all relevant stakeholders.

For product mixtures and foreseeable application scenarios, DPR would engage in this analysis during the registration process. For field mixing and for sequential and adjacent applications, the analysis would come up as part of the restricted material permit application review by the CAC. Integrating this analysis into that process will require some changes to the existing process. Under the existing process, farmers (or their representatives) submit an application for the use of specified restricted materials at identified locations. The application does not specify the timing of the use, and the permit is effective for one year. When a farmer is ready to apply the

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Confidence in the likelihood of an interactive effect is composed of two inversely related elements—as knowledge goes up, uncertainty goes down. It is beyond the scope of this report to delineate detailed guidelines for precisely where on that continuum the requirement for additional testing is triggered, and what types of tests are demanded. *This report does assert that if there is a scientifically reasonable hypothesis of interactive effects synergistically increasing human health hazard (as there is here with glutathione and DNA damage and repair), then the regulator must investigate it further.* That investigation may include any number of testing approaches from computational toxicology to traditional *in vivo* testing. ***Whatever the exact course of additional study, it must either (1) increase certainty, to a level the regulator finds acceptable, that there is no interactive effect; or (2) provide sufficient information on the type and scale of the interactive effect so that it may be considered during risk assessment.*** See section C below for further commentary about possible testing approaches.

pesticides, they submit a notice of intent (NOI) at least 24 hours prior to application, describing the particular location, pesticides and manner of application.[125,126,127] This process does not provide the CAC with information regarding pesticide use at adjacent locations, nor does it necessarily provide sufficient time for analysis of the potential interactive effects. Moreover, it is unlikely that the CAC staff will have the necessary expertise to engage in that analysis.

Consequently, the following changes to the restricted material permit application process would be necessary. DPR, in consultation with the CACs, should generate a listing of the potential interactive effects for typical field mixtures, sequential applications and adjacent applications occurring in the various counties. This would involve synthesis of existing pesticide use data and perform the analysis of the potential interactive effects by DPR. CACs would use the listing in evaluating restricted material permit applications. For atypical combinations not included in the DPR listing, the CAC would consult with DPR. Also, in reviewing an NOI, the CAC will have to examine NOIs for adjacent areas (including areas beyond the county border). Lastly, to provide adequate time for CAC review, the 24-hour period for submission of the NOI will have to be increased where combinations are expected. Clearly this would be a time and resource intensive undertaking; as a first step DPR (or perhaps the task force discussed above) would need to prioritize typical field applications of existing fumigants for evaluation over time.

Step 2: Perform Sufficient Testing or Adopt Stringent, Default Management Requirements

For adequate protection of public health, it is essential that the potential interactive effects be tested experimentally and quantified so that the results can be incorporated into computational models used to assess health risks of chemical mixtures. Of course there are limited resources available for testing and evaluation of pesticides, and expending some of those limited resources on potential interactive effects could detract from efforts to assess other potential issues. Also, the time required for testing could create troubling delays in the use of fumigants by farmers following registration. To balance those concerns against the pre-eminent need to protect human health and the environment, testing could be waived where sufficiently stringent risk management conditions are placed on the co-use of pesticides. For example, sequential application of two different pesticides at one site or adjacent sites, the timing of the second application could be adjusted to allow substantial removal of the first pesticide (e.g. a delay of five half lives would result in a nearly 97 percent reduction in active ingredient).

Here again during the registration process DPR has sufficient authority to require testing and evaluation of interactive effects of mixtures occurring in the foreseeable application of distinct pesticide products in combination. Section 6192 of DPR's regulations specifically calls for submission of data regarding the effect of the use of mixtures of two or more products in combination.[124] The CACs have no explicit authority to require testing as part of the restricted material permitting process, and thus would have to either delay action on the permit while relying upon DPR to seek testing or adopt a default management requirement to ensure that the interactive effect will not occur.[128,129] In either case, the CAC has sufficient authority to act. Under the regulations the CAC has the authority to deny a permit or impose mitigation measures in the face of substantial adverse environmental impacts.[130]

This report now turns to what to do if additional testing of potential interactive effects is called for.

C. PERFORM TESTING TO IDENTIFY THE LIKELIHOOD AND SEVERITY OF INTERACTIVE EFFECTS

In cases where additional information about potential interactive effects is needed—either because the pesticides are marketed as a mixture or because there is co-application in the field and there is a reasonable hypothesis of interactive effects—regulators will need additional experimental or other data to determine the potential impact of the interactive effects. Testing should focus on endpoints or pathways where there is a scientifically reasonable hypothesis of interactive effects. This would include conventional toxicological testing of the mixture where possible. However, in the case of sequential or adjacent applications, such testing may be problematic. As noted above, the resource and time requirements of conventional testing can be challenging. Consideration should be given to the use of tiered testing—including conventional *in vitro* tests, short term *in vivo* tests, emerging predictive toxicology approaches and modeling—and scientific judgment by experts to establish whether interactive effects are likely to occur and whether they could affect disease outcome. In that case, further animal studies may then be called for. DPR should develop guidance for such testing drawing upon recommendations developed by the task force discussed above.

Predictive toxicology approaches, those that rely on *in vitro*, *in silico*, or non-traditional *in vivo* studies to replace or supplement traditional whole animal testing, are becoming increasingly widely accepted even within regulatory decision making. The EPA is in the process of drafting and approving guidance for the use of predictive toxicology as an alternative testing approach to the traditional "six pack" for acute oral, dermal, inhalation toxicity, along with skin and eye irritation and skin sensitization.[119] Such alternative approaches can be useful here beyond just assessment of the basic six acute testing requirements. Individual pesticides and mixtures of active ingredients could be run through predictive toxicological assays. Where there is evidence of the mixture having a different result, for instance a shifting of the dose response curve between the individual chemical and the mixture, this could be used as evidence to justify inclusion of further testing of potential interactive effects such as elucidating the mechanistic pathway and/or *in vivo* testing in animals.

This would require additional screening of pesticides and commonly found pesticide mixtures. For instance, of the three fumigants assessed here, only predictive toxicology data for metam sodium hydrate and its metabolite MITC were publicly available through EPA's iCSS database.[131] These two compounds both tested positive on a number of assays that affect the cell cycle or indicate genotoxicity. Based on these findings, the other two fumigants should also be screened using these assays, and any other available assays relevant to genotoxicity. Evidence of effects on the same or similar DNA repair enzymes and DNA methyltransferases would further support the hypotheses of interactive effects for genotoxicity.

D. INCORPORATE IDENTIFIED INTERACTIVE EFFECTS INTO THE RISK ASSESSMENT

If the testing identifies interactive effects that would adversely impact human health, those effects must be incorporated into the risk assessment. The obligation to engage in this evaluation in the risk assessment is grounded in the language of the Food and Agricultural Code, and mandated by the California Environmental Quality Act. Turning first to the Food and Agricultural Code, Section 12824 establishes the standard for registration (and reevaluation of existing registrations): "The director shall endeavor to eliminate from use in the state any pesticide that endangers the agricultural or nonagricultural environment…." That section goes on to require an orderly, thorough and timely process for the necessary evaluation. DPR's regulations spell out the essential elements of the evaluation in more detail:

> During the review and evaluation of proposed pesticide labeling and data to support registration, the director shall give special attention to…each of the following factors, when applicable, in reaching a decision to register or not register the pesticide:

(a) Acute health effects such as oral toxicity, dermal toxicity, inhalation toxicity, acute eye and skin damage potential, or sensitization potential.

(b) Evidence of chronic health effects such as carcinogenicity, teratogenicity, mutagenicity, fetal toxicity, and delayed neurotoxicity.

(c) Potential for environmental damage, including interference with the attainment of applicable environmental standards (e.g., air quality standards and water quality objectives).

(d) Toxicity to aquatic biota or wildlife....

If any of these factors are anticipated to result in significant adverse impacts which cannot be avoided or adequately mitigated, registration will not be granted unless the director makes a written finding that anticipated benefits of registration clearly outweigh the risks....[142]

Thus the agency is explicitly obligated under the statute and its own regulations to consider whether a range of human health, ecological and environmental effects from use of a pesticide may result in "significant adverse impacts." (Recall that a "pesticide" is specifically defined as including a mixture of substance.) In evaluating pesticide products that contain a mixture of chemicals, DPR has historically focused on evaluating the potential adverse impacts of only one of those chemicals, usually a newly proposed active ingredient. As we have demonstrated above, when dealing with a mixture of substances in one pesticide product, substantially greater adverse impacts or even different types of adverse impacts can be caused by the interaction of the substances in the pesticide. Without evaluating these cumulative effects DPR simply cannot judge the nature and magnitude of the potential adverse impacts, and cannot fully meet its obligation to ensure that significant adverse effects are avoided, mitigated or otherwise justified.

The California Environmental Quality Act (CEQA) likewise mandates that DPR identify and evaluate significant cumulative impacts of the use of a pesticide. Enacted in 1970, CEQA aims to improve environmental decision-making by state and local agencies. It establishes a set of procedural requirements and substantive standards for decisions regarding most projects conducted or financially supported by government agencies and—most relevant here—for "projects" that must be approved by a public agency.[132,133] For these purposes a project includes the introduction of a new pesticide into the marketplace (with the agency approval taking the form of registration by DPR) as well as the ultimate use of a pesticide in the field (with the agency approval being the County Agricultural Commission's issuance of a restricted material permit). Unless it determines that the project will have no significant adverse impacts, the agency must prepare an environmental impact report (EIR) evaluating the project and feasible mitigation measures and project alternatives. Among other things, the substantive evaluation must include consideration of significant cumulative impacts.[134,135,137] DPR is not required to prepare an EIR because the pesticide registration program has been certified by the Secretary of Natural Resources as being functionally equivalent to the EIR process.[136,137] Nonetheless, DPR must still meet the substantive requirements of CEQA review, including evaluation and mitigation of significant cumulative impacts.[137,138]

Cumulative impacts are defined as "two or more individual effects which, when considered together, are considerable or which compound or increase other environmental impacts."[139] The regulations implemented by the Natural Resources Agency under CEQA (known as the CEQA Guidelines) note that the individual effects may be changes to the environment from a single project.[140] The concept clearly includes the interactive effects from two or more substances contained in a single pesticide product (or the intentional application of two products together in the field). Those interactive effects associated with the combination of substances in a single "project"—be it sale of a pesticide product with two substances or intentional mixing of two pesticides together at the application site—by definition "compound or increase" the environmental impacts resulting from the individual substances alone.

Likewise, effects from sequential or adjacent use of different pesticides also fall within the definition of cumulative effects. As the CEQA Guidelines note, "[t]he cumulative impact from several projects is the change in the environment which results from the incremental impact of the project when added to other closely related past, present, and reasonably foreseeable probable future projects."[141] Consider the case in which DPR is evaluating one pesticide with knowledge that it may be used in combination with another pesticide, or applied after it or at an adjacent site. In such cases, those other projects are reasonably foreseeable, and the agency must consider the cumulative effects of exposures to all the pesticides. Of course some combinations or sequential applications may not be foreseeable by DPR during the registration process. In that case, the County Agricultural Commission would be required to evaluate the cumulative effects as part of the restricted material permitting process.

E. RISK MANAGEMENT: ACCOUNT FOR POTENTIAL INTERACTIVE EFFECTS WHEN REGULATING PESTICIDE USE

The agency would proceed with a risk management decision the way they normally would, taking into account information on interactive effects generated as part of the risk assessment.

Addressing interactive effects offers the opportunity to use standard or modified risk management and mitigation approaches, including:

1. ***Labeling:*** Pesticides that are sold as mixtures or commonly used together or sequentially could bear labels warning of the increased risks associated with interactions between the multiple pesticides. Such labels could include additional restrictions for use associated with registration and permitting decisions, instructions avoiding co-exposure of commonly used pesticides, or instructions requiring temporal or geographic separation between applications of multiple pesticides.
2. ***Set Lower Exposure Limits:*** Interactive effects may justify decreasing the exposure limits for certain pesticides. In such cases, probabilistic approaches can be applied to data generated by required testing to adjust exposure limits. In cases in which there is a scientifically reasonable hypothesis of interactive effects that could adversely affect human health, but there is inadequate data to develop a reliable probabilistic model to quantify the impact of those interactive effects, then DPR should apply an uncertainty factor for database deficiency to the toxicity or carcinogenicity potency estimates. The value of the uncertainty factor for interactive effects should be developed by DPR in consultation with OEHHA.
3. ***Restrict Use Through Permitting:*** The State of California already enforces some restrictions on pesticide use based on the application of other pesticides.[78] This practice could be expanded.

F. PROCEDURAL IMPROVEMENTS: OPPORTUNITIES FOR AGENCIES TO IMPROVE ASSESSMENT OF INTERACTIVE EFFECTS

In addition to considering interactive effects when conducting individual risk assessments, agencies should also develop and implement tools and protocols to assist in assessing interactive effects. First, agencies can maintain a database of molecular and cellular effects of each pesticide that incorporates new information and is used to make informed decisions about the plausibility of interactive effects. For instance, an entry could be made for each pesticide as part of the registration or re-registration process that includes evidence of effects that relate to the hallmarks of cancer, other known mechanisms of toxicity, structural similarity to known CMGs, and other information that could be used to compare commonly co-applied pesticides to assess the likelihood of interactive effects. Second, agencies can develop guidelines for what constitutes a scientifically reasonable hypothesis of interactive effects by drawing on their staff and expert panels.

VIII. CONCLUSION

This report attempts to demonstrate three points:

1. It is possible these pesticides interact to increase human health hazard
2. People in California are exposed to these pesticides together
3. DPR is required to assess this risk and protect public health, but isn't doing so

The study focused on interactive effects that would affect cancer potency. Interactive effects from these and other pesticides may also increase the risk of other human health problems, including those related to developmental, reproductive, and neurotoxicity, but such interactive effects were not investigated in this report. Additionally, this study focused on three commonly used fumigant pesticides, but interactive effects could occur among other pesticides.

Assessing the interactive effects of pesticides will be complex. As our society seeks to balance the human and environmental harm caused by pesticides with the economic benefits they provide, both the interactive effects and the costs of assessing them must be considered.

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