GUIDANCE ON USE OF WEIGHT OF EVIDENCE WHEN EVALUATING THE HUMAN CARCINOGENIC POTENTIAL OF PESTICIDES

For Use by Cancer Assessment Review Committee Members

EPA OFFICE OF PESTICIDE PROGRAMS

JUNE 2023

PREFACE

This Weight of Evidence (WoE) guidance is intended to be used by members of the Office of Pesticide Program's (OPP's) Cancer Assessment Review Committee (CARC). The application of WoE analysis is an integrative and interpretive process routinely used by EPA to evaluate human health (USEPA 1991a; 1996; 2002; 2005a, 2011) and ecological (USEPA 2016a) toxicity in a manner that considers all relevant scientific and technical information.

The purpose of this WoE guidance is to aid CARC members in identifying, weighing, and documenting the lines of evidence used to reach conclusions about the human carcinogenic potential of chemicals as described in the Agency's 2005 Guidelines for Carcinogenic Risk Assessment (USEPA 2005a). Readers should refer to the 2005 guidelines for details and supplemental information related to this guidance document.

This document provides general guidance and is not binding on either EPA or any outside parties. The use of language such as "will", "is", "may", "can" or "should" in this document does not connote any requirement for either EPA or any outside parties.

BACKGROUND

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) authorizes EPA to register pesticides and require supporting studies as stipulated under 40 Code of Federal Regulations (CFR) Part 158 to meet statutory safety standards. Part 158 also establishes data requirements for pesticide tolerances under section 408 of the Federal Food, Drug, and Cosmetic Act (FFDCA). The studies in Part 158 provide the scientific basis for characterizing the potential risks associated with pesticide exposure for which EPA test guidelines have been established[1]. Guideline rodent carcinogenicity studies are required in two species (mice and rats) and two sexes (males and females) for most pesticides used on food and non-food pesticides that could lead to long-term exposures in humans. The rodent carcinogenicity studies along with data from other required toxicology studies[2] and from open literature studies are used by the Agency to evaluate the carcinogenic potential of a pesticide and to inform the appropriate cancer descriptor (which is frequently referred to as the cancer classification) and method of quantification for the cancer risk assessment, when applicable.

The CARC is an internal expert consultation committee housed in the Health Effects Division (HED) in OPP. The CARC provides the internal forum for scientists to present and defend their conclusions concerning the carcinogenic potential of a pesticide chemical and interpretation of findings from studies that may inform the carcinogenic risk assessment. The CARC serves as a scientific peer review group and evaluates the weight of evidence (WoE) for carcinogenic potential to classify the chemical in accordance with the EPA's March 2005 *Guidelines for Carcinogen Risk Assessment* (hereafter referred to in this guidance document as the 2005 Cancer Guidelines) and the 2005 *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens.* If CARC determines quantification of cancer risk should be performed, the CARC will recommend the appropriate method for expressing and

[1] Office of Chemical Safety and Pollution Prevention (OCSPP) Health Effects Test Guidelines: https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines

[2] § 158.500 Toxicology data requirements table. https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158/subpart-F

quantitatively characterizing the potential carcinogenic risk for the human populations exposed to the pesticide.

WEIGHT OF EVIDENCE EVALUATION

The Agency has a long history of using WoE approaches to support scientific conclusions. The application of WoE analysis is an integrative and interpretive process routinely used by EPA to evaluate human health (USEPA 1991a; 1996; 2002; 2005a, 2011) and ecological (USEPA 2016a) toxicity in a manner that considers all relevant scientific and technical information. The use of WoE evaluation for potential human carcinogenicity was introduced in the Agency's draft 1999 Guidelines for Carcinogen Risk Assessment (USEPA, 1999) where it describes a WoE evaluation as a "collective evaluation of all pertinent information so that a full impact of biological plausibility and coherence is adequately considered". The 1999 draft guidelines further states:

> "Judgment about the weight of evidence involves considerations of the quality and adequacy of data and consistency of responses induced by the agent in question. The weight of evidence judgment requires combined input of relevant disciplines. Initial views of one kind of evidence may change significantly when other information is brought to the interpretation. For example, a positive animal carcinogenicity finding may be diminished by other key data; a weak association in epidemiologic studies may be bolstered by consideration of other key data and animal findings."

> "Generally, no single weighing factor on either side determines the overall weight. The factors are not scored mechanically by adding pluses and minuses; they are judged in combination."

The 2005 Cancer Guidelines emphasize the importance of weighing all the evidence in reaching conclusions about the human carcinogenic potential of agents. This is accomplished in an integrative step after assessing the individual lines of evidence such as:

- tumor findings, or lack thereof, in laboratory animals and humans,
- chemical and physical properties,
- structure-activity relationships (SARs) as compared with other carcinogenic agents or suitable analogs, and
- in vitro and/or in vivo studies addressing potential carcinogenic processes and mode(s) of action (MOA), including genotoxic and non-genotoxic MOAs.

These lines of evidence inform cancer hazard and risk as well as possible mode(s) of action. Weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed, to the extent that these are revealed in the toxicological and other biologically important features of the agent.

The conclusions regarding a pesticide's potential for carcinogenicity in humans are the result of an integration of the lines of evidence based on scientific judgement and determine the appropriate descriptor to be used as part of the carcinogenic hazard narrative.

Data Sources

Because of the number of Part 158 toxicology studies required for the registration of a conventional pesticides, there is typically a large database available to inform the lines of evidence in the WoE analysis for most food and non-food use pesticide active ingredients. These studies include oral short-term and chronic toxicity studies in multiple species, metabolism studies, genotoxicity battery, neurotoxicity, immunotoxicity, developmental, and reproductive toxicity studies. Studies evaluating other routes of exposure such as dermal and inhalation pathways may also be available. These studies may provide information on potential toxicities related to carcinogenesis, evidence of proliferative or preneoplastic lesions, or pathway specific effects that inform the overall evaluation. Guideline, non-guideline, and literature studies are reviewed and evaluated by OPP scientists using national and international test guidelines (e.g., Organization for Economic Cooperation and Development (OECD),

OCSPP and OPP test guidelines) and/or guidances (e.g., OPP's guidance for considering and using open literature toxicity studies to support human health risk assessment (USEPA, 2012)) to ensure the data are reliable and of sufficient quality.

Lines of Evidence in the WoE Analysis

Each line of evidence evaluated in the WoE analysis is briefly described below. Additional details can be found in Section 2 of the 2005 Cancer Guidelines (EPA, 2005a).

1. Analysis of Tumor Data

Tumor findings may be assessed in studies with laboratory animals and/or using human data. For new pesticides evaluated by CARC, the primary source of tumor data are rodent carcinogenicity studies in both the rat and mouse. Generally, a pesticide needs to be registered and on the market for years before any human epidemiological studies become available. However, human epidemiology data may be available when re-evaluating the carcinogenic potential of a previously registered pesticide.

a) Human Data

Epidemiological studies may provide direct evidence on whether human exposure to a chemical has the potential to cause cancer. Studies of high quality and adequate statistical power are preferable and remove the need to account for extrapolation from animals to humans or extrapolation from high to low doses. Epidemiological studies can also be integrated with experimental evidence when evaluating or clarifying the carcinogenic potential of a chemical for risk assessment. The key considerations in evaluating epidemiologic studies are study design, exposure assessment, outcome assessment, confounding control, statistical analyses, and risk of other bias (USEPA 2005a; 2016b).

Generally, the weight of human evidence increases with the number of adequate studies that show comparable results on populations exposed to the same agent under different conditions. The analysis considers all relevant studies of acceptable quality, whether showing positive associations or null results, or even protective effects. In weighing positive studies against null studies, possible reasons for inconsistent results should be sought, and results of studies that are judged to be of high quality are given more weight than those from studies judged to be methodologically less sound. See the 2005 Guidelines (Section 2.2.1) for detailed guidance on the analysis of human tumor data from epidemiology studies.

b) Animal Data

The Agency considers many factors when interpreting the results of carcinogenicity studies conducted with laboratory animals. The 2005 EPA Guidelines for Carcinogen Risk Assessment are intended as a guidance only and do not provide a checklist for determining whether tumor findings are related to treatment. These guidelines emphasize the importance of weighing multiple lines of evidence in reaching conclusions regarding the human carcinogenic potential of chemicals. Evaluation of observed tumor findings takes into consideration the dose response as well as biological and statistical significance.

- ***Dosing considerations***

The doses used in the rodent carcinogenicity studies should be based on relevant toxicological information to ensure that test animals are adequately challenged to assess carcinogenic potential without exhibiting signs of excessive toxicity. It is recommended that caution is taken when assessing tumors observed at an excessively high dose that would confound the interpretation of the results to humans. The 2005 EPA Guidelines for Carcinogen Risk Assessment recommends that the highest dose level selected should elicit signs of toxicity without producing significant adverse effects on nutrition or health of the animal, substantially altering the normal life span due to effects other than tumors, or inducing inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms) (USEPA 2005a). The high dose, however, is not recommended to exceed 1,000 mg/kg/day (OCSPP 870.4200;

OCSPP 870.4300). Doses should provide relevant dose-response data for evaluating human hazard for human health risk assessment. The 2005 EPA Guidelines for Carcinogen Risk Assessment state that “weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed”. As such, CARC puts less weight on observations of increased incidence of tumors that only occur near or above the limit dose.

- ***Statistical significance of tumor findings***

The main aim of statistical evaluation is to determine whether exposure to the test agent is associated with an increase in tumor development, rather than due to chance alone. For toxicological studies submitted to the Agency for pesticide registration, including animal carcinogenicity studies, detailed reviews are performed, which summarize study findings and identify effects, such as tumors, for evaluation. As part of the review process, CARC conducts independent statistical analyses for all potentially treatment related tumors observed in the guideline animal cancer studies. Tumors are selected for statistical analyses when a study report identifies tumors as statistically significant and/or have been identified by the EPA reviewer as potentially biologically significant based on the presence of an increasing monotonic dose-response and/or relative increases from concurrent controls. The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately, but may be combined when scientifically defensible (McConnell et al., 1986). Trend tests and pairwise comparison tests are the recommended tests for determining whether chance, rather than a treatment-related effect, is a plausible explanation for an apparent increase in tumor incidence. The 2005 Cancer Guidelines states that:

> “A trend test such as the Cochran-Armitage test (Snedecor and Cochran, 1967) asks whether the results in all dose groups together increase as dose increases. A pairwise comparison test such as the Fisher exact test (Fisher, 1950) asks whether an incidence in one dose group is increased over that of the control group. By convention, for both tests a statically significant comparison is one for which *p* is less than 0.05 that the increased incidence is due to chance. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.”

In addition to the Cochran-Armitage trend test and Fischer exact test, CARC performs the Peto's prevalence test to determine the statistical significance of tumor responses when there are statistically significant differences in survival in the rodent carcinogenicity study (Peto et al., 1980).

It is important to note that a statistically significant response may or may not be biologically significant and vice versa. The selection of a significance level is a policy choice based on a trade-off between the risks of false positives and false negatives. A result with a significance level of greater or less than 5% (i.e., $p<0.05$, the most common significance level) is examined to see if the result confirms other scientific information. When the assessment departs from a simple 5% level, this should be highlighted in the risk characterization.

The effect of multiple comparisons should also be considered when reviewing long-term rodent carcinogenicity bioassays. In the evaluation of these studies, many different tumor sites and tumor types are evaluated which may increase the likelihood of a statistically significant false positive. Haseman (1983) analyzed typical animal bioassays that tested both sexes of two species and concluded that, because of multiple comparisons, a single tumor increase for a species-sex-site combination that is statistically significant at the 1% level ($p<0.01$) for common tumors or 5% for rare tumors corresponds to a 7–8% significance level for the study as a whole. Therefore, animal bioassays presenting only one significant result that falls short of the 1% level for a common tumor should be treated with caution. (USEPA 2005a).

As indicated in the 2005 EPA Guidelines for Carcinogen Risk Assessment (Section 2.2.2.1.3), the standard for determining statistical significance of tumor incidence comes from a comparison of tumors in dosed animals with those in concurrent control animals. Additional insight into the statistical and/or biological significance of a response can come from the consideration of historical control data (Tarone, 1982; Haseman, 1995; EPA, 2005a). Historical control data can add to the analysis, particularly by enabling identification of uncommon tumor types or high spontaneous incidence of a tumor in a given animal strain. Generally, statistically significant increases in tumors should not be discounted simply because incidence rates in the

treated groups are within the range of historical controls or because incidence rates in the concurrent controls are somewhat lower than average.

Historical control data are also useful to determine if concurrent control tumor incidences are consistent with previously reported tumor rates (Haseman, 1995). Given the large number of age-related tumor outcomes in long-term rodent bioassays, and thus the large number of potential statistical tests run, caution is taken when interpreting results that have marginal statistical significance or in which incidence rates in concurrent controls are unusually low in comparison with historical controls since there may be an artificial inflation of the differences between concurrent controls and treated groups. Unusually low incidence in concurrent controls may be noted when applicable and considered as part of the WoE for the tumor findings. Identification of common or uncommon situations prompts further thought about the meaning of the response in the current study in context with other observations in animal studies and with other evidence about the carcinogenic potential of the agent (EPA. 2005a).

- ***Evidence of supporting preneoplastic lesions or related non-neoplastic lesions***

Carcinogenicity rodent studies are designed to examine the production of tumors as well as preneoplastic lesions and other indications of chronic toxicity that may provide evidence of treatment-related effects and insights into the way the test agent produces tumors (EPA, 2005a). In addition, other studies in the available database (e.g., subchronic studies, reversibility studies, and mechanistic studies) may provide evidence of early proliferative lesions or other early tumor-related endpoints. As such, the presence or lack of supporting preneoplastic or other related non-neoplastic findings are noted and considered in the WoE to aid in the determination of biological significance since these lesions would not be expected for age-related tumors in carcinogenicity with continuous treatment. The CARC investigates lesions in organs where tumors were observed and demonstrated biological significance based on the presence of an increasing monotonic dose-response and/or relative increases from concurrent controls.

- ***Additional considerations when evaluating tumor data***

Other observations can strengthen or lessen the significance of tumor findings in carcinogenicity studies. Such factors include uncommon tumor types; tumors at multiple sites; tumors in multiple species, strains, or both sexes; progression of lesions from preneoplastic to benign to malignant; reduced latency of neoplastic lesions (i.e., time to tumor); presence of metastases; unusual magnitude of tumor response; and proportion of malignant tumors (EPA, 2005a). CARC considers all the above factors when determining the significance of tumor findings in animal carcinogenicity studies. Tumors identified in the rodent cancer bioassays that are not relevant to humans should be noted and characterized when integrating the lines of evidence in the WoE analysis.

2. Chemical and physical properties

The physicochemical properties of a pesticide can alter its absorption, distribution, metabolism, and excretion (ADME) in animals and humans. As such, physicochemical properties are important determinants of hazard potential and dose-response analysis. The chemical properties that should be considered include, but are not limited to, molecular weight, size, and shape; valence state; physical state (gas, liquid, solid); water or lipid solubility, which can influence retention and tissue distribution; and potential for chemical degradation or stabilization in the body. An agent's potential for chemical reaction with cellular components, particularly with DNA and proteins, is also important. The agent's molecular size and shape, electrophilicity, and charge distribution are considered to decide whether they would facilitate such reactions (USEPA 2005a).

3. Structure-Activity Relationships (SARs)

SAR is based on the understanding that substances with a similar (analogous) chemical structure may have the same biological activity. SAR may inform cancer evaluations by qualitatively comparing the structures of chemical compounds and their effects in biological systems. SAR analyses and models are often used to predict molecular properties, surrogate biological endpoints, infer MOAs and to inform the carcinogenic potential of a chemical. The

2005 Guidelines lists the following parameters as useful in comparing an agent to its structural analogues and congeners that produce tumors and affect related biological processes such as receptor binding and activation, mutagenicity, and general toxicity (Woo and Arcos, 1989):

- nature and reactivity of the electrophilic moiety or moieties present
- potential to form electrophilic reactive intermediate(s) through chemical, photochemical, or metabolic activation
- contribution of the carrier molecule to which the electrophilic moiety(ies) is attached
- physicochemical properties (e.g., physical state, solubility, octanol/water partition coefficient, half-life in aqueous solution)
- structural and substructural features (e.g., electronic, stearic, molecular geometric)
- metabolic pattern (e.g., metabolic pathways and activation and detoxification ratio)
- possible exposure route(s) of the agent

Suitable SAR analysis of non-DNA-reactive chemicals and of DNA-reactive chemicals that do not appear to bind covalently to DNA should be based on knowledge or postulation of the probable mode(s) of action of closely related carcinogenic structural analogues (e.g., receptor mediated, cytotoxicity related). Examination of the physicochemical and biochemical properties of the agent may then provide the rest of the information needed in order to make an assessment of the likelihood of the agent's activity by that MOA (USEPA 2005a).

4. Potential carcinogenic processes including genotoxic and non-genotoxic mode(s) of action

As discussed in the 2005 Cancer Guidelines, knowledge of the biochemical and biological changes that precede tumor development (which include, but are not limited to, mutagenesis, increased cell proliferation, inhibition of programmed cell death, and receptor activation) may provide important insight for determining whether a cancer hazard exists and may help inform appropriate consideration of the dose-response relationship below the range of observable tumor response.

Cancer can result from alterations in genes that control cell growth, cell division and cellular differentiation (Vogelstein et al., 1988; Hanahan and Weinberg, 2000 and 2011; Kinzler and Vogelstein, 2002). The ability of an agent to affect genotype (and hence gene products) or gene expression is of obvious importance in evaluating its influence on the carcinogenic process. Initial and key questions to examine are: Does the agent (or its metabolite) interact directly with DNA (direct mutagen), leading to mutations that bring about changes in gene products or gene expression? Does the agent bring about effects on gene expression via other nondirect DNA interaction processes? (USEPA 2005a).

- ***Evaluating potential for mutagenic*ity**

Understanding the mutagenic potential of a pesticide is critical when evaluating carcinogenic risks to humans. Genotoxicity is a broad term for any damage to the genetic material, whether the damage is transient or permanent. Transient damage refers to unintended modifications to the structure of DNA, which may or may not undergo successful repair. Permanent damage refers to heritable changes in the DNA sequence, known as mutations. Types of mutations include: 1) changes in single base pairs, partial, single or multiple genes, or chromosomes, 2) breaks in chromosomes that result in transmissible deletion, duplication or rearrangement of chromosome segments, and 3) mitotic recombination (OECD, 2015). In somatic cells, DNA-reactive chemicals can cause cancer if the mutations occur within regulatory genes that control cell growth, cell division and differentiation, such as proto-oncogenes, tumor suppressor genes and/or DNA damage response genes (OECD, 2015). Additionally, DNA damage may signal the cell to undergo apoptosis (cell death) rather than cell division and, therefore, the damage is not "fixed" as a mutation and is not passed along to daughter cells.

Evaluation of genotoxicity data requires a WoE approach that includes consideration of the various types of genetic damage that can occur. Since no single genotoxicity assay evaluates the many types of genetic alterations that can be induced by a chemical, a battery of genotoxicity tests is employed to adequately cover the genetic endpoints important for regulatory decisions. Under FIFRA, OPP requires genotoxicity tests of the technical grade

active ingredient for the registration of both food and non-food use pesticides. The current genotoxicity test battery (40 CFR Part 158.500) for pesticide registration consists of:

1) Bacterial reverse mutation test (typically conducted in bacteria strains *Salmonella typhimurium* and *Escherichia coli*),

2) in vitro mammalian (forward) gene mutation and in vitro mammalian chromosomal aberration test, and

3) in vivo test for micronucleus induction (mammalian erythrocyte micronucleus test) or in vivo chromosomal aberration test (mammalian bone marrow chromosomal aberration test)

In cases where equivocal or inconsistent results are obtained for the same endpoint in different test systems, additional testing may be required. The totality of the genetic toxicology information is evaluated using a WoE approach to determine the genotoxic potential of the pesticide. This involves the integration of in vitro and in vivo results as well as an overall evaluation of the quality, consistency, reproducibility, magnitude of response, dose-response relationship, and relevance of the findings. In the WoE analysis, studies evaluating endpoints that measure gene mutations and chromosomal aberrations (i.e., permanent DNA damage) are given more weight than endpoints reflecting DNA events that may be transient or reversible such as primary DNA damage (e.g., comet assays). In vivo studies in mammals are given the greatest weight and more weight is given to doses and routes of administration that are considered relevant for evaluating genotoxic risk based on human exposure to the pesticide. The overall concern for mutagenicity for a pesticide is based on the WoE evaluation of the findings of the available genotoxicity data and considered in the overall WoE analysis for potential carcinogenicity.

- ***Mode of action (MOA) in the assessment of potential carcinogens***

MOA information is not required under Part 158 for pesticide registration. However, MOA data may be included as part of the initial data package for new active ingredient pesticides or submitted later to support a Pesticide Registration Improvement Act (PRIA) cancer reassessment action. The 2005 Cancer Guidelines defines the term MOA as:

> ...”a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A “key event” is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. Mode of action is contrasted with “mechanism of action,” which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here. There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression.”

OPP evaluates data to support a postulated/putative MOA for a particular tumor type(s) according to the harmonized International Programme on Chemical Safety (ICPS) conceptual frameworks (Sonich-Mullin et al., 2001; Meek et al., 2003) as described in the 2005 Cancer Guidelines. The 2001 ICPS framework is based partly on Bradford Hill criteria for causality and serves as a “tool which provides a structured approach to the assessment of the overall weight of evidence for the postulated mode of action” (Sonich-Mullin et al., 2001).

An outline of the framework for evaluation of animal MOA is shown below (adapted from Meek et al., 2003; U.S. EPA ,1999; Sonich-Mullin et al. 2001):

- **Postulated MOA**- i.e., theory in case, includes a brief description of the sequence of measured effects, starting with chemical exposure, to cancer formation at a given site

- **Key events**- includes a clear description of each of the key events (measurable parameters) that are thought to underlie the MOA

*Note: A MOA may also include **associative events** which are not directly linked to tumorigenesis, but are measurable effects associated with a specific key event. For example, in the constitutive androstane receptor (CAR) MOA for rodent liver tumors, associative events include induction of hepatic CYP2B enzymes and liver hypertrophy (Elcombe et al., 2013).*

- **Dose-response relationships**- Dose-response relationships identified for each key event, and comparisons presented of dose-response relationships among key events and with cancer

- **Temporal association**- sequence of key events over time that lead to tumor formation

- **Strength, consistency, and specificity of association of key events and tumor response**- complete assessment and presentation of the relationships among the key events, precursor lesions, and tumors. Portrayal of the consistency of observations across studies of different designs

- **Biological plausibility and coherence**- determination of whether key events and the sequence of events are consistent with current biological understanding, both regarding carcinogenesis in general and for the specific chemical under review

- **Other MOAs**- alternative MOAs that may be applicable for the chemical under review. Comparison of their likelihood vis-`a-vis the proposed MOA

- **Conclusion about the MOA**- overall indication of the level of confidence in the postulated MOA

- **Uncertainties, inconsistencies, and data gaps**- identification of information deficiencies in the case; description of inconsistent findings in the data at large; evaluation of uncertainties; proposal of pointed research that could significantly inform the case

As stated in the 2005 Cancer Guidelines, the use of the framework is intended to provide an analytical approach for evaluating the MOA. It is neither a checklist nor a list of required criteria. As the type and amount of information will depend on the MOA postulated, scientific judgment is important to determine if the WoE is sufficient.

In the absence of sufficiently, scientifically justifiable MOA information, animal tumor findings are assumed to be relevant to humans. MOA data may help inform this assumption on a chemical-specific basis. For pesticide chemicals where data sufficiently support the postulated MOA(s) in laboratory animals, the next step is to determine the relevance of the animal MOA in humans. In the absence of such data, the animal MOA is assumed to be human relevant. A human relevance framework for decision making regarding human relevance is described in Meek et al. (2003) and guidance on human relevance considerations and determinations are described in Section 2.4.3.4 of the 2005 Cancer Guidelines.

Toxicokinetic studies may also contribute to MOA analysis by contributing to identifying the active form(s) of an agent that is central to the MOA. Apart from contributing in this way, toxicokinetics studies may reveal effects of saturation of metabolic processes. These may not be considered key events in a MOA, but they are given separate consideration in assessing dose metrics and potential nonlinearity of the dose-response relationship (USEPA 2005a).

Some examples of tumor MOAs evaluated by CARC for pesticidal chemicals include:

Alpha$_{2u}$ globulin-associated renal tumors in the male rat - administration of certain chemicals to the male rat results in the accumulation of the low molecular weight protein α_{2u}-globulin. The accumulation of α_{2u}-globulin in regions of the proximal tubule of the male rat kidney leads to hyaline droplet-associated nephrotoxicity resulting in sustained tubular cell proliferation, and subsequent development of neoplastic lesions. Female rats and other species do not accumulate α_{2u}-globulin like the male rat. This MOA is generally not considered relevant to humans for renal toxicity including carcinogenicity (USEPA 1991b).

Androgen Dependent - The chemical disrupts the normal levels of reproductive hormones (e.g., testosterone, luteinizing hormone) which in turn stimulates the target tissue (e.g., Leydig cells, testicular tissue) to divide which may lead to hyperplasia and neoplasia. For agents to pose a hazard to humans by this MOA, sufficient exposure levels need to be encountered which produce the same level of biological effect as seen in rodents.

Cytotoxicity and Regenerative Proliferation - Continuous exposure to a chemical or its metabolite causes persistent cell death which in turn may result in a persistent regenerative proliferative response in the damaged tissue. For irreversible tissue alterations to occur in humans, including cancer by this MOA, a sufficient exposure must be encountered over a prolonged period.

Mitogenesis - Mitogenic chemicals act by promoting the clonal expansion of preneoplastic cells by stimulating cell proliferation. This MOA is frequently found in the rodent liver where it is generally associated with an increase in metabolizing enzymes. A mitogenic chemical stimulates

cell proliferation in the target organ without obvious cytotoxicity or cell death. Another important feature of this MOA is that the mitogenic effect is not persistent over time; instead, it is resolved and then is manifested within proliferative foci which are considered preneoplastic lesions. Through continuous exposure, it is these preneoplastic lesions that develop into tumors. At this time, the adverse health effects caused by this MOA are presumed to be relevant to humans.

Mutagenesis - The chemical or a metabolite can react with or bind DNA in a manner that causes mutations. It is usually positive in multiple test systems for different genetic endpoints (particularly gene mutations and structural chromosome aberrations) and in tests performed in vivo and in vitro. Adverse health effects in rodents from these chemicals are considered relevant for human health risk.

Neuroendrocrine Disruption - Chemicals that disrupt hypothalamic control of pituitary function leading to a decrease in hormone release (e.g., luteinizing hormone) and the disruption of the ovarian cycle. This may result in an increase in cell proliferation in the mammary gland due to a hyperstimulation by estrogen. In the case of chloro-s-triazines, this neuroendocrine MOA is not considered relevant to humans because it depends on a rodent specific reproductive process.

PPAR-alpha Agonism - Chemicals that bind to and activate the Peroxisome Proliferator-Activated Receptor (PPAR) stimulate biological responses in the liver (e.g., peroxisome proliferation, induction of lipid metabolizing enzymes, oxidative stress, and hepatocyte mitogenesis). Activation of PPAR–alpha results in an increase in cell proliferation and clonal expansion of preneoplastic foci in the liver. While the human relevance of this MOA has not been definitively determined, most of the evidence indicates that this MOA is not operative in the human liver.

Thyroid Hormone Disruption - Disruption of normal levels of thyroid hormones may lead to an increase of thyroid stimulating hormone (TSH) which results in an increase in cell proliferation of the thyroid gland. If exposure is continuous in the animal, thyroid follicular cell tumors can potentially develop. However, the development of thyroid cancer by this MOA in humans may be unlikely since prolonged stimulation of the thyroid gland by TSH has not been associated with tumorigenesis in humans. However, this MOA is relevant as an indicator for potential noncancer health effects (e.g., goiter, neurodevelopmental, etc) due thyroid disruption in humans. A dose-response approach based on nonlinearity of effects (i.e., RfD approach) should be used when thyroid-pituitary disruption is judged to be the sole MOA of the observed thyroid and related pituitary tumors (USEPA 1998).

WOE ANALYSIS AND CANCER CLASSIFICATION DESCRIPTORS

The narrative describing the WoE explains the pesticides carcinogenic potential and decisions on the lines of evidence considered in the overall WOE analysis. As stated in the 2005 Cancer Guidelines it should highlight the quality and quantity of available data, all key decisions and basis for major decisions, and any unfamiliar data, analyses, or assumptions. The WoE

summarizes the assessment of all lines of evidence, identifying major points of interpretation, strengths and weakness of the evidence and the analysis, as well as any uncertainties that deserve consideration. Choosing a descriptor is a matter of scientific judgment and cannot be reduced to a formula. Descriptors are assigned using all available data from the multiple lines of evidence including CARC's overall analysis of the tumor data, toxicity information from short-term or other chronic toxicity studies, the physical and chemical properties of the pesticide, SAR, and supported mode(s) of action. Generally, more weight is given to MOAs reviewed by CARC that are presented according to the IPCS framework and are fully supported by the existing data. However, the concern for mutagenicity is always evaluated by CARC and considered when selecting the appropriate cancer classification descriptor, regardless of whether the available data fully support a mutagenic MOA according to the IPCS framework.

The five recommended standard hazard descriptors for cancer classification in the 2005 Cancer Guidelines are:

- Carcinogenic to Humans
- Likely to be Carcinogenic to Humans
- Suggestive Evidence of Carcinogenic Potential
- Inadequate Information to Assess Carcinogenic Potential
- Not Likely to be Carcinogenic to Humans

Descriptors represent points along a continuum of evidence. Consequently, there are gradations and borderline cases that are clarified by the full narrative. Multiple descriptors can be used for a single agent, for example, when carcinogenesis is dose- or route-dependent. For example, if a pesticide causes point-of-contact tumors by the inhalation route, but is negative by the oral route, then the pesticide could be described as "likely to be carcinogenic" by the inhalation route, but "not likely to be carcinogenic" by the oral route. Another example is a pesticide could be "likely to be carcinogenic" above a specified dose but "not likely to be carcinogenic" below that dose because a key event in tumor formation does not occur below

that dose. The descriptors and narratives are intended to permit sufficient flexibility to accommodate new scientific understanding and new testing methods (USEPA 2005).

CARC members should refer to the 2005 Cancer Guidelines (Section 2.5) for additional details and guidance on selecting the appropriate cancer classification based on the overall WoE analysis for the pesticide.

Note: Chemicals that were classified under previous EPA guidelines (e.g., chemicals with 1986, 1996, or 1999 cancer classifications) cannot be directly compared to the 2005 cancer classification descriptors. Each system designation refers to the reviews and criteria it contains. For a chemical to be reclassified under the 2005 Cancer Guidelines, it needs to undergo a full evaluation by CARC.

REFERENCES

Elcombe CR, Peffer RC, Wolf DC, Bailey J, Bars R, Bell D, Cattley RC, Ferguson SS, Geter D, Goetz A, Goodman JI, Hester S, Jacobs A, Omiecinski CJ, Schoeny R, Xie W, Lake BG. Mode of action and human relevance analysis for nuclear receptor-mediated liver toxicity: A case study with phenobarbital as a model constitutive androstane receptor (CAR) activator. Crit Rev Toxicol. 2014 Jan;44(1):64-82.

Hanahan, D; Weinberg, RA. (2000) The hallmarks of cancer. Cell 100:57–70.

Hanahan D, Weinberg RA. (2011) Hallmarks of cancer: the next generation. Cell 4;144(5):646-74.

Haseman, JK. (1983) Issues: a reexamination of false-positive rates for carcinogenesis studies. Fundam Appl Toxicol 3:334–339. .

Haseman, JK. (1995) Data analysis: Statistical analysis and use of historical control data. Regul Toxicol Pharmacol 21:52–59.

Kinzler, KW; Vogelstein, B. (2002) Colorectal tumors. In: Vogelstein, B; Kinzler, KW, eds. The genetic basis of human cancer. New York: McGraw-Hill.

McConnell, EE; Solleveld, HA; Swenberg, JA; et al. (1986) Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst 76:283–289.

Meek, ME; Bucher, JR; Chohen, SM; Dellarco, V; Hill, RN; Lehman-McKeeman, LD; Longfellow, DG; Pastoor, T; Seed, J.; and Patton, DE. (2003) A framework for human relevance analysis of information on carcinogenic modes of action. Crit Rev Toxicol 33:591-653.

OECD (2015). Guidance Document on Revisions to OECD Genetic Toxicology Test Guidelines. August 31, 2015.

Peto R, Pike MC, Day NE, Gray RG, Lee PN, Parish S, Peto J, Richards S, Wahrendorf J. (1080). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. IARC Monogr Eval Carcinog Risk Chem Hum Suppl. 1980;(2 Suppl):311-426.

Sonich-Mullin, C; Fielder, R; Wiltse, J; Baetcke, K; Dempsey. K; Fenner-Crisp, P; Grant, D; Hartley, M; Knaap, A; Kroese, D; Mangelsdorf, I; Meek, E; Rice, JM; and Yones, M. (2001) IPCS conceptual framework for evaluating a mode of action for chemical carcinogenesis. Regul Toxicol Phamacol 34:146-152.

Snedecor, GW; Cochran, WG. (1967) Statistical methods, 6th ed. Ames, Iowa: Iowa State University Press.

Tarone, RE. (1982) The use of historical control information in testing for a trend in proportions. Biometrics 38:215–220

USEPA (1999) Guidelines for Carcinogen Risk Assessment, Review Draft, CEA-F-0644, Office of Research and Development. http://cfpub.epa.gov/ncea/raf/cancer.cfm

USEPA (1991a) Guidelines for Developmental Toxicity Risk Assessment. Federal Register 56(234):63798-63826. http://www.epa.gov/raf/publications/pdfs/DEVTOX.PDF

USEPA (1991b) Alpha-2u-globulin: association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum, Washington, DC. EPA/625/3-91/019F. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=3000480X.TXT

USEPA (1996) Guidelines for Reproductive Toxicity Risk Assessment. Federal Register 61(212):56274-56322. http://www.epa.gov/raf/publications/guidelines-reproductive-tox-risk-assessment.htm

USEPA (1998) Assessment of Thyroid Follicular Cell Tumors. Risk Assessment Forum, Washington DC EPA/630/R-97/002. https://www.epa.gov/sites/default/files/2014-11/documents/thyroid.pdf

USEPA (1999) Guidelines for Carcinogen Risk Assessment, Review Draft, CEA-F-0644, Office of Research and Development. http://cfpub.epa.gov/ncea/raf/cancer.cfm

USEPA (2002) A Review of the Reference Dose and Reference Concentration Processes. Risk Assessment Forum, Washington DC EPA/630/P-02/002F December 2002 Final Report. http://www.epa.gov/raf/publications/pdfs/rfd-final.pdf

USEPA (2005a) Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, Washington, DC. EPA/630/P-03/001F. http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF

USEPA (2005b) Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. Risk Assessment Forum, Washington, DC. EPA/630/R-03/003F. https://www.epa.gov/sites/default/files/2013-09/documents/childrens_supplement_final.pdf

USEPA (2011) Endocrine Disruptor Screening Program. Weight of Evidence: Evaluating Results of the EDSP Tier 1 Screening to Identify the Need for Tier 2 Testing. https://www.regulations.gov/document/EPA-HQ-OPPT-2010-0877-0021

USEPA (2012). Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment. https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/guidance-considering-and-using-open-literature

USEPA (2016a). Weight of Evidence in Ecological Assessment. EPA/100/R-16/001. Washington, D.C. December. https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100SFXR.TXT

USEPA (2016b). Office of Pesticide Programs' Framework for Incorporating Human Epidemiologic and Incident Data in Risk Assessments for Pesticides. https://www.regulations.gov/document/EPA-HQ-OPP-2008-0023-0058

Vogelstein, B; Fearon, ER; Hamilton, SR; et al. (1988) Genetic alterations during colorectal-tumor development. N Eng J Med 319:525–532.

Woo, YT; Arcos, JC. (1989) Role of structure-activity relationship analysis in evaluation of pesticides for potential carcinogenicity. In: Ragsdale, NN; Menzer, RE, eds. Carcinogenicity and pesticides: principles, issues, and relationship. ACS Symposium Series No. 414. San Diego: Academic Press; pp. 175–200.