

Sessions on the toxicology of agricultural exposures and cancer

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At the International Symposium on Agricultural Exposures and Cancer, held in Oxford in 2002, three sessions dealt with toxicologic issues. Summaries of each session follow.

Carcinogenicity testing

The first presentation, by James Parry (1), was based on recommendations for genotoxicity testing from the Advisory Committee on the Mutagenicity of Chemicals of the Department of Health in the United Kingdom (UK). Because there is a wide array of test assays (bacteria to mammalian) available with which to evaluate genotoxicity, and often a confusing pattern of positive and negative results, a more focused approach is needed to evaluate the genotoxicity of pesticides. The methods chosen should be (i) reliable, (ii) well-validated, (iii) widely used, and (iv) sensitive. The genotoxicity assessment should include the following end points: the induction of gene (or point) mutations (mutagenicity), chromosome structural aberrations (clastogenicity), and chromosome numerical changes (aneugenicity). One consideration in evaluating the tests is whether or not there are factors that may affect the linearity of the dose–response. Factors that predispose to a nonlinear dose–response include multiple cellular targets and cellular protection mechanisms.

According to Tim Pastoor (2), assumptions concerning the animal cancer bioassay have included the views that (i) cancer in rodents predicts cancer in humans and (ii) the use of daily, lifetime exposure to a pesticide, two species, both genders, and inclusion of the maximum tolerated dose (MTD) maximizes the potential for detecting tumors. Cancer bioassay data from the United States (US) Environmental Protection Agency (EPA), the National Toxicology Program (NTP), and the International Agency for Research on Cancer (IARC) were summarized. Suggestions for improving the bioassay included the inclusion of new molecular biological methods, the determination of a plausible mode of action (when possible), the use of pharmacokinetic data that

permits comparison of different models, and a consideration of human patterns of exposure (episodic) versus the bioassay (constant or lifetime).

It was pointed out that the strength of evidence approach is critical when the possibility of a causal association between pesticide exposure and cancer risk in humans is being evaluated. Biological plausibility is the anchor of the strength of evidence approach. Studies build on observations, which are used for model building, hypothesis formation, and testing and lead to additional studies and new hypotheses. The interest in gene–environment interactions has increased the complexity of and requirements for better methods for assessing relationships between gene polymorphisms, the environment, their potential synergism, and the relationship to cancer diagnosis in humans. Challenges in cancer risk assessment include the need to consider latency and species effects, an understanding of the probable mode of action in rodent bioassays and the relevancy to humans, and whether an approach using the maximum tolerated dose affects the interpretation of the tumor response in rodents. Challenges of current epidemiologic approaches include the use of conservative estimates of exposure and the lack of consideration of individual physiochemical properties.

The participant discussion produced the following important questions: “Does the use of the maximum tolerated dose as the upper dose in rodent cancer bioassays produce metabolic or toxicologic effects that compromise the interpretation of the data?” and “How can we better use pharmacokinetic modeling in rodent bioassays to predict human cancer risk?” The cancer bioassay was designed as a hazard assessment tool to predict overall cancer response; therefore the lack of concordance of tumor sites between species is generally accepted. While there is interest in establishing the mode of action for each carcinogen, stringent validation of the proposed mechanism must be included, and the fact that more than one mode of action may be identified must be acknowledged. While the cancer potential of pesticide-active ingredients is required, the oncogenicity of intermediates

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and metabolites has frequently not received enough attention. The interpretation of human cancer data also presents challenges. Epidemiologic data serve as a reality check, but, for most pesticides, cancer incidence and mortality data are not available. The lack of high-quality mechanistic data on humans increases our reliance on animal modeling and molecular data. Often, time limitations and publication pressures affect the ability of the epidemiologist to conduct a thorough analysis of the data. Unfortunately, while the quality of the data should be the criterion for publication, negative cancer data in epidemiologic studies on humans is not considered publishable. As we unravel the complexity of the cancer process, such as gene–environment interactions, this attitude may influence and increase the complexity of epidemiologic study design. Biological plausibility is the anchor for both the human and experimental data used to evaluate the cancer risk of pesticides. Scientists need to take advantage of and validate emerging risk assessment methods. Better use can also be made of existing data on humans and animals. This work should include better cooperation and increased transparency between academia, industry, and federal agencies in sharing data used in risk assessments for cancer in relation to pesticides.

Statistical considerations and formulant testing

According to Robert Sielken (3), traditional dose–response data (expressed in parts per million) may not provide the best information for cancer risk assessment. A new approach, dose–response modeling, considers other definitions to describe dose, dose metrics, and predictor variables and uses statistics to sort through the likelihood of different mechanisms that may be important in causing cancer in rodent models and to determine whether the rodent mechanism of action is relevant for humans. The statistical model was demonstrated for a particular agrochemical using time-to-tumor-analysis data for the onset of fibroadenomas and adenocarcinoma. The application of statistical dose–response modeling established that the relevance of the mechanism of action for the onset of fibroadenomas, but not apparently carcinomas, was associated with mammary secretory activity at increasing doses. The analysis concluded that the particular mechanism of action is associated with the rat estrus cycle and therefore not relevant for menstruation and humans.

Pesticide formulations may contain a wide variety of chemical substances that perform useful functions, as solvents, spreaders, stickers, and emulsifying, wetting and antifoaming agents, to facilitate application and enhance the efficacy of pesticides under agrochemical field conditions. The pesticide formulation ingredients are often referred to as being the “inert” portion of the

entire formulated product because these substances do not usually possess pesticide biological activity. However, some of these formulation materials may have intrinsic toxicologic properties that need to be taken into account when the overall safety of a pesticide formulation is evaluated. Concern for occupational and consumer safety has fostered increased product stewardship and the development of reduced risk formulations, such as less dusty products, the use of stable granules, aqueous-based (or water-soluble) products, safer solvents, child-proof packages, and bait stations.

The exact composition and nature of a pesticide formulation, both the active ingredients and formulation inerts, are generally proprietary and therefore designated as confidential business information by manufacturers of chemical inerts, pesticide manufacturers, and pesticide product registrants. This situation has led to concern about the exposure to and toxicology of pesticide formulation inerts in chemical substances, mainly that these mixtures may not have been evaluated as extensively as the pesticide active ingredients. In addressing this issue, Neil Carmichael (4) stated that it should not be overlooked that considerable toxicology information may be available for some of the components since individual pesticide formulation ingredients may also be industrial chemicals that have widespread uses outside the pesticide industry. In addition, in assessing risk, it should be taken into account that exposures to end-use pesticide formulations are intermittent and short-term rather than chronic. Thus it is likely that the available toxicology may include premanufacturing notification (PMN) toxicology or acute and subchronic testing data. In addition, pesticide formulation inerts are ubiquitous and may also be regulated by food and drug authorities since the same substances may also serve as ingredients in pharmaceutical and cosmetic formulations or as food additives.

It was pointed out that, in evaluations of the relevance of animal data to human epidemiology, comparative factors, metabolism, and pharmacokinetics should also be considered. Moreover, it is important to identify confounding factors that may cause false positives in epidemiologic studies. Given the possibility that there may be an unknown mechanism of action for cancers, it would be useful to understand what is happening at the molecular level through the use of genomic technology, toxicogenetics and metabolomics. However, there must be biological coherence in the application of these new technologies to gain knowledge on the cancer mechanism of action. It was also suggested that mutational signatures that are relevant for causing cancer be considered.

In the participant discussion, it was suggested that the proposed statistical methodology should also have broad applicability beyond cancer risk assessment to evaluate the likelihood of noncancer mechanisms of

action. Manufacturing toxicologic and exposure data for formulation inerts are not readily available for epidemiologic studies. However, for epidemiologic studies, formulations in use today may not be relevant or comparable with what may have been in use 30 years ago. Amassing and evaluating the known toxicology data are challenges because of the multiple uses, manufacturers and regulatory jurisdictions either within or across regulatory agencies. For example, in the United States, both the Environmental Protection Agency (Office of Pesticide Programs and the Office of Prevention Pollution and Toxic Substances), and the Food and Drug Administration may be involved in the regulation of pesticide inert ingredients. New legislative mandates, the 1996 Food Quality Protection Act and other testing programs, and the voluntary High Productions Volume testing initiatives will lead to the development of new requirements for toxicology testing data for inert chemicals. Until resolved, the lack of transparency about the composition of pesticide formulations, due to proprietary trade secrets of confidential business information and data compensation or intellectual property issues may continue to foster concerns about the availability of pesticide inert and formulation toxicology.

Special aspects

The presentation of Susan Barlow (5) emphasized research interest in the effects of endocrine disruptors on the key hormone systems, such as reproductive, neurobehavioral, immune function, and the development of cancer, and their target tissues and possible links to human health effects. The possible gap of agricultural chemicals in endocrine-mediated carcinogenicity needs to be explored. Several pesticides have been shown in the laboratory to interact with endocrine receptors *in vitro* or to have endocrine-mediated effects *in vivo*. The limitations of this evaluation process includes the lack of adequate data on human exposures to relevant chemicals and the lack of exposure data during critical periods of development, which influence later functioning in life and make it difficult to draw causative associations between exposure and effect. Thus far, there are few known examples of adverse effects in humans after high exposures to endocrine-disrupting chemicals, and, in the case of cancer, these relate to exposure to human pharmaceuticals, rather than to low-level exposures to environmental chemicals.

Randy Rose's presentation (6) was based on the importance of characterizing the metabolic enzymes involved in human metabolism for some pesticides in order to identify potential pesticide interaction that can be related to human health problems. Dr Rose stressed that the following topics must be investigated: (i) identification of specific isoforms involved in the metabolism

of various pesticides, suggesting potential interactions, including polymorphisms, (ii) the use of human hepatocytes and the bDNA (branched deoxyribonucleic acid) assay, providing some important detoxication enzymes, and (iii) the ability of some of these pesticides to induce or inhibit specific metabolic enzymes.

It was pointed out that the IARC evaluation of carcinogenicity evidence includes human studies, animal carcinogenicity studies, and mechanistic data. The substances can be classified as follows: 1 for being carcinogenic to humans, 2A for being probably carcinogenic to humans, 2B for being possibly carcinogenic to humans, and 3 for not being classifiable with respect to carcinogenicity to humans. Many examples of substances and mechanistic data were presented. The crucial factors in the interpretation of epidemiologic studies according to IARC are the results of the most informative studies, statistical power, the quality of exposure data, dose-response analyses, and the internal consistency of the results. The conclusions from the IARC evaluation are that (i) large (prospective) studies with good exposure data still provide the best data for assessing carcinogenicity in humans, (ii) the contribution of mechanistic studies on humans has so far been limited, and (iii) most human mechanistic information is based on exposure biomarkers. In addition, the approach was addressed from the exposure point of view. The role of epidemiology data was emphasized, along with the fact that human epidemiology studies provide very useful information for hazard identification and sometimes quantitative information for data characterization. Some steps in exposure assessment were pointed out and included the tier approach, the use of a generic database, refinement, and specific studies. The important question of what level of resolution do epidemiologists really want was also introduced.

In the participant discussion, the following important questions were brought up: Can the agent present a carcinogenic hazard to humans, and, if so, under what circumstances? What is the potential relationship between an endocrine disruptor and carcinogenesis? At what levels of exposure do adverse effects occur? What are the conditions of human exposure? What is the character of the risk? How well do data support conclusions about the nature and extent of the risk?

The discussion covered the major default assumptions commonly employed in cancer risk assessment, and the following issues were considered to be of great importance: How does the metabolic pathway relate across species? How do toxicokinetic processes relate across species, and what is the correlation between the observed dose-response relationship and the relationship at lower doses?

Finally, many aspects of toxicology in cancer risk assessment must be investigated in order to provide tools

with which to characterize the nature and magnitude of the potential health effects posed by environmental agents, including several pesticides, under various conditions of exposure.

References

1. Parry JM. Assessing the potential mutagenicity of pesticides. *Scand J Work Environ Health* 2005;31 suppl 1:123–8.
2. Pastoor T, Stevens J. Historical perspective of the cancer bioassay. *Scand J Work Environ Health* 2005;31 suppl 1:129–40.
3. Sielken Jr RL, Valdez-Flores C, Holden LR, Breckenridge C, Stevens J. Statistical inferences about the mechanism of action in carcinogenicity studies. *Scand J Work Environ Health* 2005;31 suppl 1:151–5.
4. Carmichael NG. Toxicity of agrochemical formulations. *Scand J Work Environ Health* 2005;31 suppl 1:146–50.
5. Barlow SM. Agricultural chemicals and endocrine-mediated chronic toxicity or carcinogenicity. *Scand J Work Environ Health* 2005;31 suppl 1:141–5.
6. Rose RL, Tang J, Choi J, Cao Y, Usmani A, Cherrington N, Hodgson E. Pesticide metabolism in humans, including polymorphisms. *Scand J Work Environ Health* 2005;31 suppl 1:156–63.