Pesticide Testing for Registration: Toxicity, Environmental Behaviour, and Epidemiology

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Abstract

The registrant of a pesticide must present a broad array of required data to the government before the product can be registered. This paper discusses the types of toxicology and related testing that is necessary. The requirements include (i) toxicity data from acute, subacute, subchronic and chronic studies using test organisms (e.g., rabbits, rats, and mice), (ii) fate of the pesticide in the organism including the metabolic conversions and derivatives, excretion, possible storage, and the rates at which these processes take place, (iii) impact of the product on wildlife, fish and invertebrates, and (iv) the environmental residues of the pesticide (e.g., in plants and animals, drinking water and soils) and bioaccumulation. The paper also discusses the role of epidemiological studies in the registration process and evaluation of possible effects in humans**.**

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Foreword

Vegetation management is an important reforestation activity for controlling competing vegetation or brush encroachment of young tree seedlings. The activity is necessary to get tree seedlings to free-growing status in most new forest sites established in areas that have been harvested or denuded by wildfire, insects and disease.

There are a number of options for managing forest vegetation. The treatment options include prescribed fire, herbicides, manual removal with hand and power tools (e.g., girdling and slashing tools, chain saws and brush saws), placement of mulch mats, mechanical techniques with heavy machinery, and biological methods. The use of livestock (e.g., sheep) is currently the common biological control technique employed in reforestation areas in British Columbia. Biological methods with insects or specific pathogens is used on forest rangelands for noxious weed control but not commonly used for vegetation control in young forest stands.

The selection of a treatment option involves a decision-making process based on integrated vegetation management concepts that include evaluation of the need for treatment, consideration of all the approved treatment methods and choosing the most appropriate treatment method, monitoring and evaluation. Factors considered in selecting a particular method are the ability of the method to meet the required reforestation objectives, the impact of the treatment at the specific site on human safety and the environment (e.g., recreational resources, fish and wildlife and their habitat, range resources and water supply), as well as the economics of the treatment.

This publication is one of a series of papers that evaluates the potential health effects on forest workers using the commonly employed methods of vegetation control. Other papers in the series are listed at the end of this paper. The emphasis is on risks associated with exposure to chemicals during the use of two most important methods for controlling competing vegetation in regenerated (natural or planted) forest areas. These methods are the use of herbicides and manual removal or control with handheld-motorized (power) equipment.

The herbicides discussed are those that have been commonly used in forestry in Canada. The database on health effects of herbicides is extensive and permits reliable estimates of risk. For components of chain saw exhaust and fuels, there is also voluminous background of toxicological information, but exposure data in forestry is limited. Nonetheless, there is enough information to develop preliminary assessments of potential health effects. While there appears to be a high incidence of physical injury associated with manual methods of brush control, there is virtually no validated data on which to base estimates of risk. The existing data are those of workers compensation boards and insurance companies but such data are generally difficult to obtain or are not specifically enough to characterize the kind of activity that leads to injury.

The information in these reports should provide the basis for important decisions about the way vegetation management in forestry should be carried out, and the use of some forestry activities as a source of assisted employment.

Contents

Introduction

The array of tests required for registration is determined by regulatory authorities. All testing must be done under good laboratory practices protocols that are standardized across the entire community of regulated chemicals. In evaluation of data there is close cooperation and exchange of information between Canadian and U.S. regulators and those of the rest of the developed world. Testing or research outside the usual regulatory guidelines may be required and independent peer-reviewed research may be used as part of the pesticide profile.

Older pesticides are being re-registered to assure that current requirements are being met. Requirements of 50 years ago were remarkably lax, and as late as 25 years ago standardization of testing and auditing of laboratories was yet to be implemented. The herbicides of concern here have all been through the reregistration process of the USEPA, although the reregistration Eligibility Decision Document for 2,4-D has not been released.

The descriptions below are general and the order of appearance does not represent the order in which they are conducted. Much of the varied study that must be done is carried out concurrently after the preliminary work has been done.

General Toxicity

Most toxicity tests are conducted with laboratory animals, although more and more preliminary tests on isolated cell and tissue cultures and microorganisms are being developed that can decrease the need for intact animals. Detection of ability to cause genetic change or mutation has always been largely dependent on such methods. Whole animal assays can probably never be completely replaced because no other system can simulate the integrated communication and regulation within a complete organism.

The terms, “acute,” “subacute,” “subchronic” and “chronic” refer to the duration of the test. There is no precise definition of times, so there is a tendency for overlap. Generally, “acute” means very short term, usually a day or less of treatment, although animals may be observed for several days after treatment. “Subacute” may be used for periods up to three or four weeks, “subchronic” six months, and “chronic” for periods up to two years or more.

Acute toxicity

Among the first assays of a candidate pesticide will be a determination of the effects of a range of single doses, usually given by stomach tube to small groups of animals. This assay provides an initial measure of the level that causes observable symptoms, the nature of the effects, and the levels that have no effect, and that cause death. A highly toxic pesticide proceeds beyond this stage with considerable reservation, although if it is extremely effective as a pesticide, research into ways of using it safely may go forward. Conversely, a pesticide with extremely low toxicity at this early stage is not assumed safe. It is quite possible that important effects occur at lower doses over long periods of exposure.

At some point in the testing process, short-term inhalation studies will be conducted, often using aerosols generated in exposure chambers. Acute toxicity to the skin and eyes also must be done, along with evaluation of allergic responses on the skin. The most important human exposures are at the skin.

Subacute toxicity

Sometimes studies are carried out over a three- or five-day period, primarily to aid in determining appropriate dosages for longer term tests. Skin responses and systemic effects are often conducted with rats or rabbits over a 21-day skin exposure; the test material is applied to a shaved area and bandaged to assure that the material stays in place. Rabbits are more

sensitive to skin irritants than are other species. Typically the treatments are for four hours each day, with the substance washed off after each session. Allergic responses are studied in guinea pigs, which are more sensitive to such effects.

Subchronic toxicity

This kind of study typically is run over periods of three to six months, with two species, often rats and dogs. Findings from acute studies and range-finding studies on a few animals over the trial period are used to determine suitable daily doses for each species for long-term assays. The idea is to use several doses, the highest of which is sufficient to produce a detectable effect of some kind, and a lowest dose rates which should produce no effects at all. The dose response for each effect can thereby be defined. The animals are examined periodically, including blood and urine chemistry and at the end of the study are subjected to full clinical and pathological evaluation that will describe any effects that have occurred. An important part of this kind of test includes such simple measurements as weight gain, food consumption, behaviour and general appearance. If poor palatability can be rejected as a cause of decreased food intake, weight change may indicate subtle effects not detected with laboratory analyses.

The findings also provide dose-response information for designing other long-term tests, such as those needed for study of reproductive effects, birth defects and cancer potential. The 90-day tests may also show a need for conducting a similar study in still another species, or to run the test again with emphasis on some particular observation.

Chronic toxicity

Long term toxicity studies are usually carried out with both sexes in two rodent species in conjunction with lifetime cancer studies. A rodent lifetime is about two years, although some mouse assays terminate at 18 months. Typically, at six months and one year and at the end of the study, a full range of clinical chemistry, haematology, and pathology evaluations are made, as well as continual observations of weight gain, food consumption and general condition. Occasionally, effects are found at interim measurements that disappear by the end of the program because of adaptation.

Reproduction

The basic test for effects on reproduction is a two- or three-generation assay, usually conducted with rats. Groups of males and females of breeding age are selected and placed on test diets containing various concentrations of the pesticide for an extended period, then mated. The test diet is continued through gestation and weaning. Fertility, numbers and weights of pups, evidence of failed pregnancies and other indices are observed through weaning of the pups, which are then placed on the same diets. The pups are raised to breeding age and their offspring go through the same cycle. Some assays use one litter per generation, others use two. As in other assays the dose-response relationship is critical. Most direct reproductive effects caused by chemicals are threshold related; there is a dose below which effects do not occur.

Detection of Birth Defects and Effects on Foetal Development

Causation of birth defects (teratogenesis) by chemicals is considered to be a threshold-based effect, and occurs only in the period during gestation when organs and other structures are forming. In the human, this period is from about the second week after fertilization through the third month. In contrast to humans, mice, rats and rabbits are born very early in their development, and the period of organ formation extends up to a few days before birth. Direct

chemical effects outside this time period may intoxicate the foetus and delay development, but are not likely to produce structural defects. The multigeneration assays discussed above also consider developmental effects over the post-natal period; development of the central nervous system continues for some time after birth.

Rats and rabbits are the usual species used. The chemical is administered in a range of doses either in the diet or by stomach tube throughout the sensitive period. Dose rates are set so as to produce either observable defects or maternal toxicity in some form at the high end of the range, decreasing to a level that produces no effect. There are rigid protocols for examining the foetuses, which are taken by Caesarian section just prior to the normal birth time. The frequency and pattern of defects are compared to control groups and to the history of the strain of animals used. The examination also includes a search for evidence of other pathology, such as foetal loss after implantation in the wall of the uterus.

If no evidence of effect can be found at doses up to levels that cause intoxication of the mother, the chemical is not considered to be teratogenic. Maternal toxicity is known to cause birth defects as a secondary effect. That prospect is not pertinent to practical concerns because such doses are far beyond human exposures.

Detection of Genetic Defects: Damage to DNA, Mutations and Chromosomal Effects

Does the chemical in question have the ability to interact in any way with genetic material? Genetic toxicity testing is important to health protection for two reasons. Obviously the human population must be protected; there is already a significant background of naturally occurring, defined genetic defects. Of even greater importance, the genetic changes that initiate the cancer process are mutations, and a chemical that is a potent mutagen is more likely than other chemicals to also be a carcinogen. Conversely, chemicals that are unable to produce mutations in the various test systems are less likely to be direct carcinogens.

A wide variety of test systems have been validated for regulatory genetic toxicity testing. Other techniques that are not yet standardized for this purpose are used in a research context, and there is a continuing search for refinements in existing methods.

Several general kinds of genetic effects are of concern. Mutations are harmful changes that can be carried forward to the next generation of cells or to offspring of the organism. It is also possible to damage deoxyribonucleic acid (DNA) in a cell in such a way that it simply does not work and the cell eventually dies. Chemicals might interfere with the DNA repair system, which is continually correcting the thousands of errors that appear in every cell every day. A toxic impact may be exerted at the level of chromosomes, either breaking away small pieces, or causing a variety of pathological rearrangements of genetic material. These effects also occur continually, but usually at a frequency that can be tolerated without concern.

Much of the testing of chemicals for ability to cause mutations can be done in lower species, such as bacteria, yeasts and insects and with isolated cultures of mammalian cells. Because of rapid cell cycles and direct observation of mutational events, these kinds of tests can be done on the laboratory bench in a short time, without waiting to observe several generations of mammals. There are also several assays done with intact rodents.

Like all other assays, genetic toxicity and mutation tests are conducted at a range of doses if intact animals are the subjects, and several concentrations of the test chemical in the medium for microorganisms and cell cultures.

The reason the non-mammalian species are good indicators is because all living organisms use the same genetic code. The DNA of yeast, bacteria or insects carries information with the same

"alphabet" and "vocabulary" used in the DNA of mammals. It follows that damage to DNA of the test species reflects potential to cause damage to humans and other mammals. The correlation is altered by differences in the way DNA is "packaged" in the cells of bacteria and mammals, ability of the chemical to reach sensitive cells, particularly germ cells, and differences in DNA repair capability. Even a potent mutagen cannot cause inherited defects if it cannot reach germ cells, even if it is able to reach and affect cells of other organs.

Cancer Testing

Evaluation of a chemical for carcinogenicity is the most time consuming and costly of all testing. It can only be done with animals, which must be maintained on test throughout most of the lifespan. Two species are required, usually mice and rats, which have a life span of about two years. Both males and females must be evaluated. The detailed examination of tissues and analysis of data may take more than a year after the test period ends.

At least two dose rates plus untreated controls are employed. Ideally, the highest dose is the maximum the animals can tolerate without significant non-cancer injury, and the second dose is usually at half that level. The usual indicator that the maximum tolerated dose (MTD) has not been exceeded is absence of visible effects and significant tissue and cell injury with weight loss no greater than about ten percent. Other kinds of injury should be limited because non-specific tissue damage resulting from very high doses could lead to cancer that is otherwise unrelated to the chemical being administered.

Evaluation of cancer studies includes:

- Identification of the kind of tumors produced, if any,
- Dose response,
- Comparison of response with that of concurrent control groups and the historical untreated response of the species and strain of animal,
- Whether or not the chemical is able to cause mutation,
- Fate of the chemical in the body, particularly formation of carcinogenic metabolic products (see discussion of chemical metabolism, below),
- Whether the tumors were found in both sexes of both species, and
- Other factors.

The substantial background incidence of tumors in all species has been mentioned before. The background may vary in the laboratory animal population over time, so interpretation of small differences is complicated. The number of animals that can be tested has a practical limit of 100 to 200 per dose per sex, and deciding whether apparent effects are real is difficult. The cellular pathology evaluations use techniques from visual inspection through electron microscopy, but still depend on the expert interpretation of an experienced pathologist, and often panels of experts.

Fortunately, determination of carcinogenic potential also depends on the chemistry of the molecule, the history of similar substances, the behaviour and metabolism in the animal and mutagenic potential. All of these considerations are factored into the eventual evaluation.

Fate of the Pesticide in the Body (Absorption, Metabolism, Excretion)

As part of the requirement for registration, a detailed study is made of the behaviour of the chemical as it is absorbed, transported, changed and excreted. It is important to know if there is a tendency for a chemical to store or be bound in a particular tissue, because this may be an indication of where it is acting. Many chemicals are changed by the liver and other organs into more soluble forms to facilitate excretion. However,

for certain chemicals, that conversion intended as a detoxication mechanism results in a small amount of very reactive intermediate products that may be carcinogenic. It is necessary to learn whether and to what extent these kinds of reactions occur. The details of these reactions and their products can be learned by analysis of products that appear in urine and faeces, and by examining the way cell preparations from the liver and other organs change the chemical *in vitro* (literally, "in glass," on the laboratory bench).

It is also possible that products of these reactions exert toxic effects of their own, which should be distinguished from the activity of the parent compound. This is characteristic of some of the organophosphate insecticides, which are changed to a more potent form after entering the body.

Often, when the toxicology and animal metabolism studies are complete, the metabolic fate of the chemical is examined in human volunteers. Generally, the more similarity in metabolism between species, the more similar will be the patterns of toxicity. This is not a dangerous procedure because the doses used are very low and the prior information about the chemical is extensive. By the time these experiments are done, the chemical is at that same stage of development as a human therapeutic drug entering pre-clinical trials on humans.

Toxic Effects on the Immune System and the Nervous System

At the present there is not a specific registration protocol for testing pesticide effects on either the immune system or the nervous system. International committees are working on both problems. The immune system is extremely complex, and while its many different components are measurable individually, it would be very difficult and perhaps not necessary to examine all facets for each pesticide. As yet, there is no agreement on which few functions would serve as indicators of the integrity of the entire array of immune activities. Because there is so much overlap among the various protective functions, it is likely that a battery of tests will emerge soon.

Immune effects fit into two broad categories. The first includes interference with response to infectious organisms, foreign proteins and proteins of the body that have been altered through combination with toxic chemicals. Impaired immune function leads to increased susceptibility to infectious disease, and decreased ability to attack cancer cells. The second problem is simply ability of a chemical to provoke an allergic response, and this characteristic is routinely examined in the registration process, using guinea pigs as the test species.

In spite of the absence of specific function tests suitable for registration purposes, it is possible to judge whether a chemical can interfere with protection against infection. During the cancer studies, animals are maintained for nearly a lifetime on as high a dose of the chemical that they can tolerate. If this massive intake is not accompanied by increased cancer incidence and the animals do not have a higher incidence of respiratory and other common infections, it is reasonable to conclude that there has been limited interference with immunity.

For neurological effects, there are specific tests conducted for the insecticides that act on the nervous system, but they do not represent a full screen. As with immune effects, it has been difficult to devise a suitable screen, but there is indirect information that can be used, particularly clinical observations. There are behavioural tests that can be used, but most depend on highly skilled observers, and are often subjective.

The multi-generation reproduction test discussed earlier is sensitive to many kinds of interference. If no effect is seen it is likely, although not certain, that neurological injury has not occurred, because various reproductive functions are altered when the nervous system is affected. If an effect is seen in the reproduction assay,

additional study would be necessary to determine the source. None of these secondary observations can be expected to signal specific effects on the higher cerebral functions in humans, however.

Environmental Behaviour and Products of Pesticide Degradation

Pesticide movement and change in the environment must be understood in order to predict what will happen to the chemical after it is applied. Examination of pesticide behaviour in the field is based on detailed laboratory study of the basic chemistry of the substance, with particular attention to products that can be formed under various conditions, and their stability in the environment.

The next steps are lengthy studies of degradation and movement in plants, in various soils and in water under various conditions. If products are formed that are stable enough to remain in the soil for appreciable periods, they too must be characterized, and possibly evaluated for toxicity. Herbicide degradation products are usually less toxic than the parent material; this is also true of most but not all other pesticides. The products from the herbicides of interest here break down at rates comparable to the disappearance of the parent chemical and do not accumulate. The potential for transport off-site in air and water must be evaluated, as well as movement into drinking water, foodstuffs and accumulation in terrestrial and aquatic organisms.

In the forestry context, attention must be paid to deposition and persistence in food, medicinal and ceremonial plants used by First Nations residents. Residues in forage of wild and range species must be determined, to permit consideration of possible impacts.

Wildlife, Fish, and Lower Aquatic and Terrestrial Species

Studies of pesticide effects on wildlife, fish and insects are usually intended to find effects on populations, rather than individual animals. The studies used to predict possible human effects are useful in predicting effects at every level in the wild, as are the findings about environmental behaviour, which enable estimation of exposure.

Surrogate species of birds and various groups of insects are evaluated in studies of varying duration, evaluating toxicity, reproduction, food-seeking behaviour, predatory and protective responses. This work is often conducted under controlled field environmental conditions or in the laboratory rather than in the wild, because it is often impossible to separate pesticide effects from natural impacts.

Game animals and fish may be studied to learn whether pesticides will remain in the tissues to either cause future effects or be transferred to humans who consume them. The role of aquatic insects as an essential food source for fish dictates that they be evaluated under conditions approximating those under which the pesticides will be used. Information about effects on bees and other beneficial insects is also needed.

Residues in Food

Many pesticides are used on or near food crops, including some of the forestry products. Laboratory and field residue and persistence measurements on all food and forage crops must be conducted. These are done at maximum permissible application rates as well as lower use rates. These data are used to set delay periods for harvesting and to calculate potential human intake. Residue tolerances (maximum residues permitted on crops at processing and in the store) are established for each pesticide and each crop for which it is registered.

Curiously, most tolerances are based not on health risk, but on proper application of the pesticide, because the latter factor provides a lower target. Regulatory agencies continually monitor food residues. Most analyses find no detectable residues and rarely are residues near the tolerance found.

Testing Requirements

The following list is a typical array of toxicology and related testing required for registration of a pesticide. Unique characteristics of a chemical or of its use pattern may lead to additional requirements, or a decision that a given test may not be required.

Toxicity data

- acute oral toxicity
- acute dermal toxicity
- acute inhalation toxicity
- delayed neurotoxicity in hen (standard test animal)
- 90 day, rodent
- 90 day, dog
- 21 day dermal, rabbit
- 90 day dermal, rabbit
- 90 day inhalation, rat
- 90 day neurotoxicity, hen
- chronic toxicity, rat and mouse, two year (may be combined with cancer study)
- oncogenicity (cancer), rat and mouse
- chronic toxicity, dog, one or two year
- teratogenicity (birth defects), rat and rabbit
- reproduction, rat, 2 or 3 generation.
- gene mutation (Ames tests and other microorganisms)
- structural chromosomal alteration
- other genetic toxicity as specified.

Pharmacokinetics and metabolism

(Fate of the pesticide in the body, including conversion to derivatives and time courses of various processes)

- dermal absorption
- distribution in the body and duration of residues
- metabolism (chemical change by liver and other organs)
- routes and time course of excretion.

Wildlife, Fish and Invertebrates

- acute and subacute toxicity, small mammals.
- acute and subacute and reproduction toxicity, birds
- terrestrial and aquatic invertebrate toxicity and lifecycle
- coldwater and warmwater fish toxicity
- fish early life stage toxicity and life cycle
- toxicity to estuarine and marine fish, mollusks, shrimp, if necessary
- bioaccumulation in aquatic organisms (crustaceans, fish, insects, mollusks).

Environmental Chemistry and Residues

- chemical nature of residues in plants and animals
- residues in all crops on which pesticide is to be used, which could mean 50 to 100 different crops
- residues in meat of livestock species, milk, poultry, eggs, fish and shellfish
- residues in drinking and irrigation water, and residues resulting from use of irrigation water

- dissipation in soil, aquatic sediment, forest soils and litter, under several representative conditions
- accumulation in crops and other media over time.

Epidemiology

Epidemiology has a number of definitions that vary slightly according to the source and the audience. Perhaps the most technical is in "A Dictionary of Epidemiology," 4th ed. (Last, J.M., ed., Oxford University Press, 2001): "Epidemiology is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to control of health problems." A bit differently, epidemiology is defined as the study of the relationships between the various factors determining the frequency and distribution of diseases in a human community. In the context of this report, epidemiology is the study of possible associations between environmental and occupational chemicals and occurrence of diseases. The term "associations" is used in its statistical sense, which means that the relationship cannot demonstrate cause and effect. While not required for registration of pesticides, epidemiology is mentioned here because such data may be useful in evaluating risks associated with pesticides that have been in widespread use for a long time. For certain pesticides that are being re-registered epidemiology studies are reviewed, if pertinent.

An epidemiology problem may be seen in two ways. It may be an effort to find a cause for some real or apparent increase in incidence of a disease condition. Usually such studies ask a relatively specific question, such as "is the observed disease associated with a certain group of occupations, or with exposure to a group of chemicals, or some life style characteristic?" The question may be turned around: a hazardous waste site is located in a town. Are the residents suffering an unusual frequency of any diseases, or some specific effect such as miscarriage or cancer?

The initial questions are much like those to be asked in a risk analysis:

The hazard or kind of effect should be specified. What is the concern? Is it cancer, liver disease, skin irritation, reproductive problems, or some other more or less specific response that can be defined and measured? The hazard identification will have arisen either from a suspicion that there is an increased number of cases of some specific disease in the population. It is pointless to simply say, "people seem to be getting sick." Information must be collected to show that a real increase in the number of cases has occurred, or that it has not.

The population to be studied must be specified. The population may be a group of workers in a factory, or children under 15, or herbicide applicators, or residents of the forest. It might even be songbirds or fish. To consider an undefined population, such as all of the people of the province, would almost certainly be impractical, except when gathering statistics about overall disease incidence without regard to cause, which is done very well by Statistics Canada.

The potential source of the adverse effect must be specified. The source of possible impact must be specific enough to work with, like a chemical exposure, or automobile accidents, or exposure to sunlight.

For example: Is there a connection between frequency of disease "A" in a population, and exposure of members of that population to chemical "B"? Existing evidence may lead to a hypothesis that the connection does in fact exist, and the objective of the study will be to learn whether the evidence supports the hypothesis, or does not.

The science of epidemiology originated with efforts to identify the causes of infectious diseases, best characterized by the discoveries of the sources of typhoid, smallpox and yellow

fever. Investigations of bacterial and virus diseases have the advantage that each disease has an organism that can be isolated and identified, characteristic signs and symptoms that distinguish it from other diseases, and in most cases it is possible to show a cause and effect relationship when the specific disease is linked with a sufficient population of a specific organism.

Disease caused by chemicals is not often so characteristic that a causative agent can be implicated on the basis of its pattern of effects. Cancer caused by chemical exposure is rarely different from other cancers of the same kind that arise as part of the high natural background incidence of cancer that has always been with us. Finding a very few cases added to background that might be caused by some chemical agent is difficult, even when the cancer type is rare in the general population.

The best that can be accomplished is to show an *association* between some environmental or occupational characteristic and an increase over the background rate of the disease. Spurious, coincidental associations occur; some are obviously unacceptable, others require further, more detailed study. It is probably apparent that increasing the number of affected subjects in the study population and validating the chemical exposure of each individual increase the reliability of an epidemiological study.

Probably the best example of a positive finding in the field of chemically caused disease is the association of lung cancer and other lethal diseases with smoking. While epidemiology can never prove causation of individual cases, the extensive study of many human populations with similar findings, and well-documented individual smoking histories, has produced evidence so definitive that causation of disease by smoking on a broad scale cannot be denied.

Epidemiological studies of possible connections between disease and chemical exposure are usually conducted with one of two principal approaches. Case-control studies are done by identifying a group of individuals who have been diagnosed with a specific disease (cases), then finding control individuals who do not have the disease but who resemble the cases in as many other key factors as possible. Power of the study increases with greater numbers of controls per case. When the individuals have been identified, their backgrounds are searched for characteristics that differ in frequency between controls and cases. Usually the studies ask a relatively specific question, such as "is the observed disease associated with a certain group of occupations, or with exposure to a group of chemicals, or some life style characteristic?"

The other important epidemiologic approach is called a cohort study. The frequency of disease or mortality rate in a specified group is compared with that of the general population or a selected comparison group.

Most studies of possible associations between pesticide exposure and disease have been retrospective, looking back in time. Case-control studies are always retrospective because the individuals already have the disease. Cohort studies may also be done prospectively, with the population identified, then followed over many years to see if their experience is associated with increases (or decreases) in health problems.

There are two major prospective studies of pesticide-related questions underway at present. The Ranchhands are the Air Force personnel who applied Agent Orange, a mixture of concentrated 2,4,5-T and 2,4-D, in Vietnam. Their exposures were perhaps a thousand times greater than those of typical commercial applicators. They and a group of Air Force personnel (controls) who did not handle the herbicide have been and are being followed with rigorous periodic medical examinations, seeking any changes in disease incidence. The Agricultural Health Survey is in early stages, following the health of thousands of farmers in Iowa and North Carolina. An advantage of this approach is that it is possible to make possible real-time estimates of exposure to more than a hundred pesticides and other factors. As time passes, the medical histories of the

subjects can presumably be related to exposures to see if connections exist.

While epidemiology is not very sensitive, it has the advantage of studying the world as it exists or existed at some time. In epidemiology of cancer and possible causation by chemicals, the most important problem lies in the great lapse of time, possibly decades, between exposure to a chemical and the eventual appearance of a cancer that it might cause. It is often necessary to rely on fading memories of exposures that may have occurred 20 or 30 years before. If the case is deceased, the memory of next of kin may be the only information available. Rarely are records available to document exposure.

Because of these limitations in epidemiologic studies a set of criteria are used to evaluate a body of evidence when deciding there is a cause and effect relationship between exposure to a substance and the subsequent development of disease. It is important to note that in epidemiology a single study cannot alone be regarded as a definitive statement of causality. When evaluating a variety of research evidence the following criteria are useful:

Strength of Association Simply put, the bigger the relative risk or the stronger the statistical significance, the greater the likelihood that there is a true cause and effect mechanism at work.

Consistency (Reproducibility). The result from a single study may be a fluke. It is necessary to compare several studies before a conclusion can be reached. (There is a tendency to not publish negative studies, which may bias interpretation of studies that do appear in the published literature.)

Dose-response relationship. This is a fundamental rule of toxicology that applies to many other fields; the amount of illness or change increases with increasing exposure to the **causal agent.**

Coherence or biological plausibility. Does an apparent relationship fit with other knowledge? Does it make sense? This may be as simple as observing that an inhaled substance, like cigarette smoke, affects the first organ it encounters – in this case the lungs.

Temporal Relationship. Does the presumed cause precede the effect? Surprisingly, this aspect is sometimes missed.

Even with such problems, it is sometimes possible to relate exposure to tumour incidence with some modest reliability, usually in the industrial context where work histories may be on record, and where exposures were apparently heavy.

Glossary

Acute toxicity – (Short term toxicity) –Acute toxicity is the quality or potential of a substance to cause injury or illness from a single dose or short period of exposure. See **subacute, subchronic and chronic.**

Adjuvant – Any additive to a pesticide formulation that is not active itself, but is intended make the active ingredient work better.

Cancer – A malignant growth of potentially unlimited size that invades local tissues, and may spread to other parts of the body.

Carcinogen – A chemical capable of inducing cancer.

Carcinogenic – Capable of causing cancer**.**

Chronic toxicity – (Long-term toxicity)- Chronic toxicity is the quality or potential of A substance to cause injury or illness after repeated exposure for a long period of time. Chronic toxicity tests run for a year or more; for rodents the period may extend through the entire life span. A chronic effect persists for months or years and may arise from acute or long term exposure. See **acute, subacute, subchronic.**

Contaminant – In a formulation, usually residues or impurities from the manufacturing process present in small quantities. Contaminants must be identified to the regulatory agency, which judges whether they are of concern.

Deoxyribonucleic Acid – See **DNA.**

Degradation – Breakdown of a compound by physical, chemical or biochemical processes into basic components with properties different from those of the original compound.

Detoxication (Detoxification) – The biochemical process of changing a chemical in the body to a less toxic form or to a form that can be more easily excreted.

Dose – The amount of a chemical that actually enters the body to be distributed to all of the organs and cells. Distribution to tissues and cells is selective, and depends on the nature of the chemical and characteristics of each kind of cell.

Dose-response relationship – The central idea in toxicology and in pharmacology (which is the science dealing with beneficial effects of therapeutic drugs). As the dose (or concentration) of a chemical increases, the effect increases, and as the dose is lowered, the effect becomes less. This response pattern applies to every interaction between a chemical and a biological system, whether human, fish, bacteria or any other kind of organism or tissue. The dose-response relationship is absolutely essential to judgement of the effect of any chemical.

DNA (Deoxyribonucleic Acid) – The genetic library in each cell that contains all of the instructions for building and operating the body. Each kind of cell contains all of the information for the whole body. Only the information needed for each kind of cell is used by that cell; the rest is repressed. Liver cells do not try to be muscles, and muscles do not try to become brain cells, but they contain all of the information**.**

EC_{50} – Acronym for median effective concentration.

Environmental chemistry – The study of the physical, chemical and biological processes that govern behaviour and fate of a chemical such a pesticide after it is used.

Epidemiology – The scientific study of the cause, distribution, and control of epidemics or other disease in a region. In the context of these reports, epidemiology is the study of possible associations between environmental and occupational chemicals and occurrence of diseases. The term “associations” is used in its statistical sense, which means that the

relationship cannot demonstrate cause and effect.

Exposure – Amount of a chemical that reaches a surface from which it might be absorbed. The dose is some fraction of the exposure. Exposure does not include material that is on nearby foliage or other surfaces. It is only the material that reaches the skin (by contact), respiratory tract (by inhalation) or digestive tract (by ingestion).

Foetus – The later stage of mammalian development in the womb. In human, this refers to the unborn child during the period of uterine life from the end of the second month until birth.

Foetal toxicity – Direct effects of a toxicant on the foetus, independent of effects on the mother.

Formulation – A complete pesticide preparation as sold by a manufacturer for practical use. It includes the active ingredient and any necessary adjuvants and solvents. For use, it may or may not require further dilution or mixing with other substances. Formulation can also be defined as the process used by manufacturers in preparing a pesticide for practical use.

Half-life – The length of time required for disappearance of half of the material present in an organism or in environmental media. It is a more useful idea than "persistence" because it allows prediction of the time required to reach low target levels with out making measurements over exceedingly long periods. A better term is "Half-time," because the information only relates to a given location, and says nothing about the processes that deplete the chemical. If it evaporates or is carried away intact by water it may still exist in its original form. The term "half-life" originated with description of radioactive decay, in which elements become a totally different substance. The English language sometimes loses precision as it evolves.

Hazard – The kind of effect that a chemical can cause. Cancer, liver disease, skin irritation, reproductive problems, or some other more or less specific response that can be defined and measured. The term is also used non-specifically to signify any dangerous situation.

Herbicide – A chemical substance or cultured biological organism, used to kill or suppress the growth of plants.

Immune system – All of the structures and cells and their products that protect against infectious organisms and against cells of the body that have become altered in the very early development of cancer.

Inert ingredient – Any component of a formulation that is purposely added and does not have pesticidal activity. Includes solvents and adjuvants, not manufacturing impurities.

Irritation – A purely local or topical reaction which may include redness, blistering, swelling, burning or itching.

Lethal – Causing death.

LD_{50} – Acronymn for Median lethal dose.

Lethal concentration (LC_{50}) – Rate at which 50 percent of test animals will be killed.

LOAEL–Acronym for lowest-observed-adverse-effect level.

Lowest-observed-adverse-effect level (LOAEL) – The lowest measured amount of a chemical that produces significant increases in frequency or severity of adverse effects in exposed subjects. in the general sense it includes all biochemical, pathological, behavioral, reproductive, genetic and other measurable changes. the term may also be applied to any specific parameter under observation.

Median lethal dose (LD_{50}) – The dose of a chemical, biological agent, or other substances that causes death in 50% of defined test animals.

Metabolism – the sum total of the biochemical reactions that a chemical undergoes in an organism. The processes include biochemical (enzymatic) reactions in the cells of the body that convert nutrients to energy and structural materials of the body; reactions that change wastes so they can be removed; and reactions that convert foreign substances, such as some pesticides to forms that can be excreted.

Mutagenic – Capable of producing genetic changes.

Mutagens – Chemicals that are able to induce gene or chromosome damage that is stable and survives cell division to reach the next generation of cells. See **mutation.**

Mutation – Genetic change in DNA of a cell that can be transmitted to the next generation of cells. If in sperm or egg cells, a mutation may be transmitted to offspring. If in somatic (body) cells such as liver, muscle or other organs, a mutation may pass to daughter cells in the organ. The change may have no effect on cell function or it may damage the cell, or even imaginably improve it.

NOAEL – Acronym for **no-observed-adverse-effect level.**

No-observed-adverse-effect level (NOAEL) – The dose rate or concentration at and below which no adverse effects can be detected. (See **threshold; SEE LOAEL)** If the estimated dose of a herbicide to a worker is very low compared to the **NOAEL** for the most sensitive effect found in the laboratory, no harmful effect is to be expected.

Oncogenic – Able to cause cancer]**.**

Persistence – The duration of measurable concentrations of a pesticide in soil, foliage or other media. (See Half-life)**.**

Pesticide – Any chemical (or biological product) intended to control or kill pests. Herbicides, insecticides, fungicides are all pesticides. The term is sometimes incorrectly used to mean only insecticide, for example "pesticides and herbicides.".

Pharmacokinetic – Relating to the rate and pattern of the absorption, distribution, metabolism and excretion of drugs in an animal.

Registration – The process by which government (e.g., Canadian federal government) authorities determine that a pesticide is suitable for use. Standards of public and worker safety, environmental impact, and usefulness must all be met.

Risk – The probability (likelihood) that some adverse or undesirable effect will take place in the future, as a result of some specified activity. Risk may relate to health, finances or any other kind of undesirable impact. Real risk may be so small that it cannot be distinguished from zero, or so great that it is a certainty. In the context of pesticides, risk is the probability that use of the pesticide will result in some specified harmful effect on workers or the public. Risk assessment is the process of estimating that probability.

Safety Factor – See **Margin of Safety**.

Subacute – Extending over a few days to perhaps a month. This and related terms do not carry defined time periods; consequently there is overlap in the way they are used. See **Acute, subchronic and chronic.**

Subchronic – For experimental studies, relatively long term, but not as long as a chronic study. Typically three to six months. See **acute, subacute, and chronic.**

Teratogen – A chemical that can cause birth defects**.**

Teratogenic – Relating to or able to produce birth defects.

Threshold – The lowest dose that will produce a given effect. As a practical matter, the threshold is little different from the **NOAEL.**

Tolerance – Lesser than normal sensitivity of an individual to the adverse effect of a chemical. also, the allowable residue of a pesticide on a food or feed crop.

Toxicant – A toxic agent; a poison.

Toxicity – The whole pattern of harmful effects (illness and other undesirable effects) that a chemical can cause. It is a property of the chemical; it does not change.

Toxicology – The group of scientific disciplines that identifies and studies the adverse effects of chemicals on biological systems, whether in the laboratory or in the field.

Tumour – a new growth of cells multiplying progressively and without control. Classically, the term means a swelling.

Titles in this Series

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2 Pesticide testing for registration: toxicity, environmental behaviour, and epidemiology

3 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part I: Risk to workers associated with exposure to emissions from power saws

4 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part II: Exposure to and absorption of herbicides used in forestry

5 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part III: Risk to workers using 2,4-D formulations

6 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part IV: Risk to workers using glyphosate formulations (e.g., Vision®, Roundup®, Vantage Forestry® and Forza®)

7 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part V: Risk to workers using hexazinone formulations (Pronone®, Velpar® L)

8 Toxicology and potential health risk of chemicals that may be encountered by forest vegetation management workers. Part VI: Risk to workers using triclopyr formulations (Release®, or Garlon 4®)

9 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options: Summary

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