

US EPA ARCHIVE DOCUMENT

RECOMMENDATIONS

In 1996, Congress passed the Food Quality Protection Act, a law which mandates that EPA set pesticide tolerances so as to provide a “reasonable certainty of no harm” to infants and children.

By the time of **FQPA’s** passage, it had already been well established that children were at significant potential risk from pesticides. Pesticides are, after all, designed to be poisonous to the insects, fungi, weeds, rodents and nematodes and other “pests” which are their targets. In 1993, the National Academy of Sciences released a study showing that exposure to pesticides used on food constituted a health concern to infants and children not addressed by existing regulations.

Concern about children’s potential exposure to pesticides has only mounted with more recent data indicating that each day more than a million children under age six are eating foods contaminated with organophosphate pesticides at levels above that determined by EPA to be a safe daily dose. Moreover, organophosphates are chemicals known to be toxic to children’s developing brains. These same pesticides have been found to linger on counter tops, in carpets, even on plush children’s toys, several weeks or months after they are applied in the home. Studies have also reported an association between home pesticide use and childhood leukemia,¹ and an analysis of phone calls to poison control centers suggests that thousands of children under age six may well be poisoned with pesticides each **year.**²

FQPA provisions are clear about how to ensure that allowable levels of residues, or tolerances, for food-use pesticides protect infants and children. The law requires EPA to set tolerances ten times lower than it otherwise would (an additional tenfold or 10X child-protective safety factor) to account for children’s vulnerability and special exposure to pesticides, unless reliable data suggests the use of a different factor.

Our report clearly shows that EPA cannot avoid use of this child-protective safety factor for many food-use pesticides. For these pesticides, the Agency’s pesticide data requirements, its updated guidelines for testing pesticides in animals, and its system for deciding when to request manufacturers to do certain tests according to these guidelines, together do not produce a set of reliable for ensuring a reasonable certainty of no harm infants and children. Under the FQPA, the lack of reliable data means that EPA must not alter or depart from use of the **child-protective 10X** safety factor in setting tolerances for these pesticides.

In reaching this conclusion, our analysis looked at EPA’s latest, revised toxicity testing guidelines, even though they have not been finalized. Though an improvement over previously issued guidelines, these revised guidelines do not

address the data gaps we have identified. Further, they do not address the concerns first raised by the National Academy of Sciences in 1993. This means that when the EPA has finally determined what constitutes reliable data under the law, it must re-evaluate the degree to which these revised guidelines, and the Agency's triggers for deciding when to request tests using these guidelines, will provide the necessary data relating to infants and children.

Finally, the data gaps facing EPA in terms of pesticide toxicity to infants and children are only reinforced by the additional absence of upto-date, comprehensive data on the exposure of infants and children to food-use pesticides. This should further compel EPA to use an additional 10X children's safety factor in setting pesticide tolerances, as required by the FQPA.

For all these reasons NRDC makes the following recommendations:

1. **Strong Presumptive use of the 10X Safety Factor.** In its tolerance decisions, EPA must make strong, presumptive use of the additional tenfold children's safety factor, as is required by law in the Food Quality Protection Act, pursuant to the National Academy of Sciences report in 1993.
2. **Convene a Panel of Children's Experts.** The FQPA allows departure from use of this child-protective 10X safety factor *only if there* are reliable, **chemical-specific** data to use some other factor. EPA should immediately **convene** a blue ribbon panel, comprised of independent pediatricians, pediatric neurologists, pediatric immunologists, pediatric endocrinologists, and developmental or other biologists with expertise in effects of *in utero* or early childhood exposure to toxic chemicals. This panel should be augmented with EPA developmental toxicologists and pediatric exposure assessors. It should be charged with reviewing the state of the science on what complete and reliable set of toxicity and exposure data would be sufficient to warrant departure from use of the tenfold FQPA children's safety factor. EPA should:
 - Convene these experts under the Children's Health Protection Advisory Committee, whose charter is to assist EPA in the development of regulations, guidance and policies to address children's health. This group, currently formed and functioning, already includes many of the pediatric experts needed to answer the charge above.
 - Make the panel's deliberations transparent and public, and its members **free** of conflicts of interest.
3. **Finalize Revised Data Requirements and Testing Guidelines.** EPA should immediately finalize its revised pesticide data requirements and its most **up-to-date** toxicity testing guidelines. Though imperfect, and typically drafted prior to passage of the FQPA, these revisions are more stringent and better reflective of the state of the science than are existing requirements and guidelines.,
4. **Review Guidelines.** On receiving the determination of the blue ribbon panel, the EPA should again review its toxicity testing guidelines to ensure that they reliably assess-individually and collectively-the full range of toxic effects most relevant to the health of fetuses, infants, and other children, including

effects on the developing brain and nervous, immune, endocrine and reproductive systems, and revise the guidelines accordingly. Special attention should be paid to the number and adequacy of existing criteria, or triggers, by which EPA scientists determine when to request testing of a pesticide's effect on the developing brain and nervous system, and other critical organs.

5. **Review Exposure Databases.** On receiving the blue ribbon panel's determination, EPA should also review existing EPA, FDA, and USDA exposure data in terms of their reliability in describing the exposure of fetuses, infants and other children to potentially toxic pesticides.
6. **Use of the 10X Safety Factor Pending Reliable Data.** EPA must not depart from use of the additional, child-protective 10X factor in setting tolerances until the Agency has collected a body of toxicity and exposure data for that pesticide that meets the standard of reliability determined by the blue ribbon panel.

Awareness of the gaps in toxicity and exposure data for children on individual food-use pesticides dates back to before the NAS panel convened in 1988. Since then, EPA's testing requirements and guidelines have remained **largely** the same. As a result, the gaps in the data supplied by pesticide manufacturers have also remained mostly unchanged. What has increased is the recognition that large numbers of children are exposed to these pesticides each day-not only through food and water, but through contact with dirt and contaminated air, household countertops and toys.

It is important to reiterate that despite certain gaps in children's exposure and toxicity data, EPA generally has ample data for many pesticides-including organophosphates and carbamates-to necessitate immediate reductions in, or revocations of their tolerances. Additional data on children's toxicity and exposure to these pesticides will only add to the reasons for reducing or revoking these tolerances. There is, therefore, no reason for EPA to wait to make those decisions. The agency should use the best data available and, where there are data gaps for fetuses', infants', or children's toxicity or exposure, EPA should retain the presumptive tenfold safety factor.

Agribusiness, pesticide interests, and others have recently suggested that EPA back away **from** routine use of the additional FQPA safety factor to protect infants and children, or even delay in making decisions until more complete data are collected. As seen above, this is unnecessary and probably self-serving. At the same time, EPA's most recently available data indicates that it retained the tenfold children's safety factor in less than 10 percent of the first 91 tolerances issued under FQPA.

Strong and presumptive use of this tenfold **safety** factor is necessary both to protect infants and children, and also to ensure that ten years hence we are not still waiting for the pesticide manufacturers to provide the data showing with reasonable certainty that their products pose no harm to our children. Use of the FQPA safety factor, in other words, is needed to finally overcome the uncertainty that made this law necessary in the first place. Failure to use the additional 10X factor in this way only invites further delay in the generation of data, and prolongs the unjustifiable exposure of infants and children to risky levels of pesticides.

On October 23, 1995, EPA issued an Agency policy that it would take the health risks of infants and children into account when conducting environmental risk assessments. Exactly one year ago, President Clinton also signed an Executive Order calling on each federal agency to “ensure that its policies, programs, activities, and standards address disproportionate risks to children...” Strong, presumptive use of the **10X** safety factor will ensure that EPA complies with both Clinton Administration policies and with the law. Our children deserve no less.

OPP'S REVISED (SERIES 870) TOXICITY TESTING GUIDELINES

Since the early 1990s, EPA has been updating its toxicity testing guidelines. These are the protocols which guide how a chemical is tested for toxicity once such testing has been required or requested by EPA.

Initially, EPA envisioned that this process would direct future testing of both industrial chemicals, regulated by the **Office of Pollution Prevention and Toxics** (OPPT) under the Toxic Substances Control Act (TSCA), as well as pesticides, regulated by the Office of Pesticides Programs (OPP). In addition to writing a common set of testing guidelines for these two offices, EPA aimed to harmonize these new guidelines with the European testing guidelines of the Organization for Economic Cooperation and Development. In June 1996, EPA issued a draft set of 59 common or harmonized guidelines, called the Series 870 Health Effects Test Guidelines.¹

For statutory reasons, however, these harmonized draft guidelines will not result in a final set of identical guidelines common to the two **offices**. Instead, as required under TSCA, OPPT codified a separate set of eleven toxicity testing guidelines in August 1997.² For seven of these 11 guidelines, the **TSCA-specific** guidelines “are essentially those resulting from the harmonization process with minor changes to promote **enforceability**.”³

Unlike OPPT, the Office of Pesticide Programs (OPP) is under no statutory obligation to codify the testing guidelines it will use. It eventually must approve or finalize them, however, but has not yet been done. Its staff confirm, however, that OPP's final revised guidelines will resemble substantially the draft harmonized Series 870 guidelines.⁴ More specifically, OPP's final guidelines for developmental toxicity, reproduction and fertility effects, **carcinogenicity**, **neurotoxicity** and immunotoxicity should not greatly differ, in substance, from the **now-codified** TSCA guidelines.

OPP's revised guidelines will vary significantly **from** the toxicity testing guidelines under which toxicity data has been collected for already-registered pesticides? The revised guidelines, for example, include major revisions to individual guidelines for the testing of developmental toxicity, reproductive toxicity, immunotoxicity and dermal absorption. In general, these improvements include clearer descriptions of guideline requirements, partly to **ensure** that tests done according to the same duration of exposure (i.e. acute or chronic) are uniform. **The** new guidelines for developmental and reproductive toxicity, in **particular**, incorporate steps which better characterize the tissue endpoints to be grossly

examined in the exposed fetus, infant and maternal animals, as well as the endpoints to be looked for on microscopic (histopathology) **exam**.⁶ Because the revised guidelines will add rather than subtract requirements, OPP staff indicate that many pesticide registrants may already be performing toxicity tests according to them, in anticipation of their eventual adoption.'

TOXICITY TESTING GUIDELINES FOR FOOD-USE PESTICIDES

To determine whether a pesticide is safe for use, it is typically first tested on laboratory animals. Human testing of chemicals with unknown toxicity would be unacceptable. On the other hand, mammals such as laboratory mice or rats have many similarities to people. The scientific community accepts that virtually any chemical causing developmental defects or cancer in humans will cause these same effects in animals.' The opposite is also assumed: that chemicals causing toxic effects in animals will also cause them in humans.

Thus, in spite of their limitations, animal and *in vitro* tests (tests performed on cells or cellular components in culture) are those which have most often been shown to help predict a chemical's toxicity-including the cancer-causing effects of asbestos and tobacco. Testing in animals seems to be a necessary evil.'

Our experience with asbestos and tobacco also shows, however, that positive research results alone do not always prevent the eventual use of toxicants, especially when manufacturers fail to disclose the results of their findings.' Thus, data obtained **from** tests of laboratory animals are useful only if adequate test guidelines are followed and done without bias, if the results are publicly disclosed, and if subsequent regulation is then based on these scientifically sound results.

OPP requires several types of toxicity testing for **food-use** pesticides, as summarized in the body of this report. **OPP's** most up-to-date toxicity testing guidelines for meeting these requirements are described below.

Acute Tests of Toxicity

A chemical with acute toxicity is one which can cause adverse effects shortly after a one-dose (or **one-day**) exposure. Six kinds of acute toxicity tests are generally required for all food-use pesticides.

Individual tests in the acute battery include: 1) An Acute *oral toxicity* test in which laboratory animals ingest a single chemical dose, or a dose given over a **24-**hour period. Animals are observed for gross signs of toxicity such as increased production of tears, weight loss, incoordination, incontinence, recovery from those effects, and any other observations that can be made with the naked eye. Observations continue for around 2 weeks. At the end of the observation period,

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- Efforts have been made to reduce the number of animals needed to assess the toxicity of a chemical. These include the use of models or statistical computations to infer results, as well as the use of at least some *in vitro* tests-tests on cells in culture dishes rather than **on** whole animals. More research needs to be done, however, to design better experimental methods that assess toxicity accurately and **efficiently**, and to **make** sure that tests which do not use laboratory animals are just as predictive of expected toxic effects in humans.

autopsies are performed on any animals that have died during the course of the study, as well as on remaining animals, and any abnormalities of the internal organs are noted.

Guidelines for the other five acute tests call for similar test procedures, but using nonoral routes of exposure. All acute tests are performed on healthy, young adult animals. The other tests include: 2) *Acute dermal toxicity*, 3) *Acute inhalation toxicity test*, 4) *Acute inhalation toxicity test with histopathology*, 5) *Acute eye irritation*, and 6) *Acute dermal irritation*.

These acute studies help determine whether EPA will request more extensive toxicity testing by the manufacturer, as well as the doses to be given to animals in those subsequent tests. [For non food-use pesticides, the battery of acute tests-plus tests of mutagenicity-are the only means by which EPA can decide whether further testing is warranted.] Scientists also use the acute studies to determine both the LD50, the median dose that kills half the animals in a study, and the **dose-response** curves for the test substance (See Toxicology Terms). Data obtained from acute studies also serve as the basis for particular safety requirements, such as special product labeling, or for recommendations that the pesticide receive special handling or that it be in child-resistant packaging. EPA may decide based upon these test results, for example, whether a pesticide should only be applied by certified applicators.

TOXICOLOGY TERMS

Dose is the quantity of active substance an organism receives from its environment. Dose is often expressed in milligrams of the substance per kilogram of weight of the animal (**mg/kg**). Toxicologists must show that the toxic effects observed in an organism are related to the dose received. This concept, known as the **dose-response relationship**, helps determine whether the substance being tested is the cause of the toxic effects.

Lethal Dose 50 or LD50 is a commonly used measure that describes the relative acute toxicity of a substance. It is defined as the dose which kills half of the given test animals-laboratory rats, for example. A low LD50 indicates a highly toxic chemical. **LD50**, however, only measures lethality; it indicates nothing about toxic effects which may occur before death, such as pain, unconsciousness, etc.

Threshold is the dose at which minimal but observable toxic effects can occur. At doses below the threshold it is assumed that no toxic effects will occur, presumably because the organism can handle the sub-threshold dose without harm. Some scientists theorize that carcinogens have no threshold because they may increase the risk of cancer even at the smallest possible doses.

NOAEL (No-Observed-Adverse-Effect-Level) is literally the exposure level at which no adverse health effects are detected in the study animal. It can be obtained from graphs of the dose-response relationship. Regulatory agencies often use **NOAELs** as the basis for setting acceptable levels of a toxic substance in the environment. Pesticide tolerances on food, for example, are based on the NOAEL plus additional uncertainty factors.

Uncertainty Factors are used to establish a margin of safety around the NOAEL, given uncertainty in the underlying data. One uncertainty factor typically accounts for possible interspecies differences, i.e., the level of exposure producing no adverse effect in laboratory animals versus that in humans. Another is typically added to account for variations in susceptibility that will normally occur among people because of differences in their age, gender, genetics or other factors.

Subchronic and Chronic Toxicity Tests

Guidelines exist for several tests to determine the health effects of chemicals at longer-term exposures than those assessed by tests for acute toxicity. These include tests of 1) *90-Day oral toxicity*, 2) *Subchronic non-rodent oral toxicity – 90 days*, 3) *Subchronic dermal toxicity – 90 days*, 4) *Subchronic inhalation toxicity*, 5) *Repeated-dose dermal toxicity – 21/28 days*, and 6) *Chronic toxicity*.

Most of these tests expose experimental mice or rats to a daily dose of the test chemical during a fixed period of time, usually 90 days for a subchronic test. Ninety days is generally used because it represents about one-tenth of the animal's lifespan. The parameters observed are the same as those observed in the acute tests; they include physical, clinical and some behavioral observations of the animals. After death, an animal's internal organs are examined, weighed and, if any gross abnormalities are found, the affected tissue may be processed and examined under a microscope.

Being longer-term, the chronic toxicity test (or chronic feeding study) is designed to address some of the limitations of subchronic tests. It is conducted over a period of no less than 12 months; sometimes it is done in concert with the ~~two-~~ year test for carcinogenicity. Animals in the chronic test typically are exposed starting 8 weeks after birth, roughly equivalent to late adolescence in humans.' Like subchronic tests, the test for chronic toxicity may expose animals to the test agent via ingestion, inhalation or skin contact-the guideline dictates that the most likely route of human exposure should direct the route of exposure used in the test animal.

While generally required for food-use pesticides, subchronic and chronic tests may be discretionary for other pesticides, depending upon both their intended use and whether or not acute toxicity testing has first indicated the possibility of adverse effects. In some cases, the manufacturer may be required to perform a subchronic test in a non-rodent animal, usually a dog, specifically to confirm the results of an earlier study-positive or negative. Subchronic tests are also done in order to determine a ~~no-observed-adverse-effect~~ level, or NOAEL (See Toxicology Terms), which can reflect the relative toxicity of the chemical.

Carcinogenicity

Cancer has been a major public health concern ever since it was established that environmental chemicals can induce tumors. This historical concern has, perhaps, skewed EPA's toxicity testing guidelines towards a preponderance of possible tests to assess this effect. There are more than a dozen different guidelines for tests that assess, either directly or indirectly, whether a substance may cause cancer.

The *carcinogenicity* test, commonly referred to as the 2-year rodent bioassay, is required for food-use pesticides. It may also be required for other pesticides following positive findings on the acute and subchronic tests. Sometimes it is performed in concert with the test for chronic toxicity to conserve animals and reduce costs.

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- Rats reach sexual maturity at 40-60 days of age.

The **Carcinogenicity** test assesses whether a chemical may induce the formation of tumors in rodents exposed over a long period of time. Whole-animal testing for carcinogenicity is the most costly of any described in the test guidelines, primarily because it involves exposing at least 420 animals (half of each sex) daily for approximately two years." In addition, two different species of laboratory animals may be required for testing. Again, rodents are exposed to the test chemical beginning at 8 weeks, or soon after weaning. The route of exposure can be by mouth, nose or skin, depending on the chemical properties of the substance, and the likely route of exposure in humans. Daily exposure continues for around 24 months, unless it is judged to be inconvenient to dose the chemical according to this schedule, in which case animals are exposed for just 5 days per week.

During the period of exposure in *the Combined Chronic/Carcinogenicity* test, study animals are examined at least once a week for clinical signs of toxicity. The examination includes both the observational (including assessment of salivation, incontinence, changes in body weight, etc.) and motor activity test battery already described as part of the acute and subchronic tests. At various intervals during the exposure period, blood and urine may also be tested to complement the observational measurements; blood may be assessed, for example, for toxic effects on immune cells. For animals that die during the course of the study, and for all other animals at the end of the exposure period, internal organs are extracted and prepared for examination under the light microscope. These may include organs of the respiratory system, for example, if exposure has occurred via inhalation. During microscopic examination, the technician records any abnormalities or disease changes, such as tumor formation, in the tissue and not visible to the naked eye. Statistical analysis determines if the incidence of these changes is higher in the exposed groups as compared to the control group. If no changes above the control are found in exposed animals, the guidelines recommend that studies be done to confirm that the animals actually absorbed the test substance. It is unclear why this type of assessment could not be performed as easily prior to initiation of the **two-year** exposure study.

Multiple guidelines also exist for tests of mutagenicity, which can serve in some cases as indirect measures of a chemical's carcinogenicity. Cellular DNA is responsible for directing all aspects of cell life, including its proliferation. Many types of damage can occur in DNA, called mutations. Mutations can lead to cancer

IN VITRO TESTS WITH HUMAN CELLS

The science of maintaining human cells in culture has improved tremendously, especially with discovery of the chemical factors needed to ensure that these cells retain the properties they normally exhibit in their original state. Laboratories have ready access to many types of human cells, available through tissue banks which specialize in storing and **cataloguing** these samples to ensure consistency in the cell lines they produce. That way, investigators can easily compare their results with those obtained from other experiments performed on the same kinds of cells. It is now possible to grow almost any kind of human cell in culture, including brain cells which can be observed to form functional connections when grown in laboratory dishes."

if the genes that control cell proliferation are damaged. Generally, mutations and other kinds of damage to the cellular mechanisms that control cellular growth can be detected using laboratory procedures which are particular to that kind of damage.

Some mutagenicity testing is required for all pesticides, food-use or otherwise. However, pesticide data requirements do not specify exactly which, or how many, of the several mutagenicity tests are to be used. Individual tests are therefore not described here. Mutagenicity testing generally is done on cells maintained in culture (in *vitro* tests), although individual test guidelines also do not specify the type of cell to be used. (See box above) For *in vitro* tests, the test chemical is added to cell cultures in various concentrations. By microscopic observation, it is determined whether cellular DNA has been damaged.

Metabolic Tests

The term metabolism refers to the chemical transformations that a substance undergoes within an organism. These transformations may render the chemical more or less toxic to the exposed animal. Metabolic testing is generally required whenever chronic feeding studies or carcinogenicity studies are required, including for food-use pesticides. **OPP's** Series 870 guidelines include several possible tests to meet this requirement, all aimed at determining the fate of a chemical when it is absorbed and enters the body.

A chemical's metabolism may vary according to both its route and rate of absorption. Absorption studies therefore include assessments of the rate of penetration through the skin, the absorption of ingested chemicals through the gut, and the absorption of inhaled chemicals through the respiratory system. Once a chemical enters the body it may have various fates. It can be directly excreted via urine, excrement or expired air; it may accumulate in bile or in target organs. Or, it may be transformed by liver or other enzymes into a chemical with different chemical activity, and this metabolite may be excreted, deposited in the liver, or may travel through the bloodstream to other organs. The study of these processes, to determine the true mechanism of action of a chemical, is called pharmacokinetics.

Pharmacokinetic studies can give scientists important clues for how to design an antidote to a poison, or they may suggest ways to determine how much exposure a person has had to a particular chemical. In some cases, for instance, exposure may best be determined not by directly measuring amounts of the original chemical, but by measuring its metabolites—the new substances to which the original toxin may have been transformed. If these metabolites appear in urine or blood, they may be more accessible or convenient to measure than the original toxin. One example of a metabolite used to assess exposure to a pesticide is p-nitrophenol, which appears in urine after exposure to the insecticide methyl parathion.

Tests of Toxicity to the Nervous System

Many chemicals, including several dozen pesticides, are **known to** inflict adverse effects on the brain and nervous system. The potential impact of such neurotoxicants is especially great because nervous system injury can affect so many different human functions, from the control of one's rate of breathing to waking and

sleeping cycles, from the precise control of fine movements to sensory perception and such complex functions as learning and memory, among others. As described earlier in this report, the developing brain and nervous system are particularly vulnerable to toxic insults.

No tests of neurotoxicity are routinely required for every pesticide used on food. However, **the draft Series 870** guidelines do include six, sometimes-required animal studies designed to test different types of chemical effects on various components of the nervous system. These are: 1) *Neurotoxicity Screening Battery*, 2) *Schedule-Controlled Operant Behavior*, 3) *Peripheral Nerve Function*, 4) *Neurophysiology Sensory Evoked Potentials*, 5) *Delayed Neurotoxicity of Organophosphorus Substances Following Acute and 28-Day Exposure*; **as well as** the 6) *Developmental Neurotoxicity Study*, which is considered later in the appendix, along with other tests of developmental toxicity.

COMPONENTS OF THE **NEUROTOXICITY SCREENING BATTERY**

In the *functional observational battery* a single observer, who is unaware of the test animal's dosage group, typically observes the animal for **5–10** minutes. In this time, the animal's behavior and posture in the home cage are noted, as is its reaction to being moved to a larger observation area. There, the animal is further observed as to its appearance, alertness, and gait; specific behaviors such as rearing, urination and defecation are also observed, as are signs of autonomic function (salivation, lacrimation, piloerection). The animal's pain perception, startle response, grip strength, **hindfoot** splay, and body weight are also evaluated. Observations must be done under conditions which carefully control for external sounds, temperature and humidity of the room, as well as other distractions which may affect the results. Because observations can be rather subjective, they must be performed by experienced technicians. Laboratory and experimental consistency are mandatory.

The *motor activity* component of the battery record how much an animal moves during a given period of time. The guidelines dictate that the recording be automated, and that individual animals be monitored. They do not specify any one type of recording apparatus, nor do they dictate particular kinds of behavior to be observed. Animals are tested by putting them individually into the testing apparatus for as little as 1.5 hours. Since this method tests the animal's reaction to its new environment as much as its activity level, it has been suggested that a better test of the latter might be to monitor the animal in a familiar environment for days rather than minutes to hours.

The *neuropathology* component of the battery involves microscopic examination of tissues from the **nervous** system. Tissues of the brain and/or peripheral nerves are extracted, sliced and may be stained to better reveal their cellular structures. Selection of specific procedures and stains is "subject to professional judgment." After preparation, tissue samples are examined under **microscope** by a pathologist for neuropathological changes. This examination is done in a step-wise fashion, with assessment first being done on samples obtained from animals exposed to the highest doses, and then being compared to those obtained from controls. If no changes are observed, no further neuropathological examination is required.

The most basic, and the most commonly performed, tests described by the neurotoxicity guidelines are the *Neurotoxicity Screening Battery*. This battery consists of three parts: functional observational battery (FOB), motor activity and neuropathology. (See box above) Its guideline notes that the Neurotoxicity Screening Battery “is not intended to provide a complete evaluation of neurotoxicity, and additional [testing] may be necessary to assess completely the neurotoxic potential of a **chemical**.”¹² It is the first tier of neurotoxicity tests, however. Whether or not any additional neurotoxicity testing is done largely depends upon the results from this initial battery.

Potentially, the Battery can be performed according to an acute, **90-day**, or chronic dosing schedule. Use **of the 90-day** battery is conditionally required for food-use pesticide only if neurotoxic effects become apparent on general acute toxicity tests via the oral, dermal or inhalation route, or if testing according to the acute delayed neurotoxicity guideline (described below) is positive. The latter is itself required generally only for organophosphate pesticides or their **metabolites**.¹³

Organophosphates and carbamates both inhibit an enzyme called acetylcholinesterase (**AChE**). (See box below). Acetylcholine is a molecule which chemically transmits electrical signals between certain types of nerve cells. In the normal nervous system, acetylcholinesterase is needed to break down the acetylcholine after it is released, thereby resetting or preparing the nerve cell for its next electrical message. Even though acetylcholinesterase inhibition at high enough levels will cause neurological effects, organophosphate pesticides continue to be manufactured and used.

The Neurotoxicity Battery is conducted in young rats which have been exposed in the same manner as that described for the acute, subchronic and/or chronic studies. Three doses are used, each being some fraction of the benchmark dose, the highest dose that can be given to the animals without killing them. The effects of the pesticide on animals at various doses is compared with a group of animals concurrently dosed with the pesticide vehicle. This latter positive **control helps** to demonstrate the competency of the laboratory performing the study.

ORGANOPHOSPHATE PESTICIDES: WIDELY USED TOXINS

Organophosphates, or **OPs**, are one of the most widely used pesticide classes. They act on the nervous system by inhibiting the normal function of acetyl cholinesterase (**AChE**), an enzyme present in nerve endings. Many of the original compounds in this class were first engineered as **nerve** gases during World War 11, although currently used OP pesticides are generally less potent. Acute exposure to chemicals that inhibit **AChE** can cause illness within minutes, especially in children. Symptoms of acute exposure include muscle twitching, abdominal pain, diarrhea, profuse sweating, nausea, vomiting and pinpoint pupils. Some organophosphates can also cause a delayed response, with onset of symptoms occurring 2 to 4 weeks after the exposure. These symptoms usually start with cramps in the calves and numbness and tingling of the feet. They may progress to include weakness and flaccidity of the legs and other sensory disturbances. Because delayed effects may only appear weeks after exposure, pesticides-and particularly organophosphates-should be tested long enough to allow such effects on the nervous system to manifest.

Chemically induced changes in higher-level functions of the brain and nervous system, such as learning and memory, are generally more difficult to assess than other **neurotoxic** effects. It has been noted that the Neurotoxicity Screening Battery does not evaluate all of the possible toxic effects on the brain and nervous system. Assessments of learning and memory, for example, are clearly absent **from** the FOB component of the Neurotoxicity Battery.”

The FOB is an example of an observational approach to assessing neurobehavioral toxic effects in animals? in contrast, the *Schedule-Controlled Operant Behavior* (SCOB) guideline directs testing of the dosed animal’s ability to perform a complex task. There are many designs for SCOB testing, but generally they require rats or mice to perform an action, such as pressing a lever, in response to a cue and a reward, usually a food pellet. Some versions require the animal to perform the action a certain number of times to receive the reward, while others require that the action be performed after a fixed period of time.

It is notable that EPA’s Office of Pesticide Programs (OPP) incorporates observational testing, such as the FOB and motor activity tests, into its first tier Neurotoxicity Screening Battery, while measures of complex behavior such as the SCOB are relegated to a second tier of discretionary testing. In 1987, EPA did propose to its Scientific Advisory Panel to make operant behavior testing a first tier, or screening, study. This SAP, however, suggested it be used as a second tier, or discretionary, study, while recognizing that neurotoxic effects of some test substances may go undetected in the primary screen [i.e. Neurotoxicity Screening Battery] that would be detected by tests of learned behavior? While some investigators consider the SCOB test an excellent tool in assessing chemically induced changes in behavior, which may reflect indirectly on changes in learning and memory as well,¹⁷ others consider the SCOB not “sufficiently validated” for routine toxicity testing? Still other scientists find that validation issues do not prevent use of the SCOB as a higher-tier regulatory test? Under the resulting **tiering structure**, however, OPP has never requested SCOB testing for a **pesticide**.²⁰ It is unclear whether validation issues, or the cost and complexity of performing SCOB studies are primarily responsible.”

Not only the brain, but also nerves peripheral to the brain can be sensitive to poisons, particularly long-coursing nerves such as the ones reaching the extremities. Nucleoside drugs used to treat AIDS patients, for example, can change the velocity at which nerves conduct their electrical signals.” *The Peripheral Nerve Function* test measures changes in both the velocity and amplitude (intensity) of the electrical impulse that traverses from one end of a nerve to the other. This is done by applying an electrical current to the nerve of an anesthetized animal using one electrode, and then recording it at the end of the nerve using another electrode.

Similarly, the *Neurophysiology: Sensory Evoked Potentials* test measures the electrical activity of brain cells after they respond to a stimuli. In this test, electrodes are surgically attached to the skull of an animal. When the animal’s **senses**—including their visual or auditory sense, or sense of touch—are stimulated, an electrical signal travels to the brain and is recorded by the implanted electrodes. This test is very sensitive to any variation in the procedure, such as the position of the electrodes, body temperature, etc. It must be carefully done to produce reliable results.

Delayed Neurotoxicity of Organophosphorus Substances Following Acute and 28-Day Exposure is another test required only for organophosphate pesticides. Many organophosphate pesticides can cause a condition called **organophosphorus-induced delayed neurotoxicity**, or OPIDN, typically characterized by numbness or tingling in the extremities, followed by limb weakness one or two weeks after exposure. OPIDN tests are done in domestic hens in two steps. First, the hens are given an acute exposure to the test substance. If their normal walking gait is not observed to be hindered, if there is no inhibition of NTE and **AChE**, and if there are no microscopic changes observed in nervous system tissues, then no further testing is requested. If any effects are seen under these conditions, then a **28-day** exposure study is required.

Tests of the activity of another enzyme, neurotoxic esterase (**NTE**), are often helpful in diagnosing OPIDN. NTE is present in the blood and thus is easy to detect in humans. Although inhibition of NTE may not be the direct mechanism behind OPIDN, chemical-induced changes in neurotoxic esterase activity are almost always associated with OPIDN.

DEVELOPMENTAL TOXICITY

The Food Quality **Protection** Act specifically mandates that infants and children be protected from the effects of pesticide residues present in food. As explained earlier in this report, children are particularly vulnerable to the effects of environmental **poisons**.²³ In order to protect children adequately, pesticides should be specifically tested for their effects on developing animals before tolerance levels for residues in foods are approved; toxic effects on children cannot be predicted **from** studies done only on adult **animals**.²⁴

With neurotoxicants, a child's window of vulnerability to specific toxic effects on behavior can be quite **narrow**.²⁵ To illustrate the point, Balduini and coworkers (1991) exposed rats at different stages of pregnancy to methylazomethanol (**MAM**)—an agent known to cause a reduction in brain size among animals exposed *in utero*. After testing the litters for behavioral effects at various ages after birth, Balduini et al found that rats only showed learning deficits when they were exposed during the 18th to 19th day of gestation. If exposure occurred before or after that, no effects on learning could be observed. Animals exposed to MAM during adulthood exhibited none of its effects on learning and motor activity. Therefore, the exact time in gestation when fetal exposure occurs is absolutely critical to determining developmental toxicity, or neurotoxicity.

Among the toxicity testing required of food-use pesticides, only two **tests**—those for developmental and reproductive toxicity—involve *in utero* dosing of animals. Two of **OPP's** revised toxicity testing guidelines are designed to assess developmental effects. These are 1) *Prenatal Developmental Toxicity Study*, and 2) *Developmental Neurotoxicity Study*.²⁶

²⁶ Two others have been listed in the draft Series 870 guidelines, the *Preliminary Developmental Toxicity Screen* and the *Inhalation Developmental Toxicity Study*; **OPP staff** indicate that these were former OPPT guidelines that were mistakenly included (Personal Communication with Susan Makris, **OPP, USEPA**, October 9, 1997).

The *Prenatal Developmental Toxicity* Study is the study typically performed to satisfy food-use requirements for testing of developmental toxicity. The study assesses general toxic effects on developing animals exposed before birth. Dams (maternal animals) are exposed orally to the test substance for the length of the pregnancy. No dosing of the developing animal occurs after birth. Dams are weighed and observed daily, for mortality (death), moribundity, relevant behavioral changes, and overt signs of toxicity. Thorough physical examination of the dams is conducted concurrently with the weighing.

A day prior to anticipated birth, fetuses are removed by **cesarean** section, and the dams are sacrificed. Pups are counted, weighed, and the sex noted. Dams and any dead pups are observed macroscopically, and autopsied; any gross abnormalities of the bones or internal organs are noted. If dams abort or begin to deliver prior to the day of expected parturition, they are sacrificed. In dams not becoming pregnant, uteri are examined to determine if any loss of pregnancy or resorption of fetal tissue occurred.

The Developmental Neurotoxicity Study

The Developmental Neurotoxicity Study, designed specifically to assess the effects of pesticides on the developing brain and nervous system, is not required for all food-use pesticides. The protocol was extensively validated more than a decade ago.²⁶ As of April 1998, however, OPP had received data from developmental neurotoxicity testing for only six pesticides, even including those pesticides already known to be toxic to the **nervous system**.²⁷

In the study, pregnant females are dosed daily with the test chemical by an oral route throughout gestation, and then during lactation (in rats, until pups are 21 days of age). Newborns never receive the test substance directly. Daily measures of gross neurological function and behavior are measured daily in the pregnant animals; offspring are examined for similar parameters at specified intervals after birth. Motor activity tests are also performed on offspring when they are 13, 17, 21, 45 and 60 days old. Tests of auditory startle habituation are performed at weaning and at 60 days old, as are tests of learning and memory. The latter can be done using one of several different protocols. At the end of testing, the animals are sacrificed, the brain tissue is weighed, and a neuropathological examination is done.

The age at which developing animals are assessed for possible neurotoxic effects, once exposure has taken place, can be critical. Certain chemical effects on brain function in both animals and humans may not be apparent until long after the exposure has occurred. Children exposed to mercury *in utero*, for example, may exhibit no toxic effects at birth, only to have them surface at five years of age.²⁸ In another study, rats were exposed to triethyl tin during development. Investigators found that if they assessed the animals for learning disabilities at 3 months of age, no deficits could be detected. Animals assessed at 12 and 24 months, however, showed increasingly severe indices of learning impairment? This “unmasking” of neurotoxic effects with increasing age may be precipitated, experimentally, by challenging the affected animal with another chemical or drug after the original toxic insult?

Animals or humans who have suffered an initial “silent” insult may be more susceptible to a secondary neurotoxic event. It has been postulated that certain neurological diseases of the elderly, such as **Alzheimer's** and Parkinson's disease,

may result from the accumulation of damage to the brain over a **lifetime**.³¹ It is also possible that during childhood, the toxic death of brain cells may not affect neurological functions or behavior. But as the child ages and becomes an adult, and there is further normal death of brain cells, it may unmask any damage inflicted earlier in childhood by **toxins**.³²

Effects on Reproduction and Inheritance

The ability of pesticides, generally, to cause reproductive harm has become one of the greatest areas of concern for testing? This concern has arisen, in part, from observation of changes in the reproductive patterns of **wildlife**,³⁴ and from numerous observations that sperm counts in certain male populations may be declining? At the same time, male workers exposed to the pesticides Dibromochloropropane (DBCP) and Kepone have been found to have low sperm counts and other indications of infertility. In spite of this accumulation of evidence suggesting that pesticides may have a deleterious impact on reproduction and fertility in wildlife, and possibly humans, regulatory processes have not thus far been aimed at curtailing these **effects**.³⁷ Among **OPP's** set of revised (Series 870) toxicity testing guidelines, only *Reproduction and Fertility Effects* (listed as a subchronic guideline) is specifically designed to assess the reproductive effects of chemicals. In this test, a chemical is given to male laboratory animals before, during and after mating, and to female animals during pregnancy and lactation. Afterwards, some of the offspring are selected and exposed to the chemical in the same manner and for the same duration as the original pairs until a second generation is weaned.

In exposed animals, various measures of reproductive function are recorded and compared to non-exposed controls. The parameters studied in dosed male animals include the number, shape and motion of sperm, while in females they include the recording of the estrous **cycle—the** timing of when female rodents are most receptive to sexual contact. For offspring, the total number of births (live and stillbirth) are recorded, as are the weight, sex, and certain aspects of sexual maturation for each individual. Some offspring are killed and examined, with special emphasis being paid to the gross and microscopic appearance of the sexual organs. No animals from either generation are allowed to live beyond about 3 months of age.

Among the tests of mutagenicity described earlier, five deal specifically with the *inheritance* of a mutation, such as mutations in the sperm or eggs of the test animal, and are to be performed on adult male mice exposed to the test chemical? They either directly assess microscopic changes in the DNA from the sperm of exposed mice, or breed exposed males with unexposed females to assess the effects of the chemical on the resulting offspring. When DNA inside sperm or eggs is damaged, rather than cause cancer it may cause inherited birth defects or other effects which act to reduce the viability of the embryo. If a fetus with such DNA damage survives, its defective DNA may be passed from one generation to the next.

Even though EPA's data requirements fail to indicate when, or if, one of these five mutagenicity tests might be required, there is the possibility that the mutagenicity screening required of all pesticides may produce some limited information about whether a particular chemical can inflict reproductive harm.

Immune System Effects

The immune system includes several types of immune cells which circulate in the body and serve to fight infection and disease, as well as certain organs, such as the spleen and thymus, where immune cells develop. There is no specific immunotoxicity testing required of food-use pesticides. There is an acute test for dermal sensitization performed on most pesticides, but this only tests for skin sensitivity to contact with a chemical. It does not serve as a trigger for additional immune system testing. Yet pesticides can often affect the immune system, potentially contributing to a myriad of health effects including allergies, infections, and cancer. For example, a study conducted in Germany found that 2 out of 13 pesticides tested produced immunotoxic **effects**.³⁹

Within OPP's revised toxicity testing guidelines, there is a guideline for assessing immunotoxicity. This exposes young, healthy adult rats or mice to the test chemical for 30 days, typically via the oral route. Animals are immunized against foreign organic material in order to assess how well their immune system can react against this substance. After sacrificing the animals, certain tests are performed on blood cells which reflect the ability to mount an immune response. Some functional tests may be performed on a subpopulation of these cells to determine if they react normally.

This guideline is limited in that it largely answers yes or no to the question of whether the body can mount an immune response. In that sense, it is sensitive only to suppression of the immune system. Immune suppression is an important component of immunotoxicity. It can increase one's susceptibility to infectious diseases, or lead to tumor formation when the body's surveillance system-which normally eliminates malfunctioning or infected cells-is lacking. (This is why people with AIDS suffer from many opportunistic infections and rare cancers.)

But the immunotoxicity guideline fails to determine whether a chemical might **hypersensitize**, or induce increased reactivity of, the immune system. Certain chemicals, such as cyclosporin, are already known to increase the reactivity of the immune system more than is desired, especially when exposure occurs during development of the thymus, an organ important in the maturation of immune **cells**.⁴⁰ Childhood asthma, too, is disorder in which air passages in the lungs have become sensitized or made hyper-reactive to specific environmental irritants. Some investigators also hypothesize that exposure to environmental chemicals, such as pesticides, may be involved in observed increases in the incidence of chemical sensitivity in the United States." EPA's immunotoxicity guideline will not direct testing to uncover a pesticide's potential for triggering these sorts of responses.

Too, the immunotoxicity guideline when finalized will likely only involve limited testing of the various components of the immune system. For example, when the draft guideline for immunotoxicity was presented to OPP's Scientific Advisory Panel in October 1996, EPA proposed, and the SAP agreed, that any specific requirement for assessing the number of various lymphocytes (total T-cells, subsets of T-cells-such as CD4 and CD8 cells, total B-cells) as well as **NK** cells in peripheral blood, be dropped from the **test**.⁴² Instead, the guideline would chiefly focus on use of an antibody assay, using sheep red blood cells. EPA notes that the reason for this elimination is that counting or phenotyping the various immune cells

mentioned would be too costly, and that numbers of **NK'cells** have not been proven to be an indicator for toxicity to the immune **system**.⁴³

Even without measures of the above cells, immunotoxicity testing has almost never been requested by OPP. In fact, only two chemical pesticides-aldicarb and triphenyl-1 O-hydroxide-have ever been tested according to this guideline." OPP's Scientific Advisory Panel, on the other hand, has proffered its opinion that this testing is "sufficiently validated" to be included in routine toxicity **testing**.⁴⁵

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GAO/CED-887-7,
GAO/CED-87-21,
GAO/CED-88-149-BR,
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Appendix A

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42 USEPA, *Final Report of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel and Science Advisory Board Joint Meeting on Guideline Issues, October 29-30, 1996*, Report issued December 6, 1996.

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44 Additional biological pesticides may have received such testing. Personal communication with Sheryl Reilly, USEPA Scientist, April 9, 1998.

45 USEPA, *Final Report of the Federal Insecticide, Fungicide, and Rodenticide Act Scientific Advisory Panel and*

Science Advisory Board. Joint Meeting on Guideline Issues, October 29-30, 1996.