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Issues in Pesticide Hazards—Children; Cancer; Endocrine Effects

I. Overview of Risk Assessment
   A. A necessary first step to determining the likelihood (i.e., risk) that exposure to a pesticide might cause an adverse effect is to review the process of risk assessment.
   1. Often terms like hazard, exposure, and risk are misunderstood and occasionally used interchangeably.
      a. However, each term has a specific meaning in the risk assessment process, which is applicable to pesticide regulation and eventually to permitted uses.
   2. Furthermore, risk assessment and risk management are sometimes confused, perhaps because risk assessment has elements of both science (in the broadest sense of the process of hypothesis testing) and management (in the sense of policy, which is influenced by politics, economics, and sociological factors).
   B. Risk assessment as currently practiced by the EPA in its regulation of toxic substances (and recommended by the National Academy of Sciences) consists of four processes:
      1. Hazard identification (or assessment and characterization)
      2. Dose-response assessment
      3. Exposure characterization
      4. Risk characterization
   C. The first three processes in risk assessment are scientific in nature; i.e., experiments are conducted using sound principles of hypothesis testing to determine (characterize) toxicological hazard, the relationship between dosage and response (adverse effects), and degree of exposure in the environment. Risk characterization, on the other hand, is partly scientific and partly management.
   D. Hazard Assessment: determination of the range of possible biochemical and physiological responses to a toxicant. The process necessarily involves administering low and high doses to achieve a full range of effects.
      1. Hazard assessment studies are the most common type of experiments found in the toxicological literature.
         a. Most of the studies are motivated by the objective of determining the mechanism of toxicity.
            1. Thus, these studies are most often characterized by few doses, sometimes only one, administered to an animal.
            2. Often to achieve an easily measured effect, the doses are given by injection, either intraperitoneally or subcutaneously.
            3. Other variables in dosing include acute (single dosing), short term (perhaps during gestation only, or several weeks), subchronic (usually 13 weeks or 90 days), and chronic (life-time equivalent; the average lifespan of a rat is considered about 2 years).
b. Fewer studies are governed by the objective of discovering a dose causing no effect.
   1. Ironically, this “negative” data, although less likely to be published by members of research-oriented institutions like universities, is the information most valuable to regulatory agencies for proper risk assessment.
      a. Industry, however, conducts and submits the research that includes the lowest dose not causing an effect (No Observable Adverse Effect Level, NOAEL) for the various hazards that are required to be tested prior to pesticide registration.
   2. Similarly, few investigators ask the question directly, why did this substance cause no effect at this dose but an effect at a higher dose?
      a. Toxicokinetic studies are valuable in answering such a question.

2. Information about hazards of substances comes from three basic types of experiments:
   a. In Vitro
      1. Tests are conducted on purified or gross tissue homogenates of enzymes and receptors; cell and tissue cultures; perfused organs
      a. Dosages are difficult to relate to whole body exposures unless toxicokinetics are well understood.
      b. Mutagenicity studies, or the potential for causing genetic changes, are often in vitro studies, but can be studied following a dose to a whole animal.
         1. One example of a routine mutagenicity test is the Ames Test that examines the number of reversions of a histidine-minus bacterial strain to a histidine-plus character in the presence of a toxicant.
   b. In Vivo
      1. Live animals are administered doses by various routes, including dermal, inhalational, oral.
      2. Subsequent measurements range from biochemical effects to behavioral effects.
   c. Epidemiological
      1. Epidemiology, which evolved to study incidence of pathogenic diseases and causes of the disease, relies on Koch’s postulates.
      2. Post WWII, the traditional role of epidemiology was stretched to include chemical substances, but in contrast to positive identification of a disease organism and traceability of exposure levels, chemical epidemiology suffers from inaccurate (and imprecise, or even non-existent) exposure information.

3. Hazard assessment studies examine the widest possible range of toxicological endpoints. The required tests and guidelines for conducting them are promulgated by the EPA and are considered validated procedures for conducting studies that are to be submitted in support of a petition for pesticide registration (see “OPPTS Test Guidelines Series 870, Health Effects” http://www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/ Series/). The tests include variations of the following hazards:
   a. Death
      1. Single dose (acute exposures); estimation of LD50
b. Systemic effects
   1. Pathology of organs
   2. Weight loss
   3. Other gross abnormalities
   4. Blood chemistry
   5. Three duration exposures are used: 7 and/or 21-day dermal exposure test; 90-day subchronic oral exposure test; 2-year rodent or 1-year dog chronic oral exposure test.
      a. Note that for each day of the tests as noted above, toxicant if presented to the test animal either as a patch on the skin (dermal exposure) or ad libitum in the diet (oral exposure).

c. Neurotoxicity
   1. Functional examination of behavior
   2. Determination of effects on cholinesterase if evidence of neurotoxicological effects
   3. Pathologic examination of nerve tissues if evidence of neurotoxicity

d. Development toxicity
   1. Maternal exposure, usually via oral route, during gestation (for example, on rodents this might be 6-15 days in duration)
   2. Observations of gross morphological effects in fetus and neonates (teratology)
   3. Observations of maternal toxicity

e. Reproductive toxicity
   1. Exposure during gestation followed by continuous exposure through the next two generations;
      a. Thus, offspring are exposed and then paired for mating.
      b. Toxicological examination of parents and each succeeding generation of offspring under continuous exposure.

f. Immunotoxicity
   1. Levels and ratios of various immune system factors, such as number of different types of lymphocytes and different immunoglobulins (antibodies).
   2. However, it is very rare to see a study that does pathogen challenge after toxicant exposure. Such a study would aid in understanding the significance of these immune system cell changes.

g. Behavior
   1. Functional observational battery
      a. For example, how long can a rodent pup hang onto a rod, how long does it take to right itself, ability to learn a maze, and others.
      b. Exposure is in utero during gestation.

h. Carcinogenicity
   1. Life time, daily exposure
   2. Post-mortem examination of multiple tissues for tumors
   3. Systemic toxicity is also studied simultaneously (see above)
   4. Note that interpretation of potential for carcinogenic effects is done in the light of the results from mutagenicity studies that are also required.

i. Biochemical effects
1. Enzyme or receptor function, especially when mechanism of toxicity at the biochemical level is known (called toxicodynamics) and it is the most sensitive endpoint.
2. Example: plasma cholinesterase, red blood cell acetylcholinesterase (AChE), and brain AChE inhibition.

j. Toxicokinetics
1. Extent and rate of toxicant absorption, distribution, and elimination.
4. Note that the FQPA (Food Quality Protection Act) now requires the EPA to make a determination of whether a pesticide might affect the endocrine system.
   a. However, at this time there are still no agreed upon tests that must be submitted for risk assessment, but the EPA is developing its recommendations.
   b. Some toxicologists would make the case that developmental and reproductive toxicity testing has always served as a surrogate for endocrine system effects.

E. Dose-Response Characterization
1. A series of increasing doses are given to test populations, most often rodents but also dogs. Of course, one group of animals is given no toxicant.
   a. Note that for regulatory toxicology studies, males and females are dosed as independent groups rather than as a mixed group.
   b. Also keep in mind that the exposures are continuous and the most used information comes from the 90-day subchronic and 2-year chronic exposure studies.
2. The objective is to determine the lowest dose causing an adverse effect (LOAEL), and the NOAEL.
   a. For regulatory toxicology studies, usually three doses are given for the various required toxicity tests.
   b. An examination of many of the studies submitted to the EPA shows that the two highest doses cause effects, and the third dose usually hits upon the NOAEL.
   c. The separation between the NOAEL and LOAEL is usually approximately 10-fold or less.
   1. Note that the NOAEL that the EPA identifies for its use in risk characterization is an actually observed dosage, not a statistical estimate.
      Thus, the true NOAEL is somewhat higher than that eventually used in risk assessments.
3. From the dose-response studies, EPA determines which of the various toxicological endpoints (shown under “hazard characterization”) are the most sensitive for both acute (single, short term) exposure and chronic (lifetime equivalent) exposure.
   a. Note that EPA often uses the subchronic (90-day) test NOAEL as the acute endpoint, unless an acute (single oral exposure) neurotoxicity study has been conducted.
   b. Thus, the most sensitive toxicological endpoint is any toxicological response occurring at the lowest “acute” and chronic dose.
   1. For certain compounds, like herbicides and fungicides, toxicity in acute and short-term tests is essentially nil. In longer-term exposures, especially at the
high doses, weight loss (defined as significant if 10% or more compared to control groups) is often the only toxicologically significant endpoint.

F. Exposure Characterization
1. Estimate or direct measure of how much pesticide a person (or nontarget organism) contacts;
   a. Does not take into account toxicokinetics unless extrapolations are being made from one pathway of exposure to another;
      1. For example, if an oral exposure toxicity study is used to determine hazards and potential dose causing toxicity from dermal exposure, than the oral dosage is multiplied by the absorption efficiency.
      2. Implicit, therefore, is the assumption that all of an oral dose is absorbed into the blood and is distributed to the tissues.
2. The FQPA mandates that exposure of pesticides to consumers be aggregated from the diet (i.e., food), water, and residential (indoor or outdoor) use.
3. Food residues are directly measured. If not, than the EPA assumes the tolerance level.
4. Water residues are almost always generated from computer simulation models, even though the USGS has accumulated a database of residues monitored in major watershed basins throughout the U.S.
5. Residential exposures are either based on direct measurements, or extrapolations from a database of occupational exposure studies known as PHED (Pesticide Handler and Exposure Database).

G. Risk Characterization
1. Risk characterization is partly scientific and partly management (policy).
   a. For example, from dose-response assessments, one can observe directly a dosage that causes no adverse effect in the test animal. This dosage is known as the NOAEL (No Observable Adverse Effects Level).
      1. A level of exposure either estimated or directly measured in the environment can be compared to the NOAEL, forming a ratio that describes how much under or over the NOAEL of a contaminant or drug a person is being exposed to. This ratio is called the margin of exposure (MOE).

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\text{Margin of Exposure (MOE)} = \frac{\text{NOAEL (mg/kg/day)}}{\text{Exposure (mg/kg/day)}}
\]

2. For exposure to pesticides, the risk or likelihood of an adverse effect in the environment is characterized after deciding what an acceptable MOE would be. For consumer exposure, the EPA considers MOEs greater than 100 to be of no concern (i.e., the Level of Concern or LOC), meaning a reasonable certainty of no harm.
3. Another way to estimate risk is to compare exposure to the Reference Dose (RfD). The RfD is the NOAEL adjusted by a 100-fold safety (uncertainty) factor. Any exposure less than 100% of the RfD is below EPA levels of concern (LOC).

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\text{Reference Dose (RfD)} = \frac{\text{NOAEL}}{100}
\]
EPA Risk Estimate = \( \frac{\text{Exposure (mg/kg/day)}}{\text{RfD}} \times 100 \);
(which must be less than 100% of RfD.)

a. EPA uses the MOE approach to risk characterization when dealing with worker exposure and residential exposure. The MOE approach is also used when characterizing risk from aggregate and cumulative exposure.

b. The “percentage of RfD” approach is used when only examining the risk associated with dietary exposure.

c. Determining an appropriate safety factor is subjective, i.e., it is management. There are no scientific principles that would dictate the use of one safety factor over another; however, some have argued that safety (i.e., uncertainty) factors can be derived by examining the available data on the range of endpoint response among animals (for example, comparing the range of neonate LD50’s to the adult LD50’s; examining the differences in toxicokinetics and/or toxicodynamics among animals or between animals and humans) (Dourson, M. L., S. P. Felter, and D. Robinson. 1996. Evolution of science-based uncertainty factors in non-cancer risk assessment. Regulatory Toxicology & Pharmacology 24:108-120)

1. Nevertheless the rationale for using a standard 100-fold safety factor in translating the NOAEL into the risk parameter known as the Reference Dose (RfD) is a 10-fold factor for translation of data from rodents to humans (in case humans are more sensitive than rodents) and another 10-fold factor for response variability in the human population (in case children and seniors are more sensitive than middle aged men.)

4. Risk characterization when children are deemed more sensitive at a given dose than adults;
   a. Note that the FQPA specifically mandates EPA to make a determination of whether infants and children are more sensitive to a given dose of a pesticide than adults.
   b. If the findings are affirmative, than EPA uses up to a 10-fold additional safety factor to estimate the RfD. At this point, the RfD is transformed into the Population Adjusted Dose (or PAD).
      1. Similarly, if the MOE method is used to characterize risk, then the acceptable MOE will be 1000 rather than 100.

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\text{Population Adjusted Dose (PAD)} = \frac{\text{RfD}}{10}, \text{ which is substituted into the EPA Risk Estimate equation above.}
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2. Note that the acceptable risk is still considered below 100% of the PAD.

II. Why the Focus on Children?

A. In a nutshell, kids are not little adults. That is to say, they have major morphological, physiological, and behavioral differences that could expose them to more pesticide.

1. But we are also concerned that the mode of toxicity or the rate of metabolism might be different, creating increased hazard in comparison to adults.
2. You will recall from the previous lecture, that the National Academy of Sciences Report, Pesticides in the Diets of Infants and Children, gave rationale to the provisions put into place by passage of the FQPA.


“Children Are Not Little Adults

The FQPA states that EPA will “ensure that there is a reasonable certainty that no harm will result to infants and children from aggregate exposure” to pesticide residues. Typically, what is a reasonable certainty is left to the regulatory agency, and the question quickly turns to risk management rather than to risk assessment. The latter is where the science occurs while the former is based primarily on considerations of politics, economics, values perhaps informed by scientific principles. With regard to any type of chemical exposure, however, sound scientific principles do support special consideration of infants and children.

Pediatricians have long known that the physiology of infants, children, and adults are different, and such differences could influence the therapeutic doses of medicines as well as the doses that are hazardous. Notable differences that may influence toxicity of drugs and other chemicals include the following.”

- Infants and children have a greater surface area to body mass ratio than do adults.
- Brain size in infants and children is proportionally greater relative to body mass than in adults.
- The fetal brain is rapidly developing, laying down new nerve connections especially in the third trimester of pregnancy and than continuing rapidly during the first year after birth. The network of connections between nerve cells is believed to be dependent on chemical neurotransmitters like acetylcholine.
- The blood-brain barrier, a membrane-like tissue rich in blood vessels surrounding the brain, is less developed than in adults and not as impermeable to certain chemicals. Also, cerebral blood flow is greater in children than in adults.
- The ventilation (breathing) rate of infants is significantly greater than in adults, resulting in greater inspired air exposure per unit time.
- Filtration of the blood through the kidneys (known as renal clearance) is slower in infants than in adults.
- Enzymes known to detoxify chemicals may be at lower levels in infants and children than in adults.”

“Are Children More Sensitive to Chemicals Than Adults?

The differences in physiology notwithstanding, generalizations about chemicals being more hazardous to children are not possible. Some chemicals may cause greater toxicity in children than in adults, but the reverse situation could also be true. For example, some chemicals are activated to toxic products by a special group of enzymes called mixed function oxidases (MFOs). If levels of MFOs were low, a toxicant may not become activated sufficiently to cause an adverse reaction. On the other hand, certain enzymes, including the MFOs, also break down
toxicants. Low levels of these detoxification enzymes could result in higher levels of a chemical in the blood and possibly a greater toxic effect.

One difference between children and adults that is suggested by differences in some of the physiological parameters above is that exposure to a given concentration of chemical, whether it is in food and water or in the house, will be greater per unit of body weight in a child. For example, because renal clearance is lower in children, a given concentration may have a comparatively greater impact in children than in adults because the chemical will stay in the body longer. If ventilation rate of children is greater, they could be exposed to comparatively greater amounts of chemicals in air. But the actual effect will depend on the biochemical mechanism of toxicity, and thus on the specific chemical."

“Are Children More Sensitive to Pesticide Residues Than Adults?

For organophosphate insecticides (OPs), currently the subject of much scrutiny under the FQPA, acute (single high dose) exposure studies have generally shown that immature rats are more susceptible than adult rats, but the direction of toxicity is not universal and depends on the specific OP. Furthermore, studies with lethal doses are not predictive of exposure to the extremely miniscule residues found in food.

A more important concern than lethality is possible adverse effects of OPs on development, especially of the brain, and reproduction. The FQPA specifically states that the EPA shall assess risk of pesticide residues based on “available information concerning the special susceptibility of infants and children… including neurological differences between infants and children and adults, and effects of in utero [during pregnancy] exposure to pesticide chemicals.”

To address this mandate, EPA has been concentrating on reassessing the risks of OPs by examining old and new toxicological studies. Doses used in these studies must be sufficiently low so that the newborn test animals live. Indeed, the objective of these studies is to find a dose that causes no observed effect (the NOEL), whether it be weight loss, organ disease, or simple biochemical changes.

The FQPA also mandates EPA to ensure exposures to pesticide residues are safe by using an extra ten-fold safety factor over and above the routine 100-fold factor when determining the tolerable level of exposure to all residues in food, water, and residences. This tolerable level of exposure, also known as the Reference Dose (RfD), is estimated by dividing the safety factor into the NOEL. If data were available to indicate that infants and children were not at greater risk than adults, than the extra safety factor could be lowered or waived, effectively raising the RfD or tolerable level of exposure. Several months ago, the EPA released its preliminary decisions regarding the application of this extra factor, also known as the FQPA 10X factor. Their decisions, although still preliminary, serve as guides to answer the question of whether children are more sensitive to pesticides.

Based on the weight-of-the-evidence from neurotoxicological, developmental, and reproductive tests examining immature rats, EPA decided that for at least 18 OPs, an extra 10X factor was unnecessary to ensure safety because the sensitivity of the young and the adults was similar. An extra 3X safety factor was going to be needed for another 10 OPs because although enhanced sensitivity was not found, testing was not complete. For 12 OPs, an extra 10X factor was still going to be required because data were too incomplete to make an assessment or data from the scientific literature suggested enhanced sensitivity. Chlorpyrifos and methyl parathion fell into this latter category.
The conclusions that immature animals are more sensitive than adults to chlorpyrifos and methyl parathion rests largely on the ability of these compounds to inhibit the enzyme cholinesterase in the blood. Inhibition of blood cholinesterase is most sensitive toxicological endpoint used in the neurotoxicity studies of OPs, and often it is used to determine the NOEL. Although the function of this enzyme in the blood is not well understood, in the brain it is important for normal transmission of nerve impulses. Several published studies have shown that the levels of chlorpyrifos required to significantly inhibit blood cholinesterase decrease with age. However, the lowest levels inhibiting blood cholinesterase are not accompanied by any other observable nervous system effects.

Although young rats seem more susceptible to some OP insecticides than adults, the mechanism of toxicity, i.e., inhibition of cholinesterase, is identical. Indeed, the NAS concluded that even if immature animals, including humans, were more sensitive to lower doses of drugs and toxicants, the toxic mechanisms were usually the same. Thus, to assess the risk from adverse effects of pesticide residues requires a determination of the exposure relative to the levels determined to cause no harm.”

C. On other major physiological aspect of children’s susceptibility to toxicants in general is that timing of exposure relative to developmental stage is as important as dose (although within any one stage, dose is still important).
1. The timing of exposure is especially critical with respect to fetal development. Cell proliferation and differentiation are at the highest level, and these tissues are thus very susceptible to adverse perturbations if the dose is high enough.
2. Once organs have been formed, they are much less susceptible to adverse effects from toxicants.

III. Pesticide Hazards to Children—Endocrine Disruption
A. A Unifying Principle??
1. In the short time given for this topic, it will be impossible to go into depth on any one of the numerous subjects about endocrine system effects. However, there is now a unifying theme to all hazards from all chemical exposure (not just pesticide exposure), and this is the biochemistry and physiology of the endocrine system.
2. The unification of most of the pesticide hazards into one that hypothetically could be related to or at least called endocrine disruption is actually a hypothesis implied by the popular book called *Our Stolen Future* by Theo Colborn et al. (1996).
3. To quickly and simply explain why attention to the endocrine system is important, below is an excerpt from an essay I published in Agrichemical and Environmental News (Felsot, A. S. 1997. Endocrine disruptor worries here to stay. Agrichemical & Environmental News (September) 139:6-9; archived issues available at http://aenews.wsu.edu).

“The Endocrine System Is One Part of the Body’s “Computer” Internet

If we are ever going to have a rationale discussion about whether or not chemicals can disrupt the endocrine system, then we have to start from the basics. A useful model for understanding the endocrine system is to view it like part of a worldwide computer network. Computers are now linked together for communication all over the world through the Internet, commonly known as the World Wide Web. This interdependence is carried out through main
computer servers or nodes at key places in the world. Thus, your individual computer may actually be communicating with a particular node that then sends the messages to another node or directly to another computer. The receiving node or computer can give feedback to the sending node or computer, establishing a two-way interactive system of communication. Meanwhile, the individual computer initiating the message, has within itself its own parts, like the CPU (central processing unit), ROM (read-only memory), the hard disk, and the video screen that involve two-way communication among themselves.

Think of the endocrine system as one node of a body internet that also includes the nervous system and the immune system as the other nodes. Each of these nodes communicate within their own system and with each other. While computers within themselves and across the Internet communicate via a combination of electrical signals and telecommunication microwaves beamed to satellites and back, the body’s internet communicates information across the nodes via a system of chemical messengers.

The chemical messengers of the endocrine system are called hormones. Hormones are produced by ductless glands and released (secreted) directly into the blood stream. The most well-known hormones are sex steroids like estrogen, produced by the ovaries, and testosterone, produced by the testes. Estrogen and testosterone are also produced in the adrenal gland associated with the kidney of both sexes. Testosterone can be changed into estrogen by an enzyme called aromatase that is prevalent in brain cells. Thus, males also have low levels of estrogen as well as testosterone in the blood. Some of the other well-known hormones include those produced by the thyroid (thyroxin) and pancreas (insulin). The speed with which hormones can work, and an indication of their ability to communicate with the brain, is illustrated by how fast one flinches when they see an object coming straight toward them. This behavior is mediated by the hormone adrenaline, produced in the adrenal glands and in the brain.

In the nervous system, the messengers are called neurotransmitters and are released at the endings or junctions between nerves or between nerves and muscle or glands. These junctions are actually very tiny physical spaces into which chemical messengers are released. For example, acetylcholine is a chemical released at nerve endings that permit an electrical nervous impulse traveling across the fibers of a nerve to be transmitted to an adjacent nerve, where a new electrical signal is propagated. An intimate connection between the central nervous system (i.e., the brain and spinal cord) is made through the communication of neurotransmitters with the hypothalamus and pituitary, two endocrine glands in the brain. When stimulated, these glands release hormones that circulate throughout the body to affect other organs, including the sex glands.

The thymus gland, lying near the heart, is the master controller of the immune system, regulating the production of the myriad immune cells and antibodies. Certain immune cells produce hormones called cytokines that can interact with the brain. In response, the brain may produce hormones that affect other glands and organs, including the thymus.”

“Translating the Message

From the blood, hormones interact with cells by binding to special proteins called receptors. Many organs and glands contain receptors for one or more hormones. Receptors are located in membranes on the outer cell surfaces or on the nucleus. The nucleus of the cell contains all the genetic information, which is stored in the DNA, the information containing biochemical polymers making up the genes. The chemical messenger actually binds with the receptor like a lock and key. When enough receptors are bound by the messenger, the lock is
opened and a signal is transferred to the DNA. The DNA is “woken” up, setting in motion a chain of events that causes the genes to produce proteins necessary for proper functioning of the individual cells, tissues, and organs. Translation of the message by target cells result in physiological reactions ultimately responsible for stimulating, regulating, and maintaining proper metabolism, development, growth, reproduction, and behavior.

The hormones stimulate physiological responses at incredibly minuscule concentrations. For example, estrogen can stimulate growth of cells at levels equivalent to parts per trillion. Furthermore, the timing of the messages, is crucial to normal development, especially in the fetus. Thus, the right amount of hormone must be present at the right time for a male or female to develop normally. Studies with mice have shown that higher than normal amounts of estrogen at the wrong time during pregnancy can cause genetically male rats to behave more like females, and in some cases to develop female-like genitals.

Hormone concentration and timing is crucial not only to sexual development, but also to normal development of the brain. A malfunctioning endocrine system during fetal or infant development could potentially alter the proper functioning of the immune system in later adult life. In short, the endocrine system and its interactions with the brain and immune system are exquisitely balanced and timed.”

“A Promiscuous Flaw

The various hormones function like keys for specific receptors by virtue of their three dimensional molecular structure. Unfortunately, the receptors can also be activated by chemicals whose molecular structure mimics the natural hormones. Chemicals with this ability are called endocrine disrupters, and they can act in a wide diversity of ways as indicated by EPA’s working definition--endocrine disrupters “interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis [normal cell metabolism], reproduction, development, and/or behavior.” The list of chemicals with endocrine disrupting potential is growing but contains the usual suspects: various pesticides, persistent chlorinated organics like PCBs and dioxins, plasticizers, surfactants, and heavy metals. But the list also includes natural biochemicals: mycotoxins produced by fungi and chemicals produced by fruit and vegetables (called phytoestrogens).

EPA’s definition of an endocrine disrupter covers a wide array of possible effects. A discussion of the concerns can be found in the EPA special report, “Environmental Endocrine Disruption: An Effects Assessment and Analysis.” Most of the media attention has been focused on chemicals acting like estrogen, but this is only one mode of action. More important are the potential endpoints or diseases in humans: breast cancer and endometriosis in women; testicular and prostate cancers in men, abnormal sexual development, reduced male fertility, alteration in pituitary and thyroid gland functions, immune suppression, and neurobehavioral effects.

While many of these adverse effects in humans are only hypothesized as being associated with endocrine disrupters, some scientists are arguing that enough evidence as accumulated to conclude a cause and effect relationship between endocrine disrupters and disease in wildlife. Adverse effects include abnormal thyroid function and development in fish and birds; decreased fertility in shellfish, fish, birds, and mammals; decreased hatching success in fish, birds, and reptiles; demasculinization and feminization of fish, birds, reptiles, and mammals; defeminization and masculinization of gastropods, fish, and birds; decreased offspring survival; and alteration of immune and behavioral function in birds and mammals.”
“Dose Still Makes the Poison

In past essays I have written about the use of extremely high dosing required by the EPA for tests of carcinogenic potential of pesticides, and I have questioned the validity of the results for assessing the effects of exposure to the very tiny environmental concentrations. I am ready to make similar conclusions about the applicability of endocrine disrupter screening to real world exposures. The current laboratory testing procedures for endocrine disrupters do show that certain chemicals can activate the estrogen receptor or block activation of the testosterone receptor. However, the doses required to show these effects in a test-tube type experiment are thousands to millions of fold greater than for the natural hormones. Such observations suggest that environmental concentrations of the “endocrine disrupters” may be irrelevant to producing a biological effect.

Endocrine disrupting potential of pesticides like DDT have been tested by directly feeding it to rats. Adverse effects on sexual development have been reported, but the dosing rate was millions of times greater than what humans are normally exposed to in food. Thus, with regard to testing methodology, endocrine disrupters seem to be in the same boat as carcinogens.

Research reported by Tulane University in 1996 suggested that perhaps very low doses of endocrine disrupters were physiologically important. The researchers purportedly showed that two or more chlorinated hydrocarbon pesticides administered together could have a synergistic endocrine disruptive action at doses a thousand-fold lower than the doses causing the same activity when alone. When dieldrin and endosulfan were dosed together at levels equivalent to parts per billion, an estrogenic effect was observed; when given alone, parts per million were required to produce the same effect. However, no other laboratory was able to duplicate the results of the synergism experiment, and finally, the researchers at Tulane themselves admitted they could not repeat their own observations.

Nevertheless, Tulane’s retraction has not quieted the storm. Whether or not there are synergistic interactions among low doses, scientists still have shown that doses of two or more chemicals can be additive, albeit the concentrations necessary for an effect are extremely high. Perhaps stronger cases for widespread problems linked to endocrine disrupters will be made when the dosing becomes more realistic and thresholds for effects are determined.

Meanwhile, the FQPA requires EPA to certify screening tests for endocrine disrupters. Eventually all pesticides will be subjected to the approved battery of tests.”

B. Note that during 1999, the National Academy of Sciences, through its research arm, the National Research Council (NRC), issued a report, Hormonally Active Agents in the Environment.
1. The NRC concluded that some biochemical/physiological effects could not definitely be concluded to be due to hormone disruption. Thus, the committee decided it was better, and perhaps more accurate to exchange the term endocrine disrupters for hormonally active agents (HAAs).
   a. Thus, far, most HAAs, are first earmarked as hormonally active in in vitro tests. However, in vivo tests must be conducted to understand whether the compound is also active when toxicokinetic factors are at work.
2. One major conclusion from the NRC report was that evidence was too weak to conclude that exogenous HAAs had affected human health. The evidence was much
more suggestive that some specific wildlife populations in specific regions (for ex., the Great Lakes) may be suffering from overexposure to HAAs.

C. The issue of whether putative HAAs actually cause effects at “low doses” or cause one effect at a low dose but another effect at a high dose (a non-monotonic dose response curve) was addressed by the National Toxicology Program (National Toxicology Program. 2001. Endocrine Disrupters Low Dose Peer Review. US EPA and NIEHS Report (available on the web as PDF file; search Google for National Toxicology Program and Low Dose)

1. A peer review panel was assembled to examine raw data from requested studies and to re-analyze them statistically as well as examine the conduct of the study.
2. The panel concluded that there is contradictory evidence from one of the most studied compounds, bisphenol A. This compound has been tested as dose of micrograms per kg body weight.
   a. Bisphenol A is the unreacted monomer “contaminating” certain types of sealants, for example the sealants used to coat tin cans and in dental materials. Although it is not a pesticide, in today’s climate, studies of any chemical showing endocrine activity at low doses or a non-monotonic dose is taken as a surrogate for the potential bioactivity of all chemicals, including pesticides. (For the latter reason, it is important to pay attention to the toxicology literature for everything to understand how the public might view pesticides.)
3. For pesticides like methoxychlor and vinclozolin that have been found to have been found to be estrogen agonists or antiandrogenic, respectively, the lowest effective doses are actually quite high (in the range of mg/kg) and do not reflect human exposure (Note: this is my conclusion on the significance of the findings).

IV. Pesticides and Childhood Cancer

A. Although all pesticides are tested in chronic dietary exposure assays with rodents (2-year studies) and occasionally dogs (one-year study), these tests by their nature use very high doses relative to environmental exposures.
   B. One of the doses chosen must be the MTD (maximum tolerated dose).
      1. The MTD is chosen from subchronic or other range finding studies.
      2. It is the dose that causes no overt signs of toxicity, and a weight loss of nor more than 10%.
      3. Note that illness in a rat not apparent to a handler (observer) does not mean that organ pathology (and by implication, cell toxicity) is not occurring.
      4. At any rate, biochemical toxicologists tend to discount the validity of the high dose carcinogenicity assay, but it is still used by EPA, as well as being controversial.
      5. When conducting a risk assessment, EPA claims that it uses a weight of the evidence approach in making a decision about how to manage the risk.
         a. Since chronic toxicity is probably the biggest issue regarding exposure to environmental contaminants, and also the most contentious in terms of influencing a risk management decision, any effects on human health that can be
discerned from epidemiological studies would necessarily be weighted relatively heavy.

C. The main problem with current testing methods for carcinogenicity (of nonmutagens) is that the shape of the dose-response function at lower, untested doses is unknown. This is why some biochemical toxicologists argue for a biologically based study of carcinogenicity mechanisms for any contaminant.

1. As shown in the figure below, based on only three data points, high doses must be extrapolated to low doses for risk assessment, but the true dose response relationship cannot be quantitated.

![Graph showing no-threshold model of carcinogenesis.](image)


1. The NRC concluded that the level of both synthetic and natural substances that have tested positive for causing tumors in the typical rat carcinogenicity assays at MTDs are highly improbable to pose an risk for carcinogenicity nor any likelihood of significant adverse biologic effects.

E. Thus, most conclusions (which turn out to actually be hypotheses), about a correlation between pesticides and children, come from epidemiology studies.

1. The objective of these studies is to relate the incidence of a disease or condition with exposure to some agent (microbiological, chemical) or activity (lifestyle, behavior, product use, place of residence, etc.);
   a. Epidemiological studies of chemical effects are normally applied to chronic exposures and maladies best classified as chronic toxicity as opposed to acute toxicity (see endpoints below)

2. These studies are the most controversial because it is very difficult to control confounding factors (i.e., variables that may affect or influence the outcome of the results);
Furthermore, epidemiological studies at best can make associations between exposure to an agent and an outcome, but this is quite distinct from concluding an agent caused an outcome.

1. Epidemiological studies rely heavily on regression analysis.

2. Epidemiology grew out of the need to control infectious diseases, which have definable causes; furthermore, infectious diseases, which are essentially microbiological problems, can be directly tested using Koch’s postulates, stated as follows:
   a. The infectious agent (microbe) must be present in every case of a disease;
   b. The microbe must be isolated from the disease and grown in pure culture;
   c. The specific disease must be reproduced when a pure culture is inoculated into a healthy susceptible host;
   d. The microbe must be recoverable again from the newly infected host.

3. Unfortunately, for most chemical exposures, unless they are acute (as opposed to chronic), Koch’s postulates are not applicable.

4. Furthermore, in many chemical epidemiological studies that do not involve workers at a specific industry (or manufacturing site), exposure records are poor to nonexistent. Often exposure is deduced from interviews of “what was used” or “next-of-kin” interviews.

F. The attempt to associate chemicals as etiological agents with specific diseases has had some successes, but the widespread use of epidemiology to make solid cause and effect relationships rests on tenuous ground.

1. An example of a success is the association of scrotal cancer with the profession of chimney sweep.

2. The evidence gained from human studies is strengthened by consistency among several studies.
   a. Conflicting results among well-done, large epidemiological studies raise serious doubts about apparent associations.
   b. One limiting factor is dose quantification;
      1. Some investigators use qualitative exposure estimates that raise problems of misclassification of exposure
         a. low, high, medium
         b. use of a chemical for X number of years

G. Measures of Association (information from Draper, 1994, ACS Advance in Chemistry Series 241, *Environmental Epidemiology*)

1. Relative risk
   a. A measure of how many times greater the risk for one population is compared to another population;
      1. Commonly used with cohort type studies where large numbers of people who have been considered for their exposure at some specified base-line time and are then subsequently observed for the development of disease.
         a. If disease is related to exposure, the frequency of exposure should be greater among the diseased than among the non-diseased group.
   2. The incidence among those exposed to a risk factor (I_e), divided by the incidence among those not exposed (I_n)
a. \( RR = \frac{I_e}{I_o} \) (or, incidence of disease in exposed group divided by incidence of disease in unexposed group).

b. RR is a measure of the strength of an association.
1. The greater the RR value, the more likely that the risk factor is important in causation.

c. Generally, we tend to doubt relative risks of less than 1.5.

2. Attributable risk
   a. Measure of the impact of a risk factor
   b. Defined as the difference between the incidence in the exposed and the unexposed—or, the excess incidence among the exposed after removing the expected background incidence
      1. \( AR = I_e - I_o \)
      2. AR is the portion of the incidence among those exposed that can be attributed to that exposure

### Relative vs. Attributable Risk

<table>
<thead>
<tr>
<th>Factor</th>
<th>Lung Cancer</th>
<th>Coronary Heart Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy smokers (incidence)</td>
<td>166/100,000</td>
<td>599/100,000</td>
</tr>
<tr>
<td>Non smokers (incidence)</td>
<td>7/100,000</td>
<td>422/100,000</td>
</tr>
<tr>
<td>Relative risk (ratio)</td>
<td>23.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Attributable risk (cases/100,000)</td>
<td>159/100,000</td>
<td>177/100,000</td>
</tr>
</tbody>
</table>

3. Odds Ratio
   a. A measure of relative risk for case-control studies.
      1. Utilize samples of diseased and non-diseased persons to determine the frequency of the exposure of interest, rather than to evaluate exposure in a disease-free population and to await subsequent disease expression as would be done in a cohort study.
      2. Odds Ratio (OR) = \( \frac{\text{incidence of exposure in diseased group}}{\text{incidence of exposure in non-diseased group}} \)
         a. Thus the OR represents the frequency or gradient of exposure in the diseased group relative to the frequency of exposure in the control or non-diseased group.
            1. For example, if you hypothesize that compound X causes Non-Hodgkin’s Lymphoma (NHL, a type of cancer attacking the lymph glands) among a certain occupation using compound X, than if you compared the frequency of compound X use among NHL sufferers, you would predict a higher incidence of compound X use than in nonusers without NHL.

H. Measures of Uncertainty
   1. For measures of OR and RR, a 95% confidence interval (CI) about the average risk ratio is usually given in a report. The 95% CI represents the probability (i.e., less than 5% error) that the true risk has been captured in the stated interval if it was repeatedly sampled. For example, if an experiment was conducted 100 times, and the error rate less than 5%, you would have captured the true population risk in 95 of those experiments. The CI would differ with each independent experiment, but the
probability of capturing the true mean would still be set at 95% (or at whatever probability the experimenter determines is appropriate). If the lower end of the 95% CI goes below a relative risk or odds ratio of 1, it is appropriate to conclude that no conclusion can be reached with regard to the true population mean being greater than 1 (i.e., a risk greater than 1).

I. Cases where epidemiological studies work
   1. When a sudden chemical exposure in which an illness is produced within a very short time occurs (for ex., the chemical plant explosion in Bhopal, India, which spewed large amounts of methyl isothiocyanate (MITC)
   2. When under typical conditions and in very short periods of time, a researcher can correlate a change in a physiological function to the change in an environmental pollutant (for ex., children with asthma in areas where air pollution is caused by ozone)
   3. When an association has been made between long-term exposures and long-term functional effects (lead and children; can measure lead deposited in teeth)
   4. When occupational studies have linked relatively high levels of chemical exposure to the incidences of cancer in workers

J. Unfortunately, unless a specific occupational exposure has occurred, or a specific accident has occurred, exposures to environmental contaminants are not amenable to good epidemiological assessments essentially because they lack the quality of the situations described above.
   1. Lack of quantitative dose-response relationship at environmentally relevant doses;
   2. Lack of proper exposure assessment;
   3. Lack of specificity (disease could be caused by a lot of factors);
   4. Confounding factors not perceived.

K. A 1997 study by Daniels et al. (Environmental Health Perspectives 105:1068; “Pesticides and Childhood Cancers”) critically analyzed epidemiological studies published between 1970 and 1996 that involved associations with pesticide exposure and childhood cancer (n=31).
   1. Any associations were moderate (meaning they were less than an Odds Ratio of 2.0), but the strongest associations were seen between brain cancer and leukemia and frequent occupational exposure of parent to pesticides or home pesticide use.
   2. However, examining the data tables from brain cancer and leukemia, I noted the following:
      a. For childhood brain cancer, 40 correlations were shown (thus, some studies had more than one correlation in them). Of these 40, only 7 had lower 95% confidence intervals >1.0.
      b. For childhood leukemia, 25 correlations were shown. Only 4 had a lower 95% confidence interval greater than 1.0.

L. One more aspect to consider is biological plausibility.
   1. Many chemical epidemiological papers fail to consider the myriad of rodent chronic exposure studies that have been submitted to the EPA for consideration of pesticide risk assessment.
      a. These studies often have NOAELs for excess tumor occurrence.
      b. Furthermore, reference doses are estimated and exposure is not supposed to exceed this dose if the pesticide is to be registered.
c. Thus, it is extremely unlikely that children, adult consumers, nor workers are being exposed to concentrations above the reference dose.
d. Therefore, a comparison of the RfD to the cancer NOAELs (based on the rodent studies), reveals that compounds suggested to have an elevated odds ratio in the studied population cohorts do not have any biological plausibility for causing cancer given the extremely low exposure compared to the dose known not to cause excess tumors in rodents (Acquavella, J., J. Doe, J. Tomenson, G. Chester, J. Cowell, and L. Bloemen. 2003. Epidemiologic studies of occupational pesticide exposure and cancer: regulatory risk assessments and biologic plausibility. Ann. Epidemiol. 13:1-7.)

Relationship Between Cancer NOAEL (based on rodent studies) and Reference Dose (Acquavella et al. 2003)

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Use Rate (lb/acre)</th>
<th>Cancer NOAEL (mg/kg/day)</th>
<th>RfD (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrazine</td>
<td>1.3-2.2</td>
<td>2.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>0.001-5.4</td>
<td>14</td>
<td>0.1</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>0.2-3.7</td>
<td>1000 *</td>
<td>2</td>
</tr>
<tr>
<td>Acetochlor</td>
<td>0.001-3.0</td>
<td>26</td>
<td>0.02</td>
</tr>
<tr>
<td>2,4-D</td>
<td>0.2-2.0</td>
<td>125 *</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* No excess tumors were detected when glyphosate and 2,4-D were tested. The cancer NOAEL shown is the highest dose tested.

V. Organophosphorus Insecticides and Children

A. Of all the regulatory activity mandated by the FQPA, the reassessment of the OP insecticides has been the most controversial.
   1. This group of pesticides, by far, is the most hazardous, largely owing to comparatively low LD50s for many compounds (i.e., high acute toxicity).
   2. Many of these compounds have been around for 30 years or longer, so we have been exposed for quite some time.
   3. Using “new” risk assessment policies, especially with regard to probabilities of acute dietary risk assessment and aggregate exposure, EPA has determined that a number of popular (and cheap) OPs are too risky for kids.
      a. You will recall that too risky is an exposure level exceeding EPA’s RfD or PAD, such as the case may be.

B. Although cholinesterase inhibition is the most sensitive toxicological endpoint determined in hazard assessment and used for risk characterization (even though its physiological significance at low levels of inhibition are arguable), new research indicates that some OPs, at least chlorpyrifos, may interact with AChE (acetylcholinesterase) independently of its esteratic functions to cause disturbances in growth and connections of neurons.

C. Below are excerpts from an essay that I wrote concerning this novel mechanism of action [Felsot, A. S. 2000. The chlorpyrifos risk assessment. Part 2: The rugrat rant. Agrichemical & Environmental News (January) 165:14-17; archived at http://aenews.wsu.edu].
   1. Note that to discover such a mechanism of action is not the same as concluding an increased risk. Nevertheless, it is a biochemical mechanistic hypothesis that needs
further research, and in light of the uncertainty it creates, perhaps knowing about it justifies an increased safety factor for chlorpyrifos risk management.

“So what prevailing hypotheses make EPA so jumpy about possible increased sensitivity of children, and DAS so sure the data indicate no need to worry?”

“**In the Beginning—Brain Development**

Scientists can at least agree on how the nervous system develops. Within the first trimester of pregnancy the cells of the fetus are rapidly dividing and specializing into three basic types of tissue layers. Part of the outermost layer or ectoderm folds to form the neural tube, the precursor tissue for the entire nervous system. (1, 15). [Note: number citations refer to references shown below] 

As the primitive cells of the neural tube divide, some are destined to become neurons, which carry information, and many more become glial cells, which serve in a nutritive, supporting role to the neurons. The glial cells also serve as guides for the dividing neuroblast cells of the neural tube to migrate to the appropriate places in the primitive brain vesicles where they can differentiate into functional brain cells (15).

By the late first trimester and early second trimester the migrating neuroblasts have reached their destinations and they begin to send out branches known as dendrites and axons (1). The dendrites grow out from the neuron cell body (where the DNA-containing nucleus resides) and receive input signals from neighboring neurons. The signal is carried away from the cell body along branches known as axons. The axons come in close contact with dendrites of adjacent or distant nerve cells at junctions called synapses. Synapse formation begins around mid to late second trimester and essentially continues throughout life.

The synapses are actually very tiny spaces where the electrical nerve signal is transduced to chemical energy in the form of a neurotransmitter. Acetylcholine (ACh) is one of the prevalent neurotransmitters in the central nervous system and at the neuromuscular junctions. It is released into the synapse and diffuses to the dendrite membrane on the other side where it interacts with a receptor protein to restart the electrical signal.

How the nerve cells know where to migrate or branch out to remains a big mystery, but evidence is slowly mounting that neurotransmitters like ACh might play some role controlling nerve growth (9). Indeed, research over the last decade indicates that acetylcholinesterase (AChE), which is the enzyme responsible for breaking down ACh and thereby dampening its signal-carrying ability, may influence the growth and differentiation of the axons (17).

Given that ACh and AChE somehow control growth and development of the brain, it is logical to be concerned about substances that affect these molecules (3). Such concerns demand information about the nature of neurodevelopmental hazards of OP insecticides and their relationship to dose and potential exposures.”

“**Mechanistic & Regulatory Toxicology Studies Give Different Answers**

In the chlorpyrifos risk assessment, EPA cited several studies in the scientific literature that indicated suckling rats are indeed at least several fold times more susceptible to the acute effects of chlorpyrifos. The key word here is acute effects; the studies cited were testing either lethal doses or maximum tolerated doses (MTDs). While outright death was not observed at MTDs, and weight loss did not decrease by more than 10%, there is no doubt that brain AChE was significantly inhibited. In some studies (11, 12), nonlethal but telltale signs of poisoning had
occurred—tearing, salivation, and tremors—indicating that MTDs are still quite toxic. Many studies administered doses by subcutaneous (under the skin) or intraperitoneal (into the abdomen) injection. Such unconventional exposure routes expose an organism to a very large dose all at once, bypassing the protective layer of the skin or the much slower absorption into the bloodstream from the intestine.

Such drastic doses and methods of exposure are part of what I like to call mechanistic toxicology studies. Their objectives are to characterize physiological responses and their biochemical basis. Occasionally, doses are used that elicit no response in an attempt to track subtle changes in biochemistry. The objective of regulatory toxicological studies, on the other hand, is to find the NOEL for the most sensitive biochemical or physiological response.

The DAS studies submitted for EPA review used doses spanning from the NOEL through those known to produce an effect. Chlorpyrifos doses were given directly to a mother rat during pregnancy and for 11 days after birth to expose both the fetuses and the suckling newborns (neonates). The parental NOEL was always lower than the NOEL for the neonates (Table 1). In other words, the neonates were less susceptible than the adults for the most sensitive endpoint examined. The endpoints ranged from enzyme inhibition to brain histopathology and functional behavior, all effects that would be predictive of adverse neurodevelopment.”

“**Why Dose Matters**

In nearly every case that EPA used to support its conclusion that infants may be more susceptible than adults, doses were incredibly high relative to real world exposures. For example, in one cited study 1-4 day old rats were injected subcutaneously with 1 mg/kg/day of chlorpyrifos (18), the MTD previously observed not to cause outward signs of anticholinesterase toxicity. Nevertheless, brain AChE was significantly inhibited. More importantly, the exposure was nearly 800 times greater than aggregate (dietary and residential) exposure both modeled as well as measured at the 99.5th and 100th percentile, respectively (6).

Recent research compared neurochemical effects of chlorpyrifos and methyl parathion in neonatal and adult rats, and it sheds some light on the discrepancy in observations between the higher dose mechanistic studies and the lower dose regulatory studies (10). Each insecticide was subcutaneously injected into neonatal and adult rats for 7 or 14 days in a row with doses equivalent to about 20% of their MTDs. Brain cholinesterase and receptor binding inhibition were similar in neonatal and adult rats exposed to chlorpyrifos, but reversed to control levels more quickly in neonates. Neonates were always significantly more sensitive than adults to the effects of methyl parathion exposure.

Thus, neonatal rats respond differently to different OPs. Neonatal rats are more susceptible to adults to the lethal effects of acute high doses of chlorpyrifos, but are less sensitive to the subacute intermittent doses and as equally sensitive when exposed to subacute daily doses. In essence, the level of dose determines the differential sensitivity for chlorpyrifos but not for methyl parathion.”

“A Monkey Wrench in the Works?…

Curiously, EPA gave a cursory nod to an intriguing new area of research regarding possible effects of chlorpyrifos on neuronal cell replication and growth. Exposure of neonatal rats to subcutaneous injections of 1 or 2 mg/kg chlorpyrifos resulted in an inhibition of DNA synthesis and abnormal functioning of one component of the cell cycle control system called adenylyl cyclase (18, 19). However, significant brain cholinesterase inhibition occurred
immediately after dosing, so it is doubtful whether the effects were truly more sensitive endpoints than plasma cholinesterase (ChE) inhibition (10).

On the other hand, a hypothesis currently in vogue is that chlorpyrifos may affect neurodevelopment through inhibition of neuronal cell (neurite) branching and rate of growth (16). A recently released study used a special nerve cell culture (PC12) and showed that chlorpyrifos and its nontoxic metabolite TCP can reduce overall neurite growth without significant inhibition of ChE activity (4). The research suggests that chlorpyrifos and other anticholinesterase compounds react at a molecular site different than the one responsible for inhibiting the enzyme."

“…Or a No-Brainer?

One of the problems with cell culture studies is the difficulty of relating the concentration of a toxicant in the dish to the dose given to a rat. The PC12 cell study did show a definitive NOEL for neurite response to chlorpyrifos and TCP. The authors claimed that the concentration adversely affecting neurite growth was similar to brain levels of TCP reported in a study where pregnant rats were fed chlorpyrifos for four days at a rate of 3 mg/kg/day (6A). This oral dose is nearly 2500 times greater than the highest aggregate exposure to children (6).

The reported PC12 cell culture study may not have realistically reflected the concentration of chlorpyrifos or TCP that would be in the neonatal rat brain following exposure to a dose equivalent to the developmental neurotoxicity NOEL (see Table 1). TCP was not detected in blood from 5 day-old lactating neonatal rats whose mothers were exposed to 1 mg/kg/day chlorpyrifos during pregnancy and for 10 days after birth. Exposure of a neonate rat during lactation is most relevant to human fetal development because the newborn rat brain is equivalent to the developmental stage of a human brain during the third trimester of pregnancy (18)."

“A “Weight” and See Attitude

EPA claims that they will assess risk and make registration decisions using a “weight of the evidence” approach. While concluding that DAS’ data indicated no increased sensitivity of infants relative to adults, the agency chose to delve into the published scientific literature and find the “weight” it needed. Yet, most of the studies it did cite actually showed that neonatal rats were only more susceptible to lethal acute exposures but less susceptible at nonlethal intermittent or daily exposures. Three recently published papers have examined the same literature among other pieces of evidence and have thrown their “weight” behind removal of an extra FQPA safety factor (2, 6, 14).

With transparency seemingly ruling the EPA lately, anyone can read the chlorpyrifos risk assessment documents (http://www.epa.gov/oppsrrd1/op/status.htm) and make up their own mind. EPA has invited submission of comments for its consideration as it prepares the final re-registration decision. I’m waiting to see if the Tale of Two Sciences will continue.”

Table 1. Summary of EPA’s Interpretation of Results from Dow AgroSciences Tests to Determine Sensitivity of Infants

<table>
<thead>
<tr>
<th>Test</th>
<th>Doses (mg/kg/day)</th>
<th>Duration of Exposure</th>
<th>Parental NOEL</th>
<th>Offspring NOEL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental Toxicity</td>
<td>0, 0.1, 3, 15</td>
<td>Gestation Days (GD)  6-15</td>
<td>0.1</td>
<td>15</td>
</tr>
</tbody>
</table>
### Developmental Neurotoxicity

<table>
<thead>
<tr>
<th></th>
<th>0, 0.3, 1, 5</th>
<th>GD 6 to Lactation Day 11</th>
<th>&gt;0.3</th>
<th>1</th>
</tr>
</thead>
</table>

### Reproductive Toxicity

|            | 0, 0.1, 1, 5 | 2 Generations 12 weeks   | 0.1    | 1          |

### References


2. **A Final Comment and Observation:** The overwhelming majority of studies on non-cholinergic effects of chlorpyrifos on the neonatal rat brain have not examined the biological significance of the measured phenomena.
   a. Biological significance would be provided by studies of behavioral outcomes.
   b. Recently, a study examined behavioral effects on mice injected subcutaneously with either 1 or 3 mg/kg body weight chlorpyrifos. (Note that these are incredibly high doses given the estimated dietary and environmental exposures to chlorpyrifos at µg/kg/day levels.) (Ricceri, L., N. Markina, A. Valanzano, F. Stefano, M. F. Cometa, A. Meneguz, and G. Calamandrei. 2003. Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice. Toxicology and Applied Pharmacology 191(3):189-201)
   1. Neonatal mice were exposed either during postnatal days (PND) 1-4 or postnatal days 11-14.
      a. Acetylcholinesterase was inhibited in the PND 1-4 groups by ~25% but it was not inhibited in the PND 11-14 group.
   2. Behavioral analysis showed that early exposure failed to affect neonatal behaviors.
   3. For the PND 11-14 group both locomotory activity was elevated when tested on PND 25 days, and the low dose PND11-14 group of mice tended to show more antagonistic behaviors (i.e., aggressive behaviors) in the earlier parts of the observational period.
   4. Learning was not affected in any group.
   5. The authors concluded that developmental exposure to chlorpyrifos induced long-term behavioral alterations in the test mouse species. They concluded that their data supported the involvement of neural systems (for example, neural system development) in the delayed behavioral toxicity of chlorpyrifos.
   c. The significance of this new research is hard to understand from the viewpoint of human health risk assessment.
      1. The dose are incredibly high relative to estimated exposures.
      2. The route of administration is by injection; thus the dose transfer kinetics is unrealistic
      3. The relevance of the behavior measured is obscure; interestingly, however, learning was not affected.