Neurotoxicity, Immunotoxicity, and Endocrine Disruption with Specific Commentary on Glyphosate, Triclopyr, and Hexazinone: Final Report

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TABLE OF CONTENTS

TABLE OF CONTENTS (*continued*)

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This document was prepared under contract to the USDA Forest Service by Syracuse Environmental Research Associates (SERA), Inc. Dr. Patrick Durkin served as the primary author of the document and is solely responsible for the conclusions presented in the document. Dr. Durkin is co-founder and principal scientist of SERA. Prior to founding SERA in 1991, Dr. Durkin was employed at Syracuse Research Corporation, serving as director of the Life and Environmental Sciences Division and later as director of the Chemical Hazard Assessment Division (1972 to 1991). Dr. Durkin is a charter member of the American College of Toxicology, a charter member of the Society of Risk Analysis, a member of the American Association for Advancement of Science and the New York Academy of Science and is an Adjunct Professor at the SUNY College of Environmental Science and Forestry (1985-date). Dr. Durkin has conducted numerous risk assessments and risk assessment method development tasks for the USDA, U.S. EPA, and CDC/ATSDR. In recognition of his contributions to the development of the U.S. EPA's Ambient Water Quality Criteria, Dr. Durkin was given the U.S. Environmental Protection Agency Award for Special Achievement. Dr. Durkin has been appointed a consultant to the U.S. EPA Science Advisory Board (SAB) and is a member of the FQPA Science Advisory Panel (SAP) on cumulative risk. Dr. Durkin has also served as a reviewer of the risk assessment methods used by the Agency for Toxic Substances and Disease Registries (ATSDR), the Dutch Health Council, and for the journals Toxicology and Industrial Health as well as Risk Analysis.

Substantial assistance in the preparation of this document was provided by Dr. Gary L. Diamond. Dr. Diamond prepared the initial draft of the document and review of the studies summarized in this document that were published after the SERA risk assessments which are cited in this document. Dr. Diamond is a Senior Scientist at the Environmental Science Center of Syracuse Research Corporation. He is a member of the American Association for Advancement of Science, New York Academy of Science, Society of Toxicology. Dr. Diamond is an adjunct profession at SUNY College of Environmental Science and Forestry (1997-date) and an adjunct associate Professor in toxicology at the Department of Environmental Medicine, University of Rochester (1992-date). Dr. Diamond has served on advisory committees for the National Research Council, the U.S. EPA Science Advisory Board, and the U.S. Nuclear Regulatory Commission, as well as a peer-reviewer for the National Institute of Occupational Safety and Health. Dr. Diamond currently heads several research projects with EPA to develop exposure-biokinetic models for use in risk assessment and to develop methods for the consistent integration of bioavailability information into quantitative risk assessment. Dr. Diamond currently serves as SRC Program Manager of a contract that supports the EPA OERR lead risk assessment methodology and model development programs, and he is deputy Program Manger of a contract that supports the ecological and human health risk assessments programs of EPA Region 8.

During the preparation and review of this document, the USDA Forest Service expressed concern for some new information on the potential effects of glyphosate on immune function and requested additional review of this information – specifically the study by El-Gendy et al. 1998. To address this concern, SERA enlisted the assistance of Dr. Helen Tryphonas. Dr. Tryphonas reviewed a full text copy of the El-Gendy study and has reviewed all sections of the document relating to immunotoxicology. Dr. Tryphonas is a senior immunotoxicologist with Health Canada and an adjunct associate Professor in toxicology at the Université du Québec à Montréal. She holds a M.Sc. degree in Microbiology/Immunology and a Ph.D. degree in Environmental Toxicology/Immunology. She has authored/co-authored over 150 peer reviewed publications and is a co-author of the Canadian Network of Toxicology Center's, *Manual of Immunological Methods.* Her research areas include the immunotoxicologic effects of PCB's, toxaphene, fungal toxins, tributyltins and other chemicals of environmental concern. She is an active peer reviewer for several scientific Journals and has served as a peer reviewer of immunotoxicology studies for U.S. EPA and CDC/ATSDR, the Eastern Research Group, Inc. and Visions USA, Inc.

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

a.e.	acid equivalents
a.i.	active ingredient
AEL	adverse-effect level
ACGIH	American Conference of Governmental Industrial Hygienists
ATSDR	Agency for Toxic Substances and Disease Registry
bw	body weight
CD	cluster differentiation
CDC	Centers for Disease Control
Con A	Concanavalin A
CNS	central nervous system
EC_{50}	concentration causing 50% inhibition of a process
EDSTAC	Endocrine Disrupter Screening and Testing Advisory Committee
F	female
F_1	first filial generation
FS	Forest Service
GABA	gamma-aminobutyric acid
kg	kilogram
L	liter
lb	pound
LC_{50}	lethal concentration, 50% mortality
LD_{50}	lethal dose, 50% mortality
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LPS	lipopolysaccharide
MHC	major histocompatibility complex
mg	milligram
mg/kg/day	milligrams of agent per kilogram of body weight per day
mL	milliliter
MOS	margin of safety
MPTA	1-methyl-4-phenyl–2-3-6-tetrahydropyridine
MTD	maximum tolerated dose
NCI	National Cancer Institute
NMDA	N-methyl-D-aspartate
MSDS	material safety data sheet
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NRC	National Research Council
NTP	National Toxicology Program
OPP	Office of Pesticide Programs
p =	probability is equal to
P_1	first parental generation
PAH	polynuclear aromatic hydrocarbons

PCB	polychlorinated biphenyl
PFC	plaque forming cell
PHA	phytohemagglutinin
PNS	peripheral nervous system
ppm	parts per million
RBC	red blood cells
RED	reregistration eligibility decision
RfD	reference dose
SERA	Syracuse Environmental Research Associates
SI	stimulation index
SRBC	sheep red blood cells
TCDD	tetrachlorinated dibenzo-p-dioxin
TEBG	testosterone-estrogen binding globulins
UF	uncertainty factor
U.S.	United States
U.S. EPA	U.S. Environmental Protection Agency
USDA	United States Department of Agriculture

EXECUTIVE SUMMARY

INTRODUCTION

This paper addresses the impact of and approach to three specific toxicological endpoints considered in risk assessments – neurotoxicity, immunotoxicity, and endocrine disruption – and applies the general discussion of each of these endpoints to three herbicides used by the USDA Forest Service – glyphosate, triclopyr, and hexazinone. These three endpoints applied to these three chemicals address the broader issue of uncertainties that exist in any risk assessment. In every risk assessment, the available and often limited information must be used to make judgments concerning what levels of exposure are acceptable and whether or not a specific use of a chemical presents a plausible risk. In addition, as new information becomes available, judgments must be made concerning the need to incorporate this new information into existing risk assessments.

The U.S. EPA has conducted risk assessments for glyphosate, triclopyr, and hexazinone as part of the reregistration process and has determined that the registration for each of these herbicides should be maintained because these herbicides can be used without significant risk to humans or wildlife. Similarly, the Forest Service has commissioned risk assessments on glyphosate, triclopyr, and hexazinone to assess the risk of using these herbicides in applications that are specific to Forest Service programs. In addition, the Forest Service has recently commissioned detailed worksheets on glyphosate, triclopyr acid and salts, triclopyr-butoxyethyl ester, and hexazinone. These existing risk assessments and related documentation are central to this paper because the primary questions addressed in this paper are:

> To what extent do these existing risk assessments adequately address the issues of neurotoxicity, immunotoxicity, and endocrine disruption?
>
> Is there more recent information available on these three herbicides for these three endpoints that would significantly impact the conclusions of the existing risk assessments?

In order to put these questions into a context in which informed judgements can be made, this paper briefly summarizes the current status of the risk assessments for each of the three herbicides: glyphosate (Section 2.1), triclopyr (Section 2.2), and hexazinone (Section 2.3). The remaining sections address each endpoint: Section 3 for neurotoxicity, Section 4 for immunotoxicity, and Section 5 for endocrine disruption. Each of these sections consists of an initial general discussion of the endpoint followed by subsections on each herbicide. The general discussion defines important terminology, discusses general causes of adverse effects, describes approaches to assessing each endpoint, and identifies what types of observation would form a weight of evidence conclusion for each endpoint. In the discussion of the specific agents, observations pertinent to a weight of evidence evaluation for each endpoint, for each herbicide, are summarized.

NEUROLOGIC EFFECTS

The nervous system is the basis for learning and thinking, sensory perception and movement, behavior and emotion, and regulation of many of the important functions of the cardiovascular system and other internal organs. Chemically-induced impairment of the nervous system (*neurotoxicity)* can produce a variety of effects, collectively referred to as *neurologic effects*, which can encompass any of the above functions and behaviors. *Neurotoxicants* are chemicals that disrupt the function of nerves, either by interacting with nerves directly or by interacting with supporting cells in the nervous system. This definition of *neurotoxicant* is critical because it distinguishes agents that act directly on the nervous system *(direct neurotoxicants*) from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*). Virtually any chemical will cause signs of neurotoxicity in severely poisoned animals. However, unless these effects are caused by direct damage to nerve tissue, the agent is not a direct neurotoxicant. The assessment of direct neurotoxic potential usually relies on studies of subchronic, chronic, or acute exposures that are well below levels of exposure that produce effects on other organ systems.

Glyphosate – There is no evidence that glyphosate is a direct neurotoxicant in humans or other species. A small clinical investigation found no evidence for neurological effects among forest workers who mixed and sprayed Roundup during a workweek. The clinical case literature of acute glyphosate intoxication is reasonably extensive and does not provide evidence for glyphosate being an acute neurotoxicant in humans. Several long-term experimental studies examined various endpoints of neurotoxicity (brain morphology) in dogs, mice, or rats and did not find evidence of neurotoxicity. An acute study found no effect of glyphosate exposure on nervous system reflexes in dogs. Studies conducted in various bird species did not find evidence for neurological effects. Although these studies do not implicate glyphosate as a neurotoxicant, studies designed specifically to detect impairments in motor, sensory, or cognitive functions in animals exposed subchronically or chronically to glyphosate have not been reported. This is not surprising, since the undertaking of such studies on a substance such as glyphosate, for which the clinical and experimental toxicology experience provides no reason to suspect a neurotoxic potential, would be highly unusual.

Triclopyr – As with glyphosate, there is no evidence for triclopyr being a direct neurotoxicant in humans or other species. Acute toxicity studies conducted in various mammalian species have observed lethargy, impaired coordination, weakness, labored respiration, and tremors in animals exposed to lethal or near-lethal dose levels of triclopyr. Direct neurotoxic activity would be expected to be observed in longer-term experimental studies in which exposures were well below lethal levels. However, studies conducted in rodents, dogs, monkeys, birds, and amphibians have not provided evidence of direct neurotoxicity, even at the maximum tolerated dose. Neurologic endpoints evaluated in these studies may have been limited to brain morphology and observation of the animals for gross abnormalities in movement or balance. Nevertheless, these studies suggest that the acute neurological effects of triclopyr observed at near lethal doses may indeed be secondary to cardiovascular trauma from treatment-induced injuries to other organs, possibly kidney and liver. Studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals exposed subchronically or chronically to triclopyr have not been reported. Two studies found evidence for possible neurologic effects of triclopyr in fish.

The effects observed included lethargy, hypersensitivity to light stimuli, and avoidance behavior but were only observed at lethal or near-lethal exposure levels. In the absence of any signs of direct neurotoxicity in other species, these observations are consistent with indirect neurological effects secondary to general poisoning.

Hexazinone – There is also no evidence for hexazinone having a direct neurotoxic effect in humans or other animals. As with triclopyr, studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals or other species exposed subchronically or chronically to hexazinone have not been conducted. Again as with triclopyr, these studies have not been conducted because the clinical and experimental toxicology experience with hexazinone provide no reason to suspect a neurotoxicity potential. Thus, the effort and expense associated with the conduct of specific studies to assay for neurotoxicity do not appear to be justified. Acute toxicity studies conducted in various mammalian species and in quail have observed lethargy, impaired coordination, weakness, labored respiration, and tremors in animals exposed to lethal or near-lethal dose levels of hexazinone. These studies indicate that acutely lethal, or near-lethal exposures to hexazinone may exert neurological effects. If hexazinone were a direct neurotoxic agent, however, neurologic effects would be expected to be observed in longer-term experimental studies in which exposures were well below lethal levels. However, studies conducted in rodents, dogs, and birds have not provided evidence of neurotoxicity, even at the maximum tolerated dose. Neurologic endpoints evaluated in these studies were limited to brain, spinal cord and peripheral nerve morphology and observation of the animals for gross abnormalities in movement or balance. Nevertheless, these studies suggest that the acute neurological effects of hexazinone observed at near lethal doses may indeed be secondary to cardiovascular or respiratory trauma from treatment-induced injuries to other organs.

IMMUNOLOGIC EFFECTS

The *immune system* serves to distinguish cells of the individual (*self*) from cells of other organisms (*foreign*). This allows the body to recognize and destroy foreign cells *(immune response*) that enter the body inadvertently, and that might otherwise produce lethal disease. The immune system also continually searches the body for abnormal cells of the individual (*surveillance*), those that may have been altered by infection with viruses, or cells that have been rendered abnormal from aging, injury, or disease, including certain abnormalities associated with cancerous cells. Abnormal cells, once identified, are destroyed and replaced. This process underlies the body's recognition and healing of wounds. *Immunotoxicants* are chemical agents that disrupt the function of immune system. These agents can impair immune responses (*immune suppression*) or produce inappropriate stimulation of immune responses (*hyperreactivity*). Suppression of immune responses to microbes or abnormal cells can enhance susceptibility to infectious diseases or cancer. *Hyperreactivity* can give rise to *allergy* or hypersensitivity, in which the immune system or genetically predisposed individuals inappropriately responds to chemical agents (e.g., plant pollen, cat dander, flour gluten) that pose no threat to other individuals or *autoimmunity*, in which the immune system produces antibodies to self components leading to destruction of the organ or tissue involved.

Glyphosate – Based on results from the available studies in humans and experimental studies in rodents, glyphosate does not appear to be an immunotoxicant in humans or other animals. This

conclusion is supported not only by an extensive set of standard mammalian bioassays on toxicity but also by an *in vivo* assay specifically designed to detected humoral immune response and an *in vitro* assay specifically designed to detect cell mediated immune response. Epidemiological studies and clinical cases have not found evidence for allergic reactions or sensitization to dermal exposures to glyphosate formulations. Two human experimental studies provide evidence that Roundup is not a dermal allergen or sensitizing agent. Tests conducted in guinea pigs provide further support for glyphosate not being a dermal sensitizing agent. Several long-term experimental studies have examined the effects of exposure to glyphosate on lymphoid tissue morphology and blood leukocyte counts; treatment-related effects were not observed.

One study has reported immune suppression in one species of fish exposed to an unspecified formulation of glyphosate at an unspecified – but presumably low – concentration. While this study cannot and should not be totally dismissed, it has no significant impact on existing ecological risk assessments for glyphosate. As detailed at some length below (Section 4.2.1), this study does not provide information that is adequate for determining whether the reported immune responses were due to a direct effect on the immune system or secondary effects associated with cytotoxicity. In addition, this report is inconsistent both with the available studies in mammals – which provide no evidence of effects on the immune system – as well as a full life cycle test in fish that has been accepted by the U.S. EPA and provides an adequate NOEC for glyphosate in fish. Thus, based on results from the available studies in humans and experimental studies in rodents, the results of this single study do not alter the weight-of-evidence assessment that glyphosate does not appear to be an immunotoxicant in humans or other animals. This single study in fish would not significantly or quantitatively impact the risk assessment of glyphosate.

Triclopyr – There is very little direct information on which to assess the immunotoxic potential of triclopyr. The only studies specifically related to the immunotoxicity of triclopyr are skin sensitization studies conducted on triclopyr-BEE and the triethanolamine salt of triclopyr. For both of these forms of triclopyr, skin sensitization was observed following standard protocols accepted by the U.S. EPA. While these studies provide support for asserting that triclopyr may cause skin sensitization, they provide no information useful for directly assessing immune suppressive potential of triclopyr. The toxicology of triclopyr has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs. In these reviews of the toxicity of triclopyr, morphologic abnormalities in lymphoid tissues – indicative of potential damage to the immune system – have not been reported. Since histopathologic evaluations of lymphoid tissues and evaluation of blood leukocyte counts are standard procedures in most rodent bioassays and since positive effects in these tissues would typically be reported prominently, it is reasonable to assert that these effects were not noted in the many standard bioassays of triclopyr. Equally important is the fact that the most sensitive effect for triclopyr is well characterized and involves damage of proximal tubular tissue of the kidneys. This is the endpoint selected by U.S. EPA as the basis for the RfD and is the same endpoint used in the SERA risk assessment for triclopyr. Protecting against this critical effect using the existing RfD is considered to be protective of all toxic effects and there is no specific information on the potential immunologic effects of triclopyr that raises significant questions concerning the protectiveness and adequacy of the current RfD.

Hexazinone – As with triclopyr, there is very little direct information on which to assess the immunotoxic potential of hexazinone. Also as with triclopyr as well as virtually all registered pesticides, hexazinone has been tested for skin sensitization. Unlike triclopyr, however, hexazinone caused no signs of skin sensitization in guinea pigs. Just as the positive sensitization of triclopyr does not increase concern for immune suppressive activity, so the data on the negative effects of hexazinone as a sensitizer do not decrease or in any way impact concern for potential immune suppression. As with triclopyr, the only information with which to assess the potential immune suppressive effects of hexazinone is largely indirect. Like triclopyr, hexazinone has been subject to a large number of standard toxicity studies required for pesticide registration by the U.S. EPA. Although these studies are not designed to specifically detect changes in immune function, significant effects on immune function would likely be evidenced by observable changes in lymphoid tissue as well as changes in differential blood cell counts and an increase in the incidence of animals with infections. No such effects are reported by U.S. EPA in the RED and such effects were not encountered in the risk assessment prepared by SERA. The only changes in blood noted in any of the toxicity studies involve blood enzymes that are indicative of damage to liver cells.

The current RfD for hexazinone (0.05 mg/kg/day) is based on a two-year feeding study in dogs with an NOAEL of 5 mg/kg/day. This is based on the most sensitive effect – histological evidence and biochemical indicators of liver damage. While this study and other chronic studies on hexazinone cannot rule out the possibility of immunologic effects, they provide no evidence that such effects occurred. Again as with triclopyr, if such immunologic effects had occurred, changes in differential blood cell counts and/or pathological changes in lymphoid tissues would be expected along with some indication of increased susceptibility to infection. No such effects have been noted. Thus, there is no plausible basis for asserting that the current RfD established by U.S. EPA should be revised to accommodate concern for potential effects on the immune system.

ENDOCRINE DISRUPTION

The *endocrine system* participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands*, *hormones*, and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. Endocrine disruptors can exert effects by affecting the availability of a hormone to its target tissue(s) and/or affecting the response of target tissues to the hormone. These effects can enhance the action of natural hormones, or can diminish or abolish these actions. Effects may be transient or permanent, and may occur soon after exposure to the agent or may occur long after exposure ceases. Evidence of endocrine disruption relies on the corroborated demonstration, usually in animal models, of 1) a dose-related abnormality in the structure of endocrine glands (histopathologic change); and/or 2) a dose-related effect of the chemical on endocrine function, including hormone synthesis, secretion, transport and elimination, receptor

binding, or postreceptor processes that give rise to a response in a target tissue; and 3) demonstration that the above effect on endocrine function gives rise to an adverse effect in the organism or population.

Glyphosate – Three specific tests on the potential effects of glyphosate on the endocrine system have been conducted and all of these tests reported no effects. The conclusion that glyphosate is not an endocrine disruptor is reenforced by epidemiological studies that have examined relationships between occupational farm exposures to glyphosate formulations and risk of spontaneous miscarriage, fecundity, sperm quality, and serum reproductive hormone concentrations. The studies have not found positive associations between exposure to glyphosate formulations and any reproductive or endocrine outcomes. The clinical case literature does not provide evidence for glyphosate being an endocrine active agent. Several long-term experimental studies have examined the effects of exposure to glyphosate on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects were not observed. In addition, extensive testing in experimental animals and wildlife provides reasonably strong evidence that glyphosate is not an endocrine disruptor. The existing risk assessments on glyphosate have based the dose-response assessment for glyphosate on reproductive effects in experimental mammals. The mammalian database for glyphosate is admittedly complex and open to differing interpretations. This is illustrated by the existence of two different RfD's for glyphosate that have been derived by the U.S. EPA. Nonetheless, the approach taken in the SERA risk assessment used by the Forest Service is highly conservative and no recent information has been encountered suggesting that this risk assessment is not adequately protective of any reproductive effects that might be associated with glyphosate exposure.

Triclopyr – Extensive testing in experimental animals provides reasonably strong evidence that triclopyr is not an endocrine disruptor. Although no human data are available relating to endocrine disruption, several long-term experimental studies in dogs, rats, and mice have examined the effects of exposure to triclopyr on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects on these endpoints were not observed. Triclopyr did not produce morphological abnormalities in frog embryos at exposures below the LC_{50}. Triclopyr has not undergone evaluation for its potential to interact or interfere with the estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding, or postreceptor processing).

Hexazinone – As with triclopyr, hexazinone has not undergone evaluation for its potential to interact or interfere with the estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding or postreceptor processing). Again, however, extensive testing in experimental animals provides reasonably strong evidence against hexazinone being an endocrine disruptor. Epidemiological studies of health outcomes of hexazinone have not been reported, nor is there clinical case literature on human hexazinone intoxication. Nonetheless, several long-term experimental studies in dogs, mice, and rats have examined the effects of exposure to hexazinone on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects on these endpoints were

not observed. In addition, hexazinone did not produce morphological abnormalities in frog embryos at exposures below the LC_{50}.

CONCLUSIONS

Neurotoxicity, immunotoxicity, and endocrine disruption are three classes of effects that are important in any risk assessment. There are a large number of different tests that can be conducted for each of these endpoints. Of the three herbicides under review in this document, glyphosate has the most extensive database and, for the effects under consideration, fewer directly relevant studies are available on triclopyr and hexazinone. Nonetheless, each of these herbicides has been subject to a number of standard toxicity studies that are required by the U.S. EPA for pesticide registration. In addition, there is a substantial amount of information on glyphosate, triclopyr, and hexazinone in the open literature. This information has been reviewed by the Forest Service and incorporated into publicly available risk assessments. Based on these risk assessments and the review of the more recent literature conducted in the preparation of this paper, there is no scientific basis for asserting that glyphosate, triclopyr, or hexazinone cause specific toxic effects on the nervous system, immune system, or endocrine function. Based on this review, no significant changes are needed in the current risk assessments for glyphosate, triclopyr, or hexazinone prepared by the U.S. EPA or currently being used by the USDA Forest Service with respect to conclusions about risks of endocrine disruption, immunotoxicity, or neurotoxicity

1. INTRODUCTION

This paper addresses the impact of and approach to three specific toxicologic endpoints considered in risk assessments – neurotoxicity, immunotoxicity, and endocrine disruption – and applies the general discussion of each of these endpoints to three herbicides used by the USDA Forest Service – glyphosate, triclopyr, and hexazinone.

These three endpoints applied to these three chemicals address the broader issue of uncertainties that exist in any risk assessment. Chemicals may cause a large number of different effects. For example, most standard texts in toxicology (e.g., Klaassen et al. 1996) consist of many chapters covering how chemicals enter and are handled by the body (i.e., pharmacokinetics and metabolism), several specific types of effects (e.g., carcinogenicity, mutagenicity, birth defects, and developmental effects) as well as effects based on anatomical classification (e.g., effects on the blood, immune system, liver, kidney, respiratory system, nervous system, circulatory system, skin, eyes, and endocrine system). For each of these basic groups of effects, a large number of specific tests are available that provide different types of information concerning the potential for a specific chemical to cause a specific effect. Virtually no chemical has been tested for each class of effects in each of the many specific tests that are available. Thus, in every risk assessment, the available and often limited information must be used to make judgments concerning what levels of exposure are acceptable and whether or not a specific use of a chemical presents a plausible risk. In addition, as new information becomes available, judgments must be made concerning the need to incorporate this new information into existing risk assessments.

The process of making these judgments involves hazard identification and dose-response assessment. Hazard identification is the process of identifying what, if any, effects a compound is likely to induce in an exposed population. Dose-response assessment is the process of estimating levels of exposure that are not likely to be associated with any adverse effects. These two steps, along with exposure and risk characterization, comprise the risk assessment process (NRC 1983). In practice, risk assessments do not quantitatively address each endpoint that a chemical might cause. To do this would be extremely resource intensive and would make each risk assessment more complicated than necessary. Instead, the hazard identification process is used to identify the *critical effect*, the adverse effect that occurs at the lowest dose level. In many U.S. EPA risk assessments, this is referred to as the LOAEL (lowest observed effect level). Then, the dose-response assessment focuses on identifying the NOAEL (no observed effect level) or NOEC (no observed effect concentration) for the critical effect. In human health risk assessments, NOAEL's are divided by an uncertainty factor to calculated reference doses (RfD's) that are considered to be level exposure that are not likely to result in adverse effects in any member of the population.

Consistent with the recommendation of NRC (1983) that various groups within the federal government adopt common risk assessment methodologies, standard dose-response assessments used in Forest Service risk assessments are generally based on reference values, such as RfDs derived by the U.S. EPA. This approach avoids a duplication of effort, capitalizes on the expertise of other organizations, and decreases the size, complexity, and cost of risk assessments.

The specific methods for conducting, and examples of, risk assessments are available from many sources (e.g., Dourson and Stara JF 1983; U.S. EPA 2000; U.S. EPA. 2001).

The U.S. EPA has conducted risk assessments for glyphosate (U.S. EPA 1993), triclopyr (U.S. EPA 1998), and hexazinone (U.S. EPA 1994) as part of the reregistration process and has determined that the registration for each of these herbicides should be maintained because these herbicides can be used without significant risk to humans or wildlife. Similarly, the Forest Service has commissioned risk assessments on glyphosate (SERA 1996), triclopyr (SERA 1995), and hexazinone (SERA 1997) to assess the risk of using these herbicides in applications that are specific to Forest Service programs. In addition, the Forest Service has recently commissioned detailed worksheets on glyphosate (SERA 2001a), triclopyr acid and salts (SERA 2001b), triclopyr-butoxyethyl ester (SERA 2001c), and hexazinone (SERA 2001d). These worksheets summarize the most recent available risk assessment values for each of the herbicides and provide detailed exposure scenarios and risk characterizations for human health and ecological effects using methods currently employed in risk assessments conducted for the Forest Service (SERA 2001e).

These existing risk assessments and related documentation are central to this paper because the primary questions addressed in this paper are:

> To what extent do these existing risk assessments adequately address the issues of neurotoxicity, immunotoxicity, and endocrine disruption?
>
> Is there more recent information available on these three herbicides for these three endpoints that would significantly impact the conclusions of the existing risk assessments?

In order to put these questions into a context in which informed judgements can be made, the following section briefly summarizes the current status of the risk assessments for each of the three herbicides: glyphosate (Section 2.1), triclopyr (Section 2.2), and hexazinone (Section 2.3). These discussions are based largely on the U.S. EPA and SERA risk assessments cited above. The remaining sections then address each endpoint: Section 3 for neurotoxicity, Section 4 for immunotoxicity, and Section 5 for endocrine disruption. Each of these sections consists of an initial general discussion of the endpoint followed by subsections on each herbicide. The general discussion defines important terminology, discusses general causes of adverse effects, describes approaches to assessing each endpoint, and identifies what types of observation would form a weight of evidence conclusion for each endpoint. In the discussion of the specific agents, observations pertinent to a weight of evidence evaluation for each endpoint, for each herbicide, are summarized.

2. EXISTING RISK ASSESSMENTS

2.1. Glyphosate

There is a large and complex literature on the toxicity of glyphosate involving many studies in experimental mammals as well as a number of reports on toxicity in humans (SERA 1996; U.S. EPA 1993a,b; Williams et al. 2000). The U.S. EPA has derived two RfD's for glyphosate: one derived by the U.S. EPA Office of Pesticides in the RED (U.S. EPA 1993a) and the other derived by the Agency RfD workgroup and summarized on IRIS (U.S. EPA 1993b). The key study concerning these RfD's for glyphosate as well as the potential reproductive effects of glyphosate (Section 5.2.1) is the 3-generation study in rats conducted by Monsanto and used as the basis for the U.S. EPA (1993b) RfD on IRIS. This study is referred to as Schroeder (1981) in the Williams et al. (2000) review and 30 mg/kg/day is cited by Williams et al. (2000) as the NOAEL. In both the U.S. EPA RED (U.S. EPA 1993a) and IRIS (U.S. EPA 1993b), 30 mg/kg/day is classified as a NOAEL for systemic toxic effects but as a LOAEL for reproductive effects because focal tubular dilation was noted in the kidneys of high-dose male pups.

The U.S. EPA Agency wide RfD for glyphosate (U.S. EPA, 1993b) was set at 0.1 mg/kg/day based on the NOAEL of 10 mg/kg/day for reproductive effects from the Schroeder (1981) study. The study forming the basis for this RfD is summarized by the U.S. EPA (1993b) in the documentation of the RfD as follows:

> *Rats (CD Sprague-Dawley) were administered glyphosate continuously for three successive generations. Dietary concentrations of glyphosate were adjusted weekly during growth, and between mating rest periods to achieve dose levels of 0, 3, 10, and 30 mg/kg/day. Each generation (F0, F1, F2) consisted of 12 male and 24 female rats. Each parent generation was mated to produce two litters. Offspring from the second litters of the F0 and F1 parents (F1b and F2b litters, respectively) were selected to be parents for subsequent generations. Offspring not included in the selection procedure and offspring from the first litter intervals of each generation (F1a, F2a, F3a) were given a gross postmortem examination and discarded. Randomly selected offspring from the second litters of the F2 generation (F3b litters) were given a gross postmortem examination and selected tissues taken and saved. Subsequently tissues from control and high-dose F3b offspring were evaluated microscopically (10/sex/group). Tissues from control and high-dose parent generations (F0, F1, and F2) were also evaluated.*
>
> *No treatment-related effects on fertility were noted, nor were any systemic effects in adult rats apparent. Male pups from the F3b mating of the high dose group (30*

mg/kg/day) showed an increase in the incidence of unilateral renal tubular dilation. Based on this finding, the NOEL and LEL for this study are 10 and 30 mg/kg/day, respectively.

In the RED, the U.S. EPA Office of Pesticides recommends a substantially higher RfD of 2 mg/kg/day for glyphosate (U.S. EPA 1993a). As detailed in the RED (U.S. EPA 1993a, p. 15), pregnant rabbits were dosed with glyphosate at 0, 75, 175 or 350 mg/kg/day by gavage on days 6 through 27 of gestation. Effects were noted only in the 350 mg/kg/day groups and included death in 10 of 16 does by gestation day 21. The RED classified the doses of 175 and 350 mg/kg/day, respectively, as the NOEL and LOEL for maternal toxicity. The NOEL for developmental toxicity was also set at 175 mg/kg/day. As noted by U.S. EPA (1993a, p. 15): *Due to high maternal mortality at the 350 mg/kg/day dose level, too few litters (only 6) were available to assess adequately developmental toxicity at that level*. This study is cited as Rodwell et al. (1980) in U.S. EPA (1993a) and but appears to be cited as Tasker et al. (1980b) in the review by Williams et al. (2000).

While the Agency RED clearly identifies 30 mg/kg/day as a LOAEL for reproductive toxicity rats (U.S. EPA 1993a, p. 16), the RED does not discuss the selection of the 175 mg/kg/day NOAEL in rabbits relative to the 30 mg/kg/day LOAEL in rats in the justification for the derivation of the RfD (U.S. EPA 1993a, pp. 19-20).

The existence of two RfD's complicates the dose-response assessment for glyphosate because judgements are required concerning the applicability of each RfD to the risk assessment. This is addressed in the SERA (1996) risk assessment using a categorical regression analysis (Durkin et al. 1992; Hertzberg 1989; McCullagh 1980). This approach correlates categorical responses—such as NOELs, NOAELs, AELs, and FELs—with factors that may influence the response such as dose and duration of exposure. As detailed in SERA (1995, Section 3.3.3), the available human data suggest that no frank adverse effects are likely at doses substantially above 10 mg/kg/day. Consequently, the higher RfD of 2 mg/kg/day was used in the SERA (1995) risk assessment, but only for workers. For members of the general public, the more conservative RfD of 0.1 mg/kg/day was used and is maintained in the more recent SERA (2001a) worksheets on glyphosate. Thus, in the SERA worksheets on glyphosate (SERA 2001a) and in the SERA (1996) risk assessment, the higher RfD of 2 mg/kg/day (corresponding to an NOAEL of 175 mg/kg/day) is used for worker exposure as well as acute exposures for the general public. The lower RfD of 0.1 mg/kg/day (corresponding to an NOAEL of 10 mg/kg/day) is used for longer term exposures for members of the general public.

For the ecological risk assessment, the dose response assessment is based on an acute NOAEL value of 400 mg/kg/day for acute exposures and a chronic NOAEL of 10 mg/kg/day for longer-term exposures (SERA 2001a). These are identical to the values used in the SERA (1996) risk assessment. As also noted in the SERA (1996) risk assessment, no significant or systematic differences are apparent in the acute oral toxicity of glyphosate to large and small animals. Thus, the same toxicity values apply to both large and small mammals and birds.

For fish, the risks vary substantially with the formulation. For Rodeo and any other formulation of glyphosate without a surfactant, the reference concentration is 1 mg/L. For Roundup and other glyphosate concentrations with a surfactant, the reference concentration is 0.1 mg/L. SERA (2001a) uses only the lower value because none of the hazard quotients for this value exceed unity. Because of the very weak duration-response relationship for glyphosate, these values are used for both acute and chronic exposures. Because the available data on aquatic invertebrates are similar to those with fish (SERA 1996), reference concentrations for fish are used for invertebrates (SERA 2001a).

2.2. Triclopyr

The U.S. EPA RED on triclopyr (U.S. EPA 1998) was finalized in October, 1998, after the completion of the SERA risk assessment (SERA 1995). The SERA (1995) risk assessment cited and used a U.S. EPA RfD of 0.005 mg/kg/day. This RfD was based on a study in which the triclopyr triethylamine salt was administered in the diet to dogs at levels that resulted in daily doses of 0.5, 2.5, or 5.0 mg/kg/day over a 1-year period. The two higher doses were classified as adverse effect levels based on dose-related increases in serum urea nitrogen and creatinine, indicative of decreased glomerular filtration. The lowest dose was classified as a NOAEL. This dose was divided by 100, a factor of 10 to account for uncertainties in species-to-species extrapolation and another factor of 10 to encompass sensitive individuals in the population.

The U.S. EPA (1998) RED for triclopyr uses an RfD of 0.05 mg/kg/day. This RfD is based upon the 2-generation reproduction toxicity study in rats (Vedula et al. 1995) with a NOEL of 5.0 mg/kg/day, the lowest dose tested. As detailed in the RED (U.S. EPA, 1998, p. 16), an increased incidence of proximal tubular degeneration of the kidneys was observed in P1 and P2 parental rats at the next dose level (25 mg/kg/day). As in the previous RfD, this dose was divided by 100, a factor of 10 to account for uncertainties in species-to-species extrapolation and another factor of 10 to encompass sensitive individuals in the population.

For acute exposures, the U.S. EPA RED uses a NOEL of 30 mg/kg/day for maternal and developmental effects from a developmental toxicity study in rabbits (U.S. EPA, 1998, p. 17). Using the acceptable MOE of 100 for acute exposures specified in the U.S. EPA RED, this corresponds to an acute RfD of 0.3 mg/kg/day.

For the ecological risk assessment involving mammals, a NOAEL value of 30 mg/kg/day is used for acute exposures and 5 mg/kg/day for longer-term exposures (SERA 2001b,c). These values are based on the NOAEL's selected by U.S. EPA in the RED (U.S. EPA 1998) for the human health risk assessment. Since birds do not appear to be more sensitive to triclopyr than mammals (SERA 1995), these values are also used in assessing risk to birds. As noted in the SERA (1995) risk assessment, no substantial or systematic differences are apparent in the acute toxicity of triclopyr to large and small animals. Thus, the same toxicity values are applicable to both large and small mammals and birds.

For fish, the risks vary substantially with the formulation. For Garlon 3A (triclopyr acid), the reference concentration is 50 mg/L (SERA 2001b). For Garlon 4 (triclopyr-BEE), the reference concentration (NOEC) is 0.6 mg/L (SERA 2001c). Chronic toxicity values are not derived in the

SERA (1995) risk assessment. The RED (U.S. EPA, 1998) does not provide sufficient data to propose separate chronic toxicity values for fish. Because the available data on aquatic invertebrates are similar to those with fish, reference concentrations for fish are used in the SERA worksheets for invertebrates.

2.3. Hexazinone

The U.S. EPA Agency RfD for hexazinone on IRIS [http://www.epa.gov/iris/ subst/0223.htm] is 0.033 mg/kg/day, identical to that cited in the SERA (1997) risk assessment. This RfD is based on the 2-year rat feeding study in which a dietary level of 200 ppm was associated with no observable effects and 2500 ppm was associated with decreased body weight gain and food efficiency in male rats and female rats. In this RfD, the U.S. EPA assumes that rats consume food at a rate equivalent to 5% of their body weight per day. Thus, the NOAEL for this study is 10 mg/kg bw/day (200 mg/kg food • 0.05 mg food/kg bw) and the LOAEL is 125 mg/kg/day (2500 mg/kg food • 0.05 mg food/kg bw). This RfD was derived using an uncertainty factor of 300 to account for species-to-species extrapolation (10), sensitive subgroups (10), and the lack of a chronic study on dogs (3).

The U.S. EPA/OPP RED on hexazinone (U.S. EPA 1994) is cited in the SERA (1997) risk assessment and proposes a slightly higher RfD of 0.05 mg/kg/day. This RfD is based on a 2-year feeding study in dogs that was submitted to the U.S. EPA Office of Pesticides as part of the reregistration process. In this study, doses of 41.24 and 37.57 mg/kg/day in males and females, respectively, were associated with changes in clinical chemistry and histopathology indicative of liver damage. The NOEL for these effects was 5 mg/kg/day. Based on this NOEL and using an uncertainty factor of 100 for species-to-species extrapolation (10) and sensitive subgroups (10), the Office of Pesticides derived an RfD of 0.05 mg/kg/day (U.S. EPA 1994).

No modifications or amendments to the RED (U.S. EPA 1997) have been encountered and the chronic RfD of 0.05 mg/kg/day proposed in the RED was used in the SERA (1997) risk assessment as well as the more recent SERA (2001d) worksheets. The U.S. EPA has not derived an acute RfD for hexazinone and no surrogate acute reference value was proposed in the SERA (1997) risk assessment. Thus, as in the RED (U.S. EPA 1994) and the SERA (1997) risk assessment, the chronic RfD is used for characterizing risks from both acute and longer-term exposures in the more recent SERA (2001d) worksheets.

For the ecological risk assessment, the SERA (2001d) worksheets use a NOAEL value of 100 mg/kg/day for acute exposures and 5 mg/kg/day for longer-term exposures. As discussed in Section 4.3.1 of the SERA (1997) risk assessment, the acute and chronic values for mammals are also applied to birds.

For fish, the risks of acute exposure are characterized using an acute LC_{50} value of 100 mg/L. As summarized in Section 4.3.3.1 of the SERA (1997) risk assessment, all 24-hour LC_{50} values for hexazinone in fish are greater than 100 mg/L. The only subchronic toxicity data available on hexazinone in fish is the early life stage study on fathead minnow. In this study, the NOEL was 17 mg/L and this value is used to characterize risks to fish from longer-term exposures (SERA 2001d). For aquatic invertebrates, the risk of acute exposures are characterized using an LC_{50}

value of 100 mg/L and the risk of longer-term exposures are characterized using a NOEC of 29 mg/L from a daphnia life-cycle study (Section 4.3.3.2 in SERA 1997).

3. NEUROLOGIC EFFECTS

3.1. General Considerations

3.1.1. Definitions

The nervous system is the basis for learning and thinking, sensory perception and movement, behavior and emotion, and regulation of many of the important functions of the cardiovascular system and other internal organs. Chemically-induced impairment of the nervous system (*neurotoxicity)* can produce a variety of effects, collectively referred to as *neurologic effects*, which can encompass any of the above functions and behaviors.

The nervous system can be subdivided anatomically into the *central nervous system* (CNS), which includes the brain and spinal cord, and the *peripheral nervous system* (PNS), which includes nerves connecting organs and tissues with the spinal cord and brain. The latter include the nerves that carry information to the CNS about sensation (*sensory neurons*), and nerves that carry information to muscles to control movement (*motor neurons*). From the perspective of mechanisms of neurotoxicity, the nervous system can be more meaningfully subdivided into the various functional components of nerve cells (*neurons*) that can be the targets of chemical agents. The structural organization of neurons reflects their principal function, to process, store, and convey information about the body, either within the CNS, or between the CNS and other tissues and organs. This is accomplished by a combination of chemical signaling between neurons and electrical potentials and currents within neurons. Neurons consist of 1) a *cell body*, containing the nucleus and other organelles that carry out synthesis and catabolism; 2) *dendrites*, elongated cellular processes that emanate from the cell body and that function to receive information, in the form of chemical signals, from other neurons and translate these signals into electrical potentials and currents within the cell body; 3) the *axon*, an elongated process (which can be more than a meter in length) that transmits information, in the form of electrical potentials and currents, from the cell body to nerve terminals; and 4) the *nerve terminal* which receives information encoded in electrical currents from the axon and communicates, in the form of chemical signals, to other neurons. In addition to neurons, the nervous system includes a variety of other types of cells that are critical to the function of the system. These include neuroglia (in the CNS), Schwann cells (in the PNS), and various specialized sensory receptors (in the PNS). Neuroglia and other supporting cells make up approximately 90% of the cells in the CNS (Jones, 1988).

Neurotoxicants are chemical agents that disrupt the function of neurons, either by interacting with neurons specifically, or with supporting cells in the nervous system (e.g., neuroglia, Schwann cells, sensory receptors). The above definition is central to this discussion because it distinguishes agents that act directly on the nervous system *(direct neurotoxicants*), from those agents that might produce neurologic effects that are secondary to other forms of toxicity (*indirect neurotoxicants*) (O'Donoghue, 1994). An example of the latter would be an agent that

disrupts the respiratory or cardiovascular system and, thereby, deprives the brain of oxygenated blood. Another example would be an agent that disrupts kidney function and, thereby, alters nervous system function by producing irregularities in body sodium and potassium levels.

In some respects, the term *indirect neurotoxicants* may be a misnomer – or at least somewhat confusing – in that the indirect agent, by definition, does not directly damage nerve tissue. Nonetheless, the distinction between direct and indirect neurotoxicants is important because the types of biological assays needed to fully characterize these two very different causes of neurological effects will be very different. For example, an agent that disrupts kidney function may also produce, secondarily, neurological effects that are similar to those produced by agents that disrupt potassium or sodium transport in nerve cells (lethargy, stupor, muscle tremors, convulsions). However, in a typical whole animal chronic toxicity bioassay, such effects would be observed in concert with irregularities in serum sodium and potassium levels, and other indications of impaired kidney function. The same neurologic effects observed in the absence of indicators of impaired kidney function, or impaired function of other organ systems that might secondarily result in neurological effects, would be much more provocative evidence that the agent might be a direct neurotoxicant. However, bioassays directed at detecting specific forms and mechanisms of neurotoxic activity would be needed to confirm that the agent is, indeed, a direct neurotoxicant. These might include evaluations of motor or sensory function, histopathological examination of the nervous system for assessment of exposure-related structural changes, or assessments of the toxicity of the agent in *in vitro* preparations of neurons of nervous system cells (these assays are described in greater detail below). In general, lethal exposures to toxic agents, regardless of their mechanism of toxicity, almost always give rise to neurological effects in the terminal stages of the intoxication. These effects can arise from many causes, including fluid and electrolyte imbalances, pulmonary edema, or cardiovascular collapse. Thus, it is usually the case that very little information about the direct actions of a chemical agent on the nervous system is gained from studies of acute lethal, or near-lethal intoxications. The assessment of direct neurotoxic potential usually relies on studies of subchronic, chronic, or acute exposures, well below those that produce effects on other organ systems that might imperil the nervous system.

3.1.2. Causes of Neurologic Effects

Central to the function and control of the nervous system are interactions between naturally occurring chemicals (*physiological agents*) and nerve cells. These include *neurotransmitters* (e.g., acetylcholine, norepinephrine) that serve in communication between nerve cells, *hormones* that control nerve cell growth and function (e.g., nerve growth factor, thyroid hormone), and *electrolytes* (e.g., sodium, potassium calcium) that function in generating electrical impulses in nerve cells. Neurotoxicants, more often than not, exert their effects by disrupting the actions of physiological agents, often by being structurally similar enough with them to adversely interact with the same neuron components that are their normal physiological targets (e.g., atropine, curare, ergot alkaloids, 6-hydroxydopamine, sarin), or by causing their unregulated synthesis or release from neurons (e.g., amphetamine, nicotine). Indeed, most of the modern drugs used in treatment of neurologic diseases are agents that act by mimicking the structures of physiologic agents in the nervous system. Many of the common insecticides used in managing insect pests

do the same in the target pest species, including malathion and other organophosphate inhibitors of *cholinesterase,* an enzyme that destroys the neurotransmitter, acetylcholine.

Neurotoxicants can produce neurologic effects by several general mechanisms, as detailed by Fonnum (1999):

Damage to nerve cells from free radicals,

Disruption of nerve fibers,

Disruption of myelin,

Interference with ion channels,

Interference with uptake, release, or metabolism of neurotransmitters,

and Disruption of neuroglia cells.

Each of these specific effects are discussed in the following paragraphs.

Free radicals are molecules that contain one or more unpaired electrons in the outer orbital shell. Although these radicals can initiate a chain reaction of oxidative damage in many different tissues, the brain is thought to be particularly sensitive to free radicals because of its high content of polyunsaturated lipids, its relatively low activity of enzymes that dispose of free radicals (e.g., catalase, superoxide dismutase), and its relatively high iron content, which can serve as a reactant in the production of additional free radicals. Free radicals have been implicated in the mechanisms of neurotoxicity of methylmercury (Sarafian and Verity, 1991), 3-nitropropionate (Dawson et al., 1995) and toluene (LeBel and Bondy, 1991).

The movement of nutrients and other physiological agents important in nervous system function, from the cell body where they are produced to nerve endings, occurs by a process known as *axonal transport*. Disruption of axonal transport can produce a neurological effect known as *axonopathy*. This effect is typically characterized by loss of function of the affected nerves; for example, loss of sensation if sensory nerves are affected, or loss of muscle function if the affected nerves are motor nerves. Axonopathies are usually associated with structural changes in nerve axons that can be visualized in a histopathological examination of nerve tissue. Agents that produce axonopathy include acrylamide (Sabri and Spencer, 1990), hexane (Krasavage et al., 1980), and carbon disulfide (Juntunen et al., 1977).

Axons of nerves are sheathed in a substance known as *myelin* which is produced by supporting cells in the nervous system (i.e., Schwann cells in the PNS and oligodendrocytes in the CNS). Myelin serves as an electrical insulator around the axon. Chemicals that destroy myelin produce a neurological effect known as *myelinopathy.* This effect is typically characterized by loss of function of the affected nerves, including decreased nerve conduction velocity, loss of sensation if sensory nerves are affected, or loss of muscle function if the affected nerves are motor nerves.

Myelinopathy that gives rise to extensive demyelination of axons can usually be visualized in a histopathological examination of nerve tissue. Agents that produce myelinopathy include hexachloraphene, lead, tellurium, and triethyltin (Fonnum, 1999).

Electrical potentials and currents that are critical for signaling within and between neurons result from the highly controlled movement of ions (e.g., Na^+, K^+, Ca^{2+}) through channels in neuron membranes. Movement of ions through these channels is controlled by the opening or closing of the channels in response to physiological demands on the nervous system. Activation of ion currents electrically depolarizes the nerve cell, and is known as *excitation*. Disruption of the regulation of the opening and closing of ion channels can profoundly disrupt function of the nervous system. Chemical agents can disrupt ion channel function by preventing the movement of ions through channels (e.g., tetrodotoxin, found in certain species of puffer fish), or by maintaining channels in an open state, when they should be closed. The latter agents are known as *excitotoxins* (Fonnum, 1997). These agents can maintain the nerve cell in a hyper-excited state which can lead to aberrant nerve cell function as well excessive production of free radicals and related cell damage. Examples of excitotoxins include domoate, found in the alga *Nitzchia pungens* (Stewart et al., 1990); kainate, found in certain seaweeds (Coyle and Schwarcz, 1976); ibotenate, found in *Amanita* mushrooms, and N-methylamino-L-amino alanine (Spencer et al., 1987), found in sago palms. All of these agents produce excitation by adversely interacting with glutamate receptors on neurons (Hollmann and Heinemann, 1994). Several insecticides, including aldrin, DDT, dieldrin, endosulfan, lindane, and pyrethroids, also appear to exert neurological effects by interfering with the regulated opening or closing of ion channels (Narahashi, 1992; Sieghart, 1992; Soderlund, 1995).

Neurotransmitters are physiological agents that serve in communication between neurons, usually by stimulating or inhibiting the opening of ion channels. Neurotransmitters are released from neurons to interact with membrane receptors on other neurons or target tissues when cell-to-cell communication is needed. Termination of this communication is usually very fast and results from reuptake of the neurotransmitter into the neuron or metabolism of the neurotransmitter outside of the neuron. Neurotoxicants can exert neurological effects by interfering with the regulated synthesis, metabolism, release, or reuptake of neurotransmitters. Examples of this include the organophosphate insecticides, which inhibit the metabolism of acetylcholine, an important neurotransmitter in the CNS and PNS; tetanus toxins and botulinum toxins, which block the release of neurotransmitters; á-latrotoxin, found in black widow spider venom, which stimulates the release of neurotransmitters; and a variety of agents known as *convulsants*, such as isoniazid, semicarbazide, 3-mercaptopropionate, which inhibit or stimulate the synthesis of the neurotransmitter GABA (Simpson, 1990).

Neuroglia cells, in addition to producing myelin, function in the uptake, synthesis and metabolism of neurotransmitters. They also function in the regulation of the nutrient and ion composition of the extracellular environment of the CNS. Neurotoxicants can exert neurological effects by disrupting the metabolism of neuroglia. Examples include fluoracetate, found in the plant *Dichapetalum*, and methionine sulfoxamine (Fonnum, 1997; van den Berg and van den Velden, 1970).

3.1.3. Assessment of Neurotoxicity

Evidence of neurotoxicity relies largely on the corroborated demonstration, usually in animal models, of 1) a dose-related abnormality in the structure (*morphology*) of the nervous system (*histopathologic change*); and/or 2) a dose-related effect of the chemical on neurologic function, such as impaired movement, response to sensory stimuli, learning, or memory. The occurrence of both histopathologic changes and functional deficits, in particular if the histopathologic changes occur in regions of the nervous system thought to control the observed function, would be strong evidence for neurotoxicity.

Typical subchronic or chronic animal bioassays rely on morphological and functional assessments to detect neurotoxicity. Morphological assessment usually consists of examination of the brain and spinal cord for visible changes at the naked-eye and light microscopic level. Structure of the terminal portions of the peripheral nervous system is evaluated as part of the morphological examination of endocrine and exocrine glands, muscles, and other tissues. In some assays, including the standard procedures used by the National Toxicology Program (NTP), evaluation of the spinal cord and peripheral nerves (e.g., sciatic nerve) is only performed if the study finds other indications of neurotoxicity. Behavioral assessments typically include observations of the animals in their cages for gross deficits in movement, balance, or coordination (e.g., gait, posture, visible tremor) (O'Donoghue, 1996). These are sometimes further supplemented with a more comprehensive functional observation battery consisting of various qualitative or quantitative tests of movement, gait, balance and coordination, muscle strength, and reflexes (Weiss, 1999). Beyond the realm of most typical bioassays are various, more explicit tests of motor, sensory and cognitive function that can provide a more quantitative evaluation of neurological deficits (Weiss, 1999). These would usually be conducted only if there were other indications of a possible direct neurotoxic effect of the agent. An understanding of the mechanisms producing a specific deficit in neurological function can sometimes be ascertained with neurophysiological studies in which function of specific isolated components of the nervous system are examined; neurochemical assessments in which the levels, metabolism and transport of neurotransmitters, and nutrients are examined; or imaging techniques (MRI, PET) which can provide a dynamic assessment of brain metabolism and blood flow. Such techniques are usually implemented to explore mechanisms when standard bioassays yield evidence of neurotoxicity.

3.1.4. Weight of Evidence Applied to Neurotoxicity

Observations that would form the basis of a weight of evidence for neurotoxicity occurring in a given human population are shown below (this scheme would apply to non-human, ecological species, if the reference to humans is replaced by the species of concern):

Weight of Evidence	Observation
Highest	Dose-response relationship indicates effects at anticipated exposure levels
^	Neurological effects observed in exposed humans (epidemiological, clinical cases) that can be credibly linked to exposure to the chemical of concern
^	Dose-response relationship in humans is likely
^	Structure-function relationship applies to the human nervous system
^	Functional deficit is linked mechanistically to the structural change
^	Functional and/or structural changes occur in several mammalian species
^	Chemical produces, in an animal model, an impairment of motor, sensory, or cognitive function
Lowest	Chemical produces, in an animal model, a histopathologic change in the nervous system

The highest weight would be given to observations of neurotoxicity in humans. Such observations could be derived from epidemiological studies of workers exposed in their occupations or studies of the general population exposed to environmental levels; or from clinical case studies (e.g., accidental poisonings or attempted suicides). The epidemiological observation of chemical-specific neurological effects is very difficult to achieve in practice, unless the effects are severe. For this reason, studies of other mammalian species usually form the basis for a weight of evidence. The strength of the evidence increases when a neurological effect, either a functional deficit or structural abnormality, is observed in more than one mammalian test species, is understood mechanistically to the extent that we can be reasonably certain that the system affected in the test species operates in humans (e.g., humans have a much more developed brain cortex than other mammalian species), and the dose that produces the neurological effect in the test species can be expected to be achieved in human populations. The latter distinguishes neurotoxicants that demand our concern from the enumerable chemicals that are neurotoxic at doses of little or no concern to humans. Examples of the latter include water, table salt, retinol (vitamin A), and pyridoxine (vitamin B6) (O'Donoghue, 1994).

No single, socially ethical study, or set of studies can prove that a chemical will not produce neurotoxicity in humans. However, we can apply observations made in animal models, observations made from epidemiological studies of workers or the general population, and clinical experience with cases of intoxication to assess the weight of evidence about whether or

not neurotoxicity is more or less likely to occur compared to the likelihood of other forms of toxicity. Typical subchronic or chronic bioassays are usually designed to test a dose range that includes a dose that produces an adverse effect detectable in the bioassay (*maximal tolerated dose*). Thus, if no effects of the chemical are observed on the nervous system at the maximum tolerated dose, we can conclude that, in the given test species, effects other than neurotoxicity are likely to occur at doses lower than that required to produce neurotoxicity. Corroboration of this observation in more than one mammalian species would allow a broadening of this conclusion to other mammalian species. This, in combination with epidemiological studies that show no evidence of neurological effects in workers or general populations who have been exposed to levels of the chemical expected to produce other forms of toxicity, would be strong evidence that the risk of neurotoxicity to humans is relatively low.

3.2. Neurotoxicity of Specific Herbicides

3.2.1. Glyphosate

Overview – Large-scale controlled epidemiological studies of glyphosate exposure and neurological outcomes have not been reported. A small clinical investigation found no evidence for neurological effects among forest workers who mixed and sprayed Roundup during a workweek. The clinical case literature of acute glyphosate intoxication is reasonably extensive and does not provide evidence for glyphosate being an acute neurotoxicant in humans. Several long-term experimental studies examined various endpoints of neurotoxicity (brain morphology) in dogs, mice, or rats and did not find evidence of neurotoxicity. An acute study found no effect of glyphosate exposure on nervous system reflexes in dogs. Studies conducted in various bird species did not find evidence for neurological effects. Although these studies do not implicate glyphosate as a neurotoxicant, studies designed specifically to detect impairments in motor, sensory, or cognitive functions in animals exposed subchronically or chronically to glyphosate have not been reported. This is not surprising, since the undertaking of such studies on a substance such as glyphosate, for which the clinical and experimental toxicology experience provides no reason to suspect a neurotoxic potential, would be highly unusual. The latter not withstanding, there appears to be no evidence for glyphosate being a neurotoxicant in humans or other species.

Human Data – No pattern suggestive of neurotoxicity is apparent in an extensive and detailed literature on health outcomes of accidental and intentional (e.g., suicide attempts) gross over-exposures to glyphosate or its commercial formulations (Chang et al., 1999; Dickson et al., 1988; Hung et al., 1997; Lee et al., 2000; Menkes et al., 1991; Pushnoy et al., 1998; Talbot et al., 1991; Temple and Smith, 1992; Tominack et al., 1991; Sawada et al., 1988; Sorensen and Gregersen, 1999). From hundreds of reported cases, the primary symptoms of acute, severe (including lethal) glyphosate toxicity appears to be gastrointestinal distress and injury, respiratory tract distress and injury (when the herbicide is aspirated into the trachea and bronchi), acid/base disorders (*hyperkalemic acidosis*), low blood pressure, and renal failure (*cardiovascular shock*). Fever has also been reported in some cases (Tominack et al., 1991). Cardiovascular shock has been attributed to massive loss of fluids from the vascular compartment as a result of gastrointestinal injury (*hypovolemic shock*), which is exacerbated by pulmonary edema in cases where aspiration of glyphosate (and possibly surfactant) and respiratory tract injury has occurred.

A more direct cardiac depression has also been suggested to be a possible contributor (Lin et al., 1999). Fevers are not always reported and have been attributed to impairment of the regulation of cellular energy metabolism (*metabolic uncoupling*) (Olorunsogo et al., 1979a,b).

In the hundreds of reported cases, neurological symptoms that are unrelated to respiratory tract distress and shock (confusion, drowsiness, collapse, coma) associated with severe acute glyphosate toxicity cannot be identified. In a review of 92 cases, only 11 individuals were reported as having an abnormal mental state prior to the onset of severe respiratory and/or cardiovascular complications; most of these cases received atropine or pralidoxime, neurotoxicants used as antidotes for certain organophosphate insecticides that inhibit acetylcholinesterase (in these cases, organophosphate intoxication and cholinesterase inhibition was suspected, although glyphosate is not a cholinesterase inhibitor) (Tominack et al., 1991). In a review of 93 cases, 12 were reported as having neurological symptoms (confusion, coma) two of which occurred after cardiovascular resuscitation. The causes of symptoms in 10 other cases were not distinguished from secondary respiratory tract and/or cardiovascular distress (Talbot et al., 1991). Thus, the weight of evidence suggests that any neurologic symptoms associated with glyphosate exposures were secondary to other toxic effects.

Barbosa et al. (2001) have recently reported a case of Parkinsonism in an adult male who was exposed to glyphosate. While this study does not provide a clear or at this point credible association between glyphosate and neurotoxic effects, this is an unusual report that must be examined closely. Parkinsonism is a degenerative disease of the central nervous system that impairs movement. The subject of the Barbosa et al. (2001) report was a 54-year old male who experienced an extensive dermal exposure to the herbicide while spraying a garden. The acute and transient symptoms included eye irritation (*conjenctival hyperemia*) and skin rash which progressed to blisters. One month after the exposure, the subject presented with hand tremors. He was subsequently diagnosed with Parkinsonism, based on the results of a neurological examination and brain imaging. Parkinsonism is a chronic degenerative disorder that could have been present in the patient prior to the exposure.

While the case reported by Barbosa et al. (2001) may have involved gross over-exposure to glyphosate, this over-exposure, in itself, is not dismissive of a possible neurologic risk. As noted above, extreme and sometimes fatal over-exposures to glyphosate are not generally associated with neurologic effects. In addition, there is an at least tenuous biological basis for suggesting a potential association. Glyphosate is a structural analog of glycine, a physiological agent that serves as an inhibitory neurotransmitter in the CNS. Glycine, which is also a naturally occurring amino acid and is essential for normal growth and development, has been implicated as an excitotoxin when present at high concentrations in brain tissue (Johnson and Ascher, 1987; Newell et al., 1997). Excitotoxicity has been hypothesized as a possible mechanism of Parkinsonism induced by the neurotoxicants MPTA (1-methyl-4-phenyl–2-3-6-tetrahydropyridine) and N-methylamino-L-alanine (Kanthasamy et al., 1997; Karcz et al., 1999; Spencer et al., 1987).

At this point, there is no evidence to conclude that glyphosate can produce or exacerbate Parkinsonism; indeed, the Barbosa et al. (2001) observation stands in contrast to the abundant

case literature that suggests glyphosate is not a neurotoxicant in humans. However, the risk assessment community should be alert to any follow-up studies of glyphosate interactions with the pathophysiological mechanisms that underlie excitotoxicity and Parkinsonism, such as the NMDA (N-methyl-D-aspartate) receptor/ion channel complex. Nonetheless, the possible connection between the onset of Parkinsonism and the exposure to glyphosate cannot be established from the case reported by Barbosa et al. (2001), as the apparent concurrence of the two effects could be coincidental. A coincidental association is suggested by the fact that no other cases of glyphosate-related Parkinsonism have been reported.

The only other human data on the association between glyphosate exposure and neurologic effects comes from an occupational exposure study that monitored for signs of neurotoxicity (Jauhiainen et al., 1991). In the study by Jauhiainen et al. (1991), biological monitoring was conducted on five workers using Roundup in *brush saw spraying*. This activity seems comparable to selective foliar applications using a backpack or cut surface treatments. Each worker handled an average of 9.8 L of an 8% solution of Roundup. The amount of glyphosate handled each day was approximately 0.279 kg [9.8 L • 0.08 • 0.356 kg/L] (Jauhiainen et al. 1991, p. 62, column one, top of page). Urine samples were collected at the end of each workday for 1 week during the application period, and one sample was taken 3 weeks after the applications. As part of this biomonitoring study, the subjects were asked to respond to a health questionnaire that included whether they had experienced symptoms of *"...blurred vision, fatigue, headache, tremor of the hands, and muscular spasms or twitching..."* before, after the last day of the workweek, or *"3 weeks later, when the forest workers had stopped their work with the herbicide."* Other than headaches reported in two of five individuals using Roundup, no signs related to neurotoxicity were reported. The incidence of these effects [2/5 in exposed vs 0/5 in control] is not statistically significant using the Fischer Exact test – i.e., $p = 0.2222$.

Experimental Mammals – The toxicology of glyphosate has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs (SERA 1996; U.S. EPA 1993a; Williams et al. 2000). The RED prepared by the U.S. EPA (1993a) and the review by Williams et al. (2000) both included summaries of unpublished studies that were submitted to the U.S. EPA as part of the registration and reregistration processes for glyphosate.

According to Williams et. al. (2000):

> *"Histopathologic examinations were routinely conducted on brain, spinal cord and peripheral nerves such as the sciatic nerve. In addition, the animals in these studies were regularly observed for unusual clinical signs of toxicity that would indicate any functional effect on the nervous system. The developmental studies conducted with glyphosate...included examinations to determine if there were adverse effects in the developing nervous system. There was no evidence of neurotoxicity in any of these studies."*

Williams et al. (2000) also describe a study in which neurological examinations were conducted on dogs that received a single oral dose of 59 or 366 mg/kg of Roundup (Naylor, 1988, not available for review). According to Williams et al. (2000):

> *"A detailed examination consisting of 12 different measurements of spinal, postural, supporting, and consensual reflexes was performed before treatment, during the postadministration observation period, and again on the following day. Reflexes appeared normal, and there were no clinical signs indicative of neuromuscular abnormalities."*

In subchronic studies in mice and rats (NTP, 1992), morphological examinations were conducted of brain (including basal ganglia, a site of injury in Parkinsonism); however, it is unclear from the report whether or not spinal cord and sciatic nerve were examined. In any event, the NTP (1992) study did not report abnormal findings in these tissues, nor did it report clinical signs of neurotoxicity. The NTP (1992) study observed histological changes in salivary glands in both rats and mice. These changes were less severe in animals that received glyphosate in combination with a dosage of propranolol, an antagonist of â-adrenergic neurotransmitters. Propranolol also completely prevented similar changes produced by isoproterenol, a â-adrenergic agonist. NTP (1992) concluded from these results that glyphosate may have produced the salivary gland changes by acting through an adrenergic mechanism. This conclusion has been challenged as being difficult to reconcile with the absence of â-adrenergic effects (e.g., on heart rate and blood pressure) when glyphosate was administered intravenously to dogs or rabbits (Williams et al., 2000). However, it is possible that, rather than acting by a direct adrenergic mechanism, glyphosate could have produced an adrenergic-mediated stimulation of the salivary glands through some indirect mechanism exerted during prolonged repeated dosing. Until the salivary gland effect is corroborated and better understood, it cannot be classified as a glyphosate-induced neurologic effect.

Schiffman et al. (1995) conducted a study of the effects of glyphosate on taste response in gerbils. This study appears to be the only reported investigation of the effects of glyphosate on sensory mechanisms. Glyphosate (1 or 10 mM) applied to the tongue of anesthetized gerbils decreased taste receptor response, measured as electrical impulses along the chorda tympani nerve, to various tastants such as table salt (salty), sugars (sweet), or acids (sour). The mechanism of this effect on the taste response has not been investigated. The effect could have been produced by a general biochemical alteration in the epithelial cells of the tongue, including the specialized cells that detect taste (glyphosate has been shown to produce injury to the oral cavity), by chemical injury to the tongue, or by a direct neurotoxic effect on the sensory nerve endings. Here again, the available information does not allow the effects reported in Schiffman et al. (1995) to be classified as a glyphosate-induced neurologic effect.

Wildlife – The toxicity of glyphosate has been studied in various bird families including chickens and quail, finches, and ducks. Neurological effects were not reported in these studies. A report by Monsanto (1982) found *"no behavioral or treatment-related effects"* in chickens that

received oral doses of 1,250 mg/kg, 2 times per day until a total dose of 15,000 mg/kg was administered.

3.2.2. Triclopyr

Overview – There is no evidence for triclopyr being a direct neurotoxicant in humans or other species. As with glyphosate, studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals or other species exposed subchronically or chronically to triclopyr have not been reported. Again, this is not surprising, since the undertaking of such studies on a substance for which the clinical and experimental toxicology experience provide no reason to suspect a neurotoxicity potential, would be highly unusual. Experiments conducted in fish suggest possible effects of triclopyr on behavior when exposures are at or near lethal levels. As is the case with mammals, these studies provide no evidence that triclopyr is a direct neurotoxicant.

Acute toxicity studies conducted in various mammalian species have observed lethargy, impaired coordination, weakness, labored respiration, and tremors in animals exposed to lethal or near-lethal dose levels of triclopyr. Direct neurotoxic activity would be expected to be observed in longer-term experimental studies in which exposures were well below lethal levels. However, studies conducted in rodents, dogs, monkeys, birds, and amphibians have not provided evidence of direct neurotoxicity, even at the maximum tolerated dose. Neurologic endpoints evaluated in these studies may have been limited to brain morphology and observation of the animals for gross abnormalities in movement or balance. Nevertheless, these studies suggest that the acute neurological effects of triclopyr observed at near lethal doses may indeed be secondary to cardiovascular trauma from treatment-induced injuries to other organs, possibly kidney and liver. Studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals exposed subchronically or chronically to triclopyr have not been reported. Two studies found evidence for possible neurologic effects of triclopyr in fish. The effects observed included lethargy, hypersensitivity to light stimuli, and avoidance behavior but were only observed at lethal or near-lethal exposure levels. In the absence of any signs of direct neurotoxicity in other species, these observations are consistent with indirect neurological effects secondary to general poisoning.

Human Data – Epidemiological studies or case reports involving humans exposed to triclopyr have not been reported in the literature.

Experimental Mammals – The toxicology of triclopyr has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs (SERA 1995; U.S. EPA 1998). In most standard subchronic and chronic rodent bioassays used and accepted by U.S. EPA for pesticide registration, brain morphology is assessed. The spinal cord and peripheral nerves (e.g., sciatic nerve) are usually evaluated only if there are other indications of neurotoxicity. Available summaries of these studies do not report neurological effects at doses that included the maximum tolerated dose (SERA 1995; U.S. EPA 1998). These studies included two chronic 2-year dietary studies in which rats were exposed to 3, 12, or 36 mg/kg/day triclopyr in diet (Dunn et al., 1980; Eisenbrandt et al., 1987); three subchronic studies in which rats were exposed to 3-250 mg/kg/day triclopyr in diet (Barna-Lloyd et al., 1992; Humiston et

al., 1975; Landry et al., 1984); a 2- and 3-generation study in which rats were exposed to 3-30 mg/kg/day triclopyr in the diet (Hanley et al., 1976, 1984); two 2-year studies in which mice were exposed to 3-190 mg/kg/day in the diet (Molello et al., 1979; Tsuda et al., 1992); and three subchronic studies in which dogs were exposed to 0.1-20 mg/kg/day in the diet (Quast et al., 1976, 1977, 1988). A 20-day oral exposure of monkeys to 10, 20, or 30 mg/kg/day did not result in treatment-related toxicity (Mollello et al., 1976).

The acute toxicity of triclopyr has been studied in various mammalian species (SERA 1995; U.S. EPA 1998). A consistent finding at lethal or near-lethal dose levels is lethargy, impaired coordination, weakness, labored respiration, and tremors, suggesting a neurological component to the acute toxicity of triclopyr. Similar signs and symptoms are associated with acute exposures to triclopyr acid, triclopyr BEE, and the Garlon formulations. Liver and kidney injury also occurs at dose levels that produce neurological effects; thus, the observed neurological effects may be secondary to toxicity in these organs and related electrolyte or acid/base abnormalities, and/or functional collapse of the cardiovascular system. No other evidence points to a direct effect of triclopyr on the central or peripheral nervous system.

Wildlife – The toxicity of triclopyr has been studied in various birds, fish, and amphibians (SERA 1995; U.S. EPA 1998). Neurological effects were not reported in the summaries of these studies. Johansen and Green (1990) reported that juvenile coho salmon exposed to near-lethal concentrations of Garlon 4 (0.32-0.42 mg/L, 96 hours; LC_{50} = 0.84 mg/L) showed less spontaneous activity and greater activity when enclosure lights were turned on in light-dark cycle. These changes in behavior suggest that triclopyr may have increased photoperiod sensitivity, which may reflect a direct or indirect neurological effect of the exposure. Rainbow trout exposed to Garlon 4 or Garlon 3a for 96 hours exhibited changes in behavior including erratic swimming (Garlon 4, 0.6 mg/L; Garlon 3a, 200 mg/L) and active avoidance to the triclopyr formulations in a Y-maze (Garlon 4, 20 mg/L; Garlon 3a, 800 mg/L) (Morgan et al., 1991). The behavioral effects were observed at exposure concentrations above the LC_{50} (Garlon 4, 2.4 mg/L; Garlon 3a, 400 mg/L) and may be indicative of either a direct or indirect neurological effect of triclopyr or, as discussed above, on the inerts in the triclopyr formulations. In any event, this study along with a large number of other studies on fish and other aquatic organisms are considered in the SERA (1995) risk assessment.

3.2.3. Hexazinone

Overview – There is no evidence for hexazinone having a direct neurotoxic effect in humans or other animals. As with triclopyr, studies designed specifically to detect impairments in motor, sensory, or cognitive functions in mammals or other species exposed subchronically or chronically to hexazinone have not been conducted. Again as with triclopyr, these studies have not been conducted because the clinical and experimental toxicology experience with hexazinone provide no reason to suspect a neurotoxicity potential. Thus, the effort and expense associated with the conduct of specific studies to assay for neurotoxicity do not appear to be justified.

Nonetheless, acute toxicity studies conducted in various mammalian species and in quail have observed lethargy, impaired coordination, weakness, labored respiration, and tremors in animals

exposed to lethal or near-lethal dose levels of hexazinone. These studies indicate that acutely lethal, or near-lethal exposures to hexazinone may exert either direct or indirect neurological effects. If hexazinone were a direct neurotoxic agent, however, neurologic effects would be expected to be observed in longer-term experimental studies in which exposures were well below lethal levels. However, studies conducted in rodents, dogs, and birds have not provided evidence of neurotoxicity, even at the maximum tolerated dose. Neurologic endpoints evaluated in these studies were limited to brain, spinal cord and peripheral nerve morphology and observation of the animals for gross abnormalities in movement or balance. Nevertheless, these studies suggest that the acute neurological effects of hexazinone observed at near lethal doses may indeed be secondary to cardiovascular or respiratory trauma from treatment-induced injuries to other organs.

Human Data – As with triclopyr, no epidemiological studies or case reports involving humans exposed to hexazinone have not been reported. Based on human experience with a granular formulation of hexazinone, Spencer et al. (1996) report that dust associated with the application of some batches of granular formulations may be sufficiently dense to cause eye and respiratory irritation in workers. These effects are transient and do not persist after exposure is terminated. This study contains no information suggesting that hexazinone caused any signs of direct neurotoxicity. Since this study involved exposures that were far above the RfD for hexazinone (SERA 1997, Section 3.2.2.1.), this study does at least suggest that the current RfD is protective of adverse effects including neurotoxicity.

Experimental Mammals – Neither the U.S. EPA (1994) RED or the SERA (1997) risk assessment contain any information that suggests that hexazinone caused direct neurotoxic effects. The toxicology of hexazinone has been examined in several subchronic and chronic dietary studies in mice, rats, and dogs (Kennedy and Kaplan, 1984). These studies included daily observations of the animals for outward signs of toxicity, and at the conclusion of exposure, histopathological examination of brain, eye, skeletal muscle, sciatic nerve, and spinal cord. Neurological effects were not apparent from gross observations on the animals, and no treatment-related abnormalities were found in tissues of the nervous system at doses at or below the maximum tolerated dose. The studies included: 90-day (0, 1,000, 5,000 ppm in diet), 2-year (0, 200, 1,000, 2,500 ppm in diet), and 3-generation (0, 200, 1,000, 2,500 ppm in diet) rat studies; a 2-year mouse study (0, 2,500, 100,000 ppm in diet); and a 90-day study in dogs (0, 200, 1,000, 5,000 ppm in diet) (Kennedy and Kaplan, 1984).

The acute toxicity of hexazinone has been studied in various mammalian species (Kennedy, 1984; SERA, 1997; U.S. EPA, 1994). A consistent finding at lethal or near-lethal dose levels is excessive salivation, lethargy, weakness, tremors, and convulsions, suggesting a neurological component to the acute toxicity of hexazinone. These effects are accompanied by rapid and labored breathing and pulmonary congestion; thus, the observed neurological effects appear to be secondary to respiratory distress and/or functional collapse of the cardiovascular system. Histopathological examinations of brain and eye were conducted in rats that were exposed to 300 mg/kg/day (approximately 1/5 of the LD_{50}) on 5 consecutive days, followed by 2 days without treatment and 5 more consecutive days of treatment (Kennedy, 1984). No treatment-related indications of neurotoxicity were found from gross observation of the animals, and there were no

indications of histopathological changes in the brain or eye. Thus, no evidence points to a direct effect of hexazinone on the central or peripheral nervous system in experimental mammals.

Wildlife – The toxicity of hexazinone has been studied in various birds, fish, and amphibians (Kennedy, 1984; SERA, 1997; U.S. EPA, 1994). Quail given a single oral dose of 2,510 mg/kg (approximate LD_{50}) exhibited signs of neurotoxicity, including decreased response to sound and movement, wing droop, hind limb weakness, loss of coordination, and convulsions. It is not possible to determine conclusively from the results of this study whether the observed neurological effects in quail reflect a direct effect of hexazinone on the nervous system or are indirect effects resulting from impairment of the cardiovascular or respiratory systems. Nonetheless, the lack of apparent direct neurotoxic effects in mammals suggests that these neurologic effects were secondary to other toxic effects associated with gross over-exposure to hexazinone. In any event, this information on quail is considered in both the SERA (1997) risk assessment and the U.S. EPA (1994) RED. For example, the dose of 2,510 mg/kg associated with these effects is a factor of 25 above the NOAEL value of 100 mg/kg/day for characterizing the risk of acute exposures in birds (SERA, 2001d).

4. IMMUNOLOGIC EFFECTS

4.1. General Considerations

4.1.1. Definitions

At a fundamental level, every human being consists of a highly organized collection of cells in an environment shared with cells of other living organisms. The *immune system* serves to distinguish cells of the individual (*self*) from cells of other organisms *(foreign*). This allows the body to recognize and destroy foreign cells *(immune response*) that enter the body inadvertently, and that might otherwise produce lethal disease. The immune system also continually searches the body for abnormal cells of the individual (*surveillance*), those that may have been altered by infection with viruses, or cells that have been rendered abnormal from aging, injury, or disease, including certain abnormalities associated with cancerous cells. Abnormal cells, once identified, are destroyed and replaced. This process underlies the body's recognition and healing of wounds.

The *immune system* consists of a set of first defense agents including the mononuclear phagocytic cells (macrophages in tissues, monocytes in circulation) and the natural killer cells as well as specific lymphoid tissues dispersed throughout the body. The *lymphoid tissue* is comprised of T and B lymhocytes, epithelial cells and stromal cells, and is arranged into structurally and functionally distinct organs such as the *thymus, spleen and lymph nodes* or accumulations of diffuse lymphoid tissue such as the *gut-associated lymphoid tissue* (GALT). All cells of the immune system derive from a pluripotent stem cell in the *bone marrow*. *T lymphocytes* become immunologically competent (mature) in the thymus. *B lymphocytes* mature in the GALT (mammals) or bursa of Fabricius (birds). The immune system defends its host against foreign agents by utilizing both the non-specific and specific components, its mature lymphocytes with their associated cell-surface antigens (cellular immunity), special proteins in circulation (immunoglobulins), specific *antibodies* produced by the plasma cell (humoral

immunity) in response to foreign *antigens* (bacterial, viral, parasites, foreign proteins etc.) and a number of other cell products known as *cytokines*. Cells of an individual are recognized as self by their cell-surface recognition antigens. Each individual has a unique signature of cell recognition antigens, known as the *major histocompatibility complex* (MHC). Changes of these signature antigens identifies a cell as foreign or abnormal, and triggers an unwanted immune response. The MHC together with other types of cell-surface antigens on lymphocytes (cluster differentiation antigens, CD) enable the immune system to recognize and respond to foreign or abnormal cells. In *autoimmune* diseases, this recognition system fails, and the immune system mounts an often-destructive response against self cells and tissues. Examples of autoimmunity include Hashimoto's thyroiditis due to the production of antibodies to native thyroglobulin, which is the major iodine-containing protein; autoimmune haemolytic anaemias, in which patients produce antibodies to their own red cells, and the Goodpasture's syndrome in which autoantibodies are produced to glomerular basement membrane of the kidneys leading to glomerulonephritis (kidney damage).

4.1.2. Causes of Immunologic Effects

Immunotoxicants are chemicals that disrupt the function of the immune system. Depending on the mechanism of action, immunotoxicants can either impair immune responses (immune suppression) or stimulate the immune responses (hyperreactivity). *Immune suppression* may lead to enhanced susceptibility to infectious agents or inability to clear cancerous cells from the system. Examples of such agents include corticosteroids, which are drugs used in the treatment of inflammation, and cyclosporin, a drug used to suppress the immune response in transplantation patients (Diasio and LoBuglio, 1996). Environmental pollutants that are known to be immunosuppressive include benzene, PAHs, PCBs, TCDDs, certain heavy metals (e.g. lead, mercury and cadmium), and certain organophosphate and organochlorine insecticides (Burns et al., 1996; Luster et al., 1992, 1993; Tryphonas and Feeley, 2001). *Hyperreactivity* on the other hand can lead to *allergy or hypersensitivity* in which the immune system of genetically predisposed individuals responds in an exaggerated manner to substances (allergens) such as plant pollen, cat dander, peanuts, and eggs that do not pose a threat to other non-susceptible individuals. This type of reaction involves a sensitization phase during which the individual is subjected to repeated exposures of the allergen and a subsequent encounter with the allergen which may result in a mild reaction (skin rashes or hives, congestion, sneezing etc) or a less frequent but severe reaction (*anaphylaxis*) leading to death. Hyperreactivity can also lead to autoimmunity in which the immune system produces antibodies to self antigens resulting in damage of the organ or tissue involved. Only a few agents have been shown to cause autoimmunity. These include several metals, such as gold and mercury (Bigazzi, 1992) and viruses (Denman, A.M.,1983).

4.1.3. Assessment of Immunotoxicity

Evidence of immunotoxicity relies largely on the corroborated demonstration, usually in animal models, of 1) a dose-related histopathologic change in lymphoid tissue; and/or 2) a dose-related effect of the chemical on immune response to a foreign antigen. The occurrence of both

histopathologic changes in lymphoid tissue and abnormalities in one or more types of immune responses, would be strong evidence for immunotoxicity.

Typical subchronic or chronic animal bioassays conduct morphological assessments of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus (thymus weight is usually measured as well), and blood leukocyte counts. These assessments can detect signs of inflammation or injury indicative of a direct toxic effect of the chemical on the lymphoid tissue. Changes in cellularity of lymphoid tissue and blood, indicative of a possible immune system stimulation or suppression, can also be detected.

The above evaluations can detect abnormalities in structure of lymphoid tissue and changes in lymphoid cell numbers, however, they cannot detect impairments of immune responses. Assessment of immune system responsiveness, can only be made by observing the outcome of an antigen challenge to the immune system. A variety of tests have been developed to assess the effects of chemical exposures on various types of immune responses (Luster et al., 1988, 1992, 1993). These include measuring the effects of chemical exposure on antibody-antigen reactions (*humoral immunity*), measuring changes in the activity of specific types of lymphoid cells when exposed to foreign antigens (*cell-mediated immunity*), and assessing changes in the susceptibility of exposed animals to resist infection from pathogens or proliferation of tumor cells (*host resistance*). Tests of immune responsiveness are not typically conducted as part of standard toxicity bioassays, unless there are other indications that the chemical may have immunologic potential. These indications might include histopathologic change in lymphoid tissue, changes in blood leukocyte counts, or indications of excessive infectious disease in treatment groups.

4.1.4. Weight of Evidence Applied to Immunotoxicity

Observations that would form the basis of a weight of evidence for immunotoxicity occurring in a given human population are shown below (this scheme would apply to non-human, ecological species, if the reference to humans is replaced by the species of concern):

Weight of Evidence	Observation
Highest	Dose-response relationship indicates effects at anticipated exposure levels
^	Similar effects are observed in exposed humans (epidemiological, clinical cases)
^	Dose-response relationship in humans is likely
^	Structure-function relationship applies to the human immune system
^	Functional deficit or structural change is linked mechanistically to immunosuppression, immune system hyper-reactivity, or autoimmunity
^	Functional and/or structural changes occur in several mammalian species

^	Chemical produces, in an animal model, a decrease in host resistance to infectious agents or syngeneic tumor cells
^	Chemical produces, in an animal model, a decrease or increase in humoral or cellular immune response to a foreign antigen.
^	Chemical produces, in an animal model, a dose-related histopathologic change in lymphoid tissue.
Lowest	Chemical produces, in an animal model, a dose-related change in the numbers or types of lymphoid cells in lymph tissue or blood.

The highest weight would be given to observations of immunologic effects in humans. Such observations could be derived from epidemiological studies of workers exposed in their occupations or of the general population exposed to environmental levels; or from clinical case studies (e.g., accidental poisonings or attempted suicides). The epidemiological observation of certain chemical-specific immune responses, such as impairment of host resistance or autoimmunity, are very difficult to achieve in practice because of the relatively high incidence of infectious diseases and autoimmune diseases in the general population. A large body of epidemiological studies on the relationship between chemical exposures and allergies and hypersensitivity exist. Studies of other mammalian species must usually form the basis for a weight of evidence of immunotoxic potential in humans. The strength of the evidence increases when evidence of impaired immune system function (e.g., suppression or hyper-reactivity), or a structural abnormality in lymphoid tissue is observed in more than one mammalian test species, is understood mechanistically to the extent that we can be reasonably certain that disruption of an immune system is involved in the effect, that the immune system affected in the test species operates in humans, and the dose that produces the immunological effect in the test species can be expected to be achieved in human populations. The latter distinguishes agents that demand our concern from the enumerable chemicals that may interact and produce responses of the human immune system that are beneficial or that are adverse at doses of little or no concern to humans. In the absence of direct evidence of immunologic effects of a chemical, studies of chemicals of similar structure can sometimes be informative, if sufficient knowledge exists, about the mechanisms that relate chemical structure to the immunological effects.

As with neurotoxicity, no single, socially ethical study, or set of studies can prove that a chemical will not produce immunological in humans. However, we can apply observations made in animal models, observations made from epidemiological studies of workers of the general population, and clinical experience with cases of poisonings to assess the weight of evidence about whether or not immunological effects are more or less likely to occur compared to the likelihood of other forms of toxicity. For example, evidence for a low potential for immunological effects would be subchronic and/or chronic rodent assays in which: 1) the chemical does not suppress humoral or cellular immune responses to foreign antigens (e.g., LPS mitogen, Con A mitogen, sheep red blood cells); 2) the chemical does not impair host resistance in challenges with tumor cells (e.g., PYB6 sarcoma, B16F10 melanoma), bacterial infections (e.g., *Listeria monocytogenes, Strepococcus*), virus infections (influenza), or parasitic infections (e.g., *Plasmodium yeilii)*; and 3) in a rodent assay, that includes a maximum tolerated dose, it is found that the chemical does not produce histopathologic changes in lymphoid tissue, or

suppressed or elevated blood leukocyte counts. Corroboration of these observations in more than one mammalian species would allow a broadening of this conclusion to other mammalian species. This, in combination with epidemiological studies that show no evidence of immunological effects in workers or general populations who have been exposed to levels of the chemical expected to produce other forms of toxicity, would be strong evidence that the risk of immunotoxicity in humans is relatively low.

4.2. Immunotoxicity of Specific Herbicides

4.2.1. Glyphosate

Overview – Based on results from the available studies in humans and experimental studies in rodents, glyphosate does not appear to be an immunotoxicant in humans or other animals. This conclusion is supported not only by an extensive set of standard mammalian bioassays on toxicity but also by an *in vivo* assay specifically designed to detected humoral immune response and an *in vitro* assay specifically designed to detect cell mediated immune response.

Epidemiological studies and clinical cases have not found evidence for allergic reactions or sensitization to dermal exposures to glyphosate formulations. Two human experimental studies provide evidence that Roundup is not a dermal allergen or sensitizing agent. Tests conducted in guinea pigs provide further support for glyphosate not being a dermal sensitizing agent. Several long-term experimental studies have examined the effects of exposure to glyphosate on lymphoid tissue morphology and blood leukocyte counts; treatment-related effects were not observed.

One study has reported immune suppression in one species of fish exposed to an unspecified formulation of glyphosate at an unspecified – but presumably low – concentration. While this study cannot and should not be totally dismissed, it has no significant impact on existing ecological risk assessments for glyphosate. As detailed at some length below, this study does not provide information that is adequate for determining whether the reported immune responses were due to a direct effect on the immune system or secondary effects associated with cytotoxicity. In addition, this report is inconsistent both with the available studies in mammals – which provide no evidence of effects on the immune system – as well as a full life cycle test in fish that has been accepted by the U.S. EPA and provides an adequate NOEC for glyphosate in fish.

Human Data – Two experimental studies have evaluated the ability of glyphosate formulations to induce allergy in humans. Maibach (1986) exposed volunteers to Roundup and found that direct dermal application did not produce allergic or photoallergic responses. Williams et al. (2000) describe a study in which dermal exposure to Roundup (approximately 9 or 4.1% glyphosate as the isopropylamine salt) did not produce allergy or sensitization (Shelanski et al., 1973). A study of five forest workers who participated in mixing and spraying operations (see neurotoxicity discussion for more details on the study) did not observe changes in blood leukocyte counts or symptoms of allergy (e.g., skin rash, respiratory symptoms) (Jauhiainen et al., 1991).

The clinical case literature on health outcomes of accidental and intentional acute intoxications with glyphosate has been summarized in the discussion of neurological effects (Section 3.2.1). Although cases of skin rashes following dermal exposures have been reported (Barbosa et al., 2001), these effects are thought to derive primarily from irritation rather than allergy, based on observations of Maibach (1986). The case literature does not provide evidence for immunologic effects of glyphosate or glyphosate formulations.

Experimental Mammals – Two studies have been published which specifically address the potential immunologic effects of glyphosate and both of these studies indicate that glyphosate is not immunotoxic. The only reported *in vivo* study (Blakley 1997) assayed for the effects of glyphosate on immune response to antigens. In this study, mice were exposed for 26 days to Roundup in drinking water (0, 0.35, 0.70, or 1.05 %) and then assessed the humoral (antibody) immune response to a sheep red blood cell challenge. The response in exposed mice was not different than that of control (unexposed) mice. This is consistent with *in vitro* assays using human immunocompetent cells — natural killer cells and cytotoxic T cells — which indicated that exposure to glyphosate or Roundup at concentrations ranging from 0.01 to 10 ⎵moles had no effect on immune system function (Flaherty et al. 1991). Further, there is no evidence that glyphosate or glyphosate formulations produce sensitization in acute dermal sensitization tests performed in guinea pigs (SERA, 1996; U.S. EPA, 1993a; Williams et al. 2000).

As noted in the previous discussion of neurologic effects (Section 3.2.1), the toxicology of glyphosate has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs (SERA, 1996 ; U.S. EPA, 1993a,b; Williams et al. 2000). In these reviews, no studies are reported that indicate morphologic abnormalities in lymphoid tissues. Histopathologic evaluations of lymphoid tissues and evaluation of blood leukocyte counts are standard procedures in most rodent bioassays. These studies included a 2-year dietary study, in which rats were exposed to 2,000, 8,000 or 20,000 ppm glyphosate in diet (Stout and Ruecker, 1990); 3-generation dietary study in rats at exposure levels of 3, 10, or 30 mg/kg body weight (Schroeder, 1981); and a 2-generation dietary study, in rats at exposure levels of 2,000, 10,000 or 30,000 ppm (Reyna, 1990).

Subchronic studies, in which mice and rats were exposed to 3,125, 6,250, 12,500, 25,000 or 50,000 ppm glyphosate in the diet, examined morphology of the major lymphoid tissues, including bone marrow, major lymph nodes, spleen and thymus; thymus weight; and blood leukocyte counts (NTP, 1992). No treatment-related effects were observed at or below the maximum tolerated dose (50,000 ppm).

Wildlife – El-Gendy et al. (1998) have published a study on potential effects of glyphosate on immune function in fish. This is the only study that has reported any effect on immune function in any species. In this study, Bolti fish (*Tilapia nilotica*) were exposed for up to 4 weeks to glyphosate. However, neither the formulation of glyphosate nor the specific concentration used in the study are reported. Instead, exposure level was described as *"1/1000 of the field recommended concentration"* and the formulation is given only as "*glyphosate 48% SC*".

This study examined a number of important immunologic endpoints including:

- Proliferative response of splenocytes (LT) to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) and to the B-cell mitogen lipopolysaccharide (LPS). This is an *in vitro* assay for cell-mediated immunity.

- The Plaque Forming Cell (PFC) assay following *in vitro* immunization with sheep red blood cells (SRBC). This is a key assay to determine effects on humoral (antibody in circulation) immunity.

- The quantification of serum anti-SRBC levels. This endpoint is also an assay for humoral immunity.

- The electrophoretic evaluation of serum protein fractions. This is a general parameter for detection of overt/non-specific toxicity.

However, there are several aspects of this study that pose difficulties in interpreting the data.

Firstly, it is stated that the LT assay was performed on blood samples taken at 1 hr, 24 hr, 2 and 4 weeks of treatment. It is assumed that for each of these treatment dates a new set of cultures would be set up. Therefore one would expect to have stimulation index (SI) values for the control for each of the mitogens tested at each time point. This is not the case since SI values for all three mitogens are presented only once. Furthermore, it is not clear for which time point the stated SI values are (see Table 1 in El-Gendy et al. 1998).

Secondly, the authors report data for the anti-SRBC titres (Table 3 in El-Gendy et al. 1998) at 1 hr, 24 hr, 2 and 3 weeks. Firstly, no data are presented for optimizing the number of SRBC injected. Secondly, the schedule of immunization (one injection vs multiple injections) with SRBC is not stated by the authors. It is rather odd that statistically significant depressed anti-SRBC titres are noted within one hr following treatment. Thirdly, no data are presented on the preimmunization level of anti-SRBC in the control and treated. Also only one control value is presented without stating for which time point this applies to. Further no control values are presented for each of the time points to which the treated should be compared.

Thirdly, the PFC assay is carried out in vitro using several treatment levels in ìM quantities. Data from this assay is questionable for the following reasons: It is not clear whether the assay was performed in groups of fish separate from those which were immunized for anti-SRBC *in vivo*; there is evidence from Table 2 in El-Gendy et al. 1998 that the concentrations used in this assay are cytotoxic to spleen cells. Thus, the issue of direct toxicity of the chemicals in question on cells of the immune system is a very important issue. Ideally there should be very little toxicity when one deals with immunologic assays.

Fourthly, the data on protein levels and serum fractions are inconclusive.

Lastly, and most importantly, the authors do not mention any infections of the fish and have not challenged the fish with any infectious agent to test for a potential decrease in resistance to

infection due to effects on the immune system. In terms of potential ecological effects, the failure to test for susceptibility to infections greatly reduces the utility of this study. Thus, it cannot be concluded from the data presented in this study that the effects reported on the immune system represent a direct toxic effect on the immune parameters examined. Given the reported cytotoxicity, it is plausible that the reported immune effects are the result of general cytotoxicity rather than due to specific effects on immune function.

In addition to the above noted deficiencies, the study by El-Gendy et al. (1998) is inconsistent with a full life-cycle toxicity study has been conducted in fathead minnow, a standard chronic toxicity assay that was required by and accepted by the U.S. EPA (1993a) for the reregistration of glyphosate. In this study, the NOEC was 25.7 mg/L (U.S. EPA, 1993a, p. 41). While El-Gendy et al. (1998) do not report the concentration tested in their study, the study required by U.S. EPA defines clearly a NOEC for an exposure over a life span. If glyphosate had caused any substantial impairment of immune function in this assay, signs of the immune impairment – i.e., increased infections – should have been apparent. Thus, in terms of the ecological risk assessment, the study by El-Gendy et al. (1998) has no significant impact.

4.2.2. Triclopyr

There is very little direct information on which to assess the immunotoxic potential of triclopyr. The only studies specifically related to the immunotoxicity of triclopyr are skin sensitization studies conducted on triclopyr-BEE and the triethanolamine salt of triclopyr. For both of these forms of triclopyr, skin sensitization was observed following standard protocols accepted by the U.S. EPA (1998, p. 6). While these studies provide support for asserting that triclopyr may cause skin sensitization, they provide no information useful for directly assessing immune suppressive potential of triclopyr.

As noted in the previous discussion on the neurologic effects of triclopyr (Section 3.2.2), the toxicology of triclopyr has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs (SERA 1995; U.S. EPA 1998). In these reviews of the toxicity of triclopyr, morphologic abnormalities in lymphoid tissues have not been reported. While the SERA (1995) risk assessment covered only studies in the open literature, the RED prepared by U.S. EPA (1998) does include summaries of a large number of unpublished studies. Since histopathologic evaluations of lymphoid tissues and evaluation of blood leukocyte counts are standard procedures in most rodent bioassays and since positive effects in these tissues would typically be reported prominently, it is reasonable to assert that these effects were not noted in standard bioassays of triclopyr.

Equally important is the fact that the most sensitive effect for triclopyr is well characterized and involves damage of proximal tubular tissue of the kidneys. This is the endpoint selected by U.S. EPA (1998) as the basis for the RfD and is the same endpoint used in the SERA (1995) risk assessment. As discussed in Section 1, protecting against this critical effect using the existing RfD is considered to be protective of all toxic effects. There is no specific information on triclopyr that raises significant questions concerning the protectiveness and adequacy of the current RfD.

4.2.3. Hexazinone

As with triclopyr, there is very little direct information on which to assess the immunotoxic potential of hexazinone. Also as with triclopyr as well as virtually all registered pesticides, hexazinone has been tested for skin sensitization. Unlike triclopyr, however, hexazinone caused no signs of skin sensitization in guinea pigs (U.S. EPA, 1984). A lack of activity as a skin sensitizer has also been reported in Kennedy (1984) and SERA (1997).

Just as the positive sensitization of triclopyr does not increase concern for immune suppressive activity, so the data on the negative effects of hexazinone as a sensitizer do not decrease or in any way impact concern for potential immune suppression. As with triclopyr, the only information with which to assess the potential immune suppressive effects of hexazinone is largely indirect. Like triclopyr, hexazinone has been subject to a large number of standard toxicity studies required for pesticide registration by the U.S. EPA (U.S. EPA, 1984). Although these studies are not designed to specifically detect changes in immune function, significant effects on immune function would likely be evidenced by observable changes in lymphoid tissue as well as changes in differential blood cell counts. No such effects are reported by U.S. EPA (1984) in the RED and such effects were not encountered in the risk assessment prepared by SERA (1997). The only changes in blood noted in any of the toxicity studies involve blood enzymes that are indicative of damage to liver cells.

As noted in Section 2.3, the U.S. EPA/OPP RfD for hexazinone (0.05 mg/kg/day) is based on a NOAEL of 5 mg/kg/day. This NOAEL is based on the most sensitive effect – histological evidence and biochemical indicators of liver damage. While this study and other chronic studies on hexazinone cannot rule out the possibility of immunologic effects, they provide no evidence that such effects occurred. Again as with triclopyr, if such immunologic effects had occurred, changes in differential blood cell counts and/or pathological changes in lymphoid tissues would be expected along with some indication of increased susceptibility to infection. No such effects have been noted. Thus, there is no plausible basis for asserting that the current RfD established by U.S. EPA should be revised to accommodate concern for potential effects on the immune system.

5. ENDOCRINE DISRUPTION

5.1. General Considerations

5.1.1. Definitions

The *endocrine system* participates in the control of metabolism and body composition, growth and development, reproduction, and many of the numerous physiological adjustments needed to maintain constancy of the internal environment (*homeostasis*). The *endocrine system* consists of *endocrine glands, hormones,* and *hormone receptors*. *Endocrine glands* are specialized tissues that produce and export (*secrete*) *hormones* to the bloodstream and other tissues. The major endocrine glands in the body include the adrenal, hypothalamus, pancreas, parathyroid, pituitary, thyroid, ovary, and testis. Hormones are also produced in the gastrointestinal tract, kidney, liver, and placenta. *Hormones* are chemicals produced in endocrine glands that bind to *hormone receptors* in target tissues. Binding of a hormone to its receptor results in a process known as

postreceptor activation which gives rise to a *hormone response* in the target tissue, usually an adjustment in metabolism or growth of the target tissue. Examples include the release of the hormone *testosterone* from the male testis, or *estrogen* from the female ovary, which act on receptors in various tissues to stimulate growth of sexual organs and development of male and female sexual characteristics. The target of a hormone can also be an endocrine gland, in which case, receptor binding may stimulate or inhibit hormone production and secretion. An example of this would be the hormone LH (luteinizing hormone), secreted from the pituitary gland, which acts on receptors in the testis to stimulate the secretion of testosterone. This system of endocrine glands, that are responsive to hormones released from other endocrine glands, provides a complex network of control systems for turning on and turning off hormone stimulation of tissues in response to physiological demands, or at appropriate stages of the life span, or reproductive cycle. Examples of this are the dramatic changes in growth and development that occur as the fetus develops in the uterus and as individuals sexually mature during puberty. Repeated cycles of turning on and turning off hormone stimulation of the ovary and uterus occur approximately each month in females to produce the menstrual cycle.

An *endocrine disruptor* is an exogenous agent (from outside of the body) that produces adverse effects on an organism or population of organisms by interfering with endocrine function (Kavlock et al., 1996). The endocrine system is highly regulated to achieve hormone activities in amounts needed to respond to physiological demands. *Endocrine disruption* is a state of uncontrolled hormone action, in which hormone responses are absent or insufficient when needed, or occur inappropriately when they are not needed. These can result in abnormalities in growth and development, reproduction, body composition, homeostasis, and behavior. Endocrine disruptors are not considered to be a major cause of endocrine disorders in humans. However, a variety of inherited endocrine diseases are known to be caused by abnormalities in endocrine glands, hormone transport, or hormone receptors. Certain endocrine diseases are thought to be caused by autoimmune disorders in which the body attacks and destroys its own endocrine glands, for example, Graves' disease, in which the body mounts an immune response against the thyroid gland (Davies, 2000).

Some of our most important drugs are endocrine disruptors. Examples of these include thyroid blocking agents used in the treatment of hyperthyroidism (e.g., thiopropyluracil); corticosteroids used in the treatment of inflammation, and as diuretics in the treatment of edema and hypertension; estrogens used in female birth control and to manage symptoms of menopause; hypoglycemics used in the treatment of certain forms of diabetes mellitus; and various adrenergic agonists and antagonists used in the treatment of allergic reactions, asthma, heart disease, and hypertension (Hardman and Limbird, 1996). Endocrine-active agents are also in our diet, including iodine, needed for the production of thyroid hormone, and phytoestrogens, estrogenic compounds found in many edible plants.

5.1.2. Causes of Endocrine Disruption

Endocrine disruptors can exert effects by affecting the availability of a hormone to its target tissue(s) and/or affecting the response of target tissues to the hormone (EDSTAC, 1998). These effects can enhance the action of natural hormones, or can diminish or abolish these actions.

Effects may be transient or permanent, and may occur soon after exposure to the agent or may occur long after exposure ceases (*latent*).

Interference with hormone synthesis. Hormones are synthesized in endocrine glands by a series of chemical reactions mediated by protein catalysts known as *enzymes*. In these enzyme reactions, a physiological precursor agent is chemically changed to a hormone end product. An agent that inhibits the activity of an enzyme involved in hormone synthesis can impair the production of hormone and decrease the amount of hormone available to produce responses in target tissues. An example of a hormone synthesis inhibitor is propylthiouracil, a drug used to inhibit synthesis of thyroid hormone in the treatment of hyperthyroidism (Meirer and Burger, 2000). Hormone synthesis inhibition may contribute to endocrine disruptive effects of the fungicide fenarimol, which inhibits the synthesis of estrogen (Hirsch et al., 1987), and the dithiocarbamate fungicides, which inhibit catecholamine synthesis (dopamine-â-hydroxylase, Goldman et al., 1994).

Interference with hormone storage. In some endocrine glands, hormones are stored in vesicles within endocrine gland cells. Inside the vesicles, hormones are isolated from enzymes that might otherwise degrade the hormones, rendering them no longer capable of binding to hormone receptors. Agents that disrupt the hormone storage process can increase degradation of hormone, resulting in less hormone available for secretion. The drugs, reserpine and amphetamine, are examples of agents that interfere with storage of the hormone epinephrine in the adrenal gland (Hardman and Limbird, 1996).

Interference with hormone secretion. The first step in exporting hormones to target tissues is the secretion of the hormone out of the endocrine cell. Secretion is usually a highly regulated process than involves *signaling* processes that activate the secretion process. Signaling often involves activation of ion channels to allow ion (e.g., calcium, potassium) currents to flow through the endocrine cell membrane, as well as other enzyme reactions in the cell. Examples of agents that interfere with these signaling processes include certain metal cations which disrupt calcium ion movement into endocrine cells (Cooper et al., 1987).

Interference with hormone transport in the bloodstream. Hormones are often transported in the bloodstream bound to specialized proteins that protect the hormone from degradation and excretion before it arrives at the target tissue. Examples include the reproductive hormones testosterone and estrogen, the adrenal corticosteroids, and thyroid hormones, all of which have binding proteins for transport in the bloodstream. Interference with the production and levels of binding proteins or in the binding of hormone to binding proteins can alter the amount of hormone available to act on target tissues. Production and levels of hormone binding proteins are regulated by the endocrine system. For example, estrogens stimulate the production of testosterone-estrogen binding globulins (TEBG), whereas glucocorticoids inhibit the production of TEBG (U.S. EPA, 1997).

Interference with hormone elimination. Hormones are degraded into inactive forms by chemical reactions mediated by enzymes. Agents that increase the rate of hormone degradation can decrease the amount of hormone available to act on target tissues. One of the important enzyme

systems that serves in the elimination of steroid hormones (e.g., estrogen, testosterone, corticosterone), and thyroid hormone, is the microsomal enzyme system of the liver and other tissues. This system includes cytochrome P450 and glucuronyl transferase, whose synthesis is increased (*induced)* by dioxins, polychlorinated biphenyls (PCBs), and certain chlorinated insecticides, including lindane and DDT (Connor et al., 1995; Curran and DeGroot, 1991; Safe, 1986; Sierra-Santoyo et al., 2000; Visser, 1990).

Interference with hormone action. Hormones act on target tissues by binding to specialized recognition proteins known as *hormone receptors*. The resulting hormone-receptor complex activates various other processes that lead to the physiological hormone response (*postreceptor activation*). Agents that bind to hormone receptors, but do not initiate the hormone response, can prevent binding of the natural hormone and prevent responses that would normally occur in the presence of the hormone. Such agents are known as *hormone antagonists*. Chemicals that bind to hormone receptors and initiate the same response that would naturally occur in the presence of the hormone are known as *hormone agonists*. These agents can produce uncontrolled hormone-like responses in the absence of the natural hormone. Most of the drugs that have been developed to treat endocrine disorders or to modify the response of the endocrine system are hormone antagonists or agonists (Hardman and Limbird, 1996). Examples of environmental agents that appear to be estrogen receptor antagonists or agonists include the insecticides methoxychlor, chlordecone (Kepone) and DDT, certain alkylphenols, and certain PCBs (Connor et al., 1997; White et al., 1994). The fungicide vincolozolin and DDE, a metabolite of DDT, appear to be androgen receptor antagonists (Kelce et al., 1994, 1995). Examples of agents that interfere with postreceptor activation include certain metal cations, cholera and pertussis toxins, phorbol esters, the insecticide lindane, and TCDD (Cooper et al., 1987; Gilman, 1987; Safe et al., 1991).

5.1.3. Assessment of Endocrine Disruption

Evidence of endocrine disruption relies on the corroborated demonstration, usually in animal models, of 1) a dose-related abnormality in the structure of endocrine glands (histopathologic change); and/or 2) a dose-related effect of the chemical on endocrine function, including hormone synthesis, secretion, transport and elimination, receptor binding, or postreceptor processes that give rise to a response in a target tissue; and 3) demonstration that the above effect on endocrine function gives rise to an adverse effect in the organism or population (EDSTAC, 1998). Examples of adverse effects include impairment in growth or development, reproduction, homeostasis, or behavior. This latter evidence, of an adverse effect, is particularly important since it distinguishes endocrine disruptors from chemicals that are merely endocrine-active but have little or no potential for disruption of the endocrine system. The endocrine system responds to many exogenous chemicals in ways that do not always have adverse consequences. For example, the thyroid hormone synthesis responds to changes in the amount of iodine in the body to maintain appropriate levels of thyroid hormone in the face of a constantly changing dietary iodine level (Taurog, 2000). Estrogen agonists are a normal constituent of our diet, which includes estrogens in meat products as well as phytoestrogens in plant products (e.g., soy beans) (NAS, 1999). Thus, the demonstration of endocrine-activity of a chemical agent is not sufficient evidence for concluding that the chemical is an endocrine disruptor.

Morphological examination of the major endocrine glands for histopathologic changes are usually included in well-designed subchronic or chronic rodent bioassays. However, typical rodent subchronic or chronic bioassays begin exposures after weaning, whereas, the assessment of potential adverse consequences of endocrine disruption requires the evaluation of exposures that span all of the critical stages of the lifespan at which endocrine controlled growth and development occur (EDSTAC, 1998). Organisms may be particularly sensitive to endocrine disruption during embryonic development and post-natal, and during growth and maturation (e.g., puberty). Disruption of the endocrine system during development may give rise to effects on the reproductive system that may be expressed only after maturation (U.S. EPA, 1997). For this reason, multigeneration exposures are recommended for toxicological assessment of suspected endocrine disruptors (EDSTAC, 1998). These assays, ideally, should include assessments of embryonic development, postnatal development and growth, reproductive performance, morbidity and mortality, endocrine gland morphology, and biomarkers of endocrine gland function (e.g., serum hormone levels). Such studies may be conducted in several taxa (e.g., mammals, birds, amphibians, invertebrates) for assessments of endocrine disruption potential in wildlife (EDSTAC, 1998). Dose-response relationships for endocrine disruptors may be complex; the response may increase or decrease over intervals of a dose range of a given agent. For example, testosterone can stimulate sperm production at low doses and inhibit sperm production at high doses (EDSTAC, 1998). As a result, assays conducted at a high dose range may not be predictive of responses at a lower dose. Dose ranging studies are recommended to ensure that the assays include a dose range of adequate width to include a clearly toxic dose (maximum tolerated dose) and to capture possible *low-dose effects*. If these types of assays examine an adequately wide dose range below and including the maximum tolerated dose, they can be expected to detect adverse consequences, including latent consequences, of endocrine disruption. However, they cannot be expected to provide definitive conclusions about whether the observed abnormalities do in fact result from endocrine disruption. Other studies directed at identifying endocrine mechanisms underlying the abnormalities would be needed for this purpose.

A variety of short-term *in vitro* and *in vivo* tests have also been described that assess whether the chemical interferes with hormone availability (e.g., synthesis, secretion, transport in the bloodstream) or with the target tissue response (e.g., hormone receptor binding or postreceptor processing). These assays can be used to assess the potential for endocrine disruption and have been proposed as screening assays for endocrine disruption (EDSTAC, 1998). The observation of endocrine activity of a test chemical in these short-term assays together with the observation of abnormalities in growth, development, reproduction, homeostasis, or in endocrine glands, in a multigeneration study in whole animals, would be strong evidence that the chemical is a potential endocrine disruptor.

5.1.4. Weight of Evidence For Endocrine Disruption

Observations that would form the basis of a weight of evidence for endocrine disruption occurring in a given human population are shown below (this scheme would apply to non-human, ecological species, if the reference to humans is replaced by the species of concern):

Weight of Evidence	Observation
Highest	Dose-response relationship indicates effects at anticipated exposure levels
^	Similar effects are observed in exposed humans (epidemiological, clinicalcases)
^	Dose-response relationship in humans is likely
^	Structure-function relationship applies to the human endocrine system
^	Functional deficit or structural change is linked mechanistically to the endocrine disruption
^	Functional and/or structural changes occur in several mammalian species
^	Chemical produces, in an animal model, a histopathologic change in an endocrine gland
^	The dose-related changes noted below occur when exposure to the chemical occurs at critical stages of development.
^	The chemical produces, in an animal model, a dose-related impairment or abnormality in embryonic development, postnatal development and growth, reproductive performance, morbidity or mortality, endocrine gland morphology, or a biomarker of endocrine gland function (e.g., serum hormone levels)
Lowest	The chemical increases or decreases hormone production, secretion, binding to transport proteins, receptor binding, or postreceptor processing.

The highest weight would be given to observations of endocrine-related effects in humans. Such observations could be derived from epidemiological studies of workers exposed in their occupations or of the general population exposed to environmental levels; or from clinical case studies (e.g., accidental poisonings or attempted suicides). The epidemiological observation of chemical-specific endocrine effects is very difficult to achieve in practice, unless the effects are severe. This is in part because all people are exposed to endocrine-active substances as a normal part of their diets, or as part of drug therapies, and there are numerous endocrine diseases that can produce symptoms that would be similar to the effects of an endocrine disruptor. For this reason, studies of other mammalian species must usually form the basis for a weight of evidence. The strength of the evidence increases when an endocrine effect, either a functional deficit to structural abnormality, is observed in more than one mammalian test species, is understood

mechanistically to the extent that we can be reasonably certain that disruption of an endocrine system is involved in the effect, that the endocrine system affected in the test species operates in humans, and the dose that produces the effect in the test species can be expected to be achieved in human populations. The latter distinguishes endocrine-active agents that demand our concern from the enumerable chemicals that may interact and produce responses of the human endocrine system that are beneficial or that are adverse at doses of little or no concern to humans. Examples of the latter include water, dietary iodine, dietary estrogens and phytoestrogens. In the absence of direct evidence of endocrine effects of a chemical, studies of chemicals of similar structure can sometimes be informative, if sufficient knowledge exists, about the mechanisms that relate chemical structure to the endocrine effects.

As with both neurotoxicity and immunotoxicity, observations made in animal models and from epidemiological studies as well as clinical experience with cases of intoxication must be used to assess the weight of evidence about whether or not endocrine disruption is more or less likely to occur compared to the likelihood of other forms of toxicity. For example, evidence for a low potential for endocrine disruption would be: 1) the chemical is tested in an endocrine disruption screening battery and it is found that the chemical does not increase or decrease availability of hormones (e.g., estrogens, androgens, thyroid hormones) to target tissues, hormone receptor binding, or postreceptor processing); and 2) in a multigeneration rodent assay that includes a maximum tolerated dose, it is found that the chemical does not produce abnormalities in embryonic growth and development, postnatal development and growth, reproductive performance, endocrine gland morphology, or in a biomarker of endocrine gland function (e.g., serum hormone levels). Corroboration of these observations in more than one mammalian species would allow a broadening of this conclusion to other mammalian species. This, in combination with epidemiological studies that show no evidence of endocrine-related effects in workers or general populations who have been exposed to levels of the chemical expected to produce other forms of toxicity, would be strong evidence that the risk of endocrine disruption in humans is relatively low.

5.2. Endocrine Disruption by Specific Herbicides

5.2.1. Glyphosate

Overview – Three specific tests on the potential effects of glyphosate on the endocrine system have been conducted and all of these tests reported no effects. The conclusion that glyphosate is not an endocrine disruptor is reenforced by epidemiological studies that have examined relationships between occupational farm exposures to glyphosate formulations and risk of spontaneous miscarriage, fecundity, sperm quality, and serum reproductive hormone concentrations. The studies have not found positive associations between exposure to glyphosate formulations and any reproductive or endocrine outcomes. The clinical case literature does not provide evidence for glyphosate being an endocrine active agent. Several long-term experimental studies have examined the effects of exposure to glyphosate on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects were not observed.

In addition, extensive testing in experimental animals and wildlife provides reasonably strong evidence that glyphosate is not an endocrine disruptor. The existing risk assessments on glyphosate (U.S. EPA, 1993a; SERA 1996) have based the dose-response assessment for glyphosate on reproductive effects in experimental mammals. The mammalian data base for glyphosate is admittedly complex and open to differing interpretations. This is illustrated by the existence of two different RfD's for glyphosate that have been derived by the U.S. EPA. Nonetheless, the approach taken in the SERA (1996) risk assessment used by the Forest Service is highly conservative and no recent information has been encountered suggesting that this risk assessment is not adequately protective of any reproductive effects that might be associated with glyphosate exposure.

Human Data – Numerous epidemiological studies have examined relationships between pesticide exposures, or occupation in agriculture, and reproductive outcomes; however, very few studies have attempted to characterize exposures, either qualitatively or quantitatively, to specific pesticides (Arbuckle and Sever, 1998). Of those studies that have specifically addressed potential risks from glyphosate exposures, adverse reproductive effects have not been associated with glyphosate exposure.

The Ontario Farm Health Study collected information on pregnancy outcomes and pesticide use among Ontario farm couples. Three retrospective cohort studies of this group have examined relationships between exposures to glyphosate formulations (defined as self-reported participation in mixing and/or spraying operations) and reproductive outcomes. One study analyzed self-reported spontaneous miscarriages of 3,984 pregnancies among 1,898 couples who self-reported exposures to glyphosate formulations within a period beginning two months before pregnancy and ending the month of conception (Savitz et al., 1997). Risk of miscarriage was unrelated to self-reported exposure to glyphosate formulations. A second study of spontaneous abortions among 2,110 women and 3,936 pregnancies disaggregated the herbicide exposures into pre- and post-conception and spontaneous abortions into early- (< 12 wk) and late-term (12-19 wk) abortions (Arbuckle et al., 2001). Spontaneous abortions were not associated with post-conception glyphosate formulation exposure; however, the odds ratio for abortions and post-conception exposure was 1.4 (1.0-2.1), and for late-term abortions was 1.7 (1.0-2.9). The latter odds ratios were not adjusted for maternal age which is a risk factor for spontaneous abortion. When maternal age was considered in a regression tree analysis, spontaneous abortions were found to be unrelated to glyphosate formulation use. Curtis et al. (1999) examined fecundity (time to pregnancy after discontinuation of birth control with the intent to conceive) among 1,048 farm couples (2,010 planned pregnancies) who self-reported exposures to glyphosate formulations within a period beginning 2 months prior to trying to conceive (to account for time of spermatogenesis) and ending at pregnancy. Fecundity was unrelated to glyphosate exposure.

Larsen et al. (1998a) examined relationships between use of pesticides and semen quality among farmers in Denmark. Participants in the study included 161 farmers who self-reported crop spraying with a variety of pesticides, that included Roundup (7% prevalence of use) and 87 farmers who did not use pesticides. Semen samples were collected at the start of the spraying season and 12-18 weeks after the first spraying. Evaluations included sperm count, morphology, chromatin structure and motility; and serum concentrations of reproductive hormones

(testosterone, LH, FSH). Semen quality and reproductive hormone levels were unrelated to pesticide use. In a related study, fecundity was compared among farmers who did or did not participate in pesticide spraying operations (Larsen et al., 1998b). Fecundity was determined from the number of self-reported menstrual cycles or months between discontinuation of birth control and pregnancy. Participants included 450 traditional farmers who reported that they sprayed pesticides, 72 traditional farmers who did not participate in spraying operations, and 94 organic farmers who reported not using pesticides on their crops. Fecundity was unrelated to pesticide use or participation in pesticide spraying operations.

The clinical case literature on health outcomes of accidental and intentional intoxications with glyphosate has been summarized in the discussion of neurological effects (Section 3.2.1). Endocrine effects would have to be very severe in order to be reported in association with an acute exposure (e.g., endocrine gland failure or extreme gland hyperactivity), as detection of endocrine effects usually requires substantial longer-term follow up of cases. No endocrine effects have been reported that can be associated with exposures to glyphosate.

Experimental Mammals – Glyphosate has not undergone an extensive evaluation for its potential to interact or interfere with the estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding or postreceptor processing as recommended by EDSTAC, 1998). Only three specific tests on the potential effects of glyphosate on the endocrine system have been conducted and all of these tests reported no effects. Glyphosate was inactive as an estrogen receptor agonist (*estrogenic activity*) in MCF-7 human breast cancer cells (Lin and Garry, 2000) or in yeast transformed to express rainbow trout estrogen receptor and an estrogenic reporter gene (Petit et al., 1997). In a third assay, glyphosate did not inhibit steroid synthesis in MA-10 mouse Leydig tumor cells by disrupting expression of the steroidogenic acute regulatory (StAR) protein (Walsh et al., 2000). This protein mediates the rate-limiting step in the mitochondrial synthesis of steroid hormones (the transfer of cholesterol to the inner mitochondrial membrane). In the Walsh et al. (2000) study, however, Roundup did inhibit steroid synthesis, probably due to the effects of the surfactant on membrane function. All of these assays are *in vitro* – i.e., not conducted in whole animals. Thus, such studies are used qualitatively in the hazard identification to assess whether there is a plausible biologic mechanism for asserting that endocrine disruption is plausible. Because they are *in vitro* assays, measures of *dose* and quantitative use of the information in dose/response assessment is not appropriate. For glyphosate, these studies to not indicate a basis for suggesting that glyphosate is an endocrine disruptor.

As noted in the discussion on the neurologic effects of glyphosate (Section 3.2.1), the toxicology of glyphosate and commercial formulations has been examined in subchronic, chronic, and multigeneration studies in rodents and in subchronic studies in dogs (SERA 1996; U.S. EPA 1993a; Williams et al. 2000) and these studies can be used to more quantitatively address potential gross effects on the endocrine system, including reproductive function. As summarized in Section 2.1, the U.S. EPA has derived two different RfD's for glyphosate and both of these are based on reproductive toxicity. The U.S. EPA Agency wide RfD for glyphosate of 0.1 mg/kg/day is based on a NOAEL of 10 mg/kg/day from a three generation dietary reproduction study in rats with a corresponding LOAEL of 30 mg/kg/day (U.S. EPA,

1993b) and the U.S. EPA Office of Pesticides has derived an RfD of 2 mg/kg/day based on a gavage teratology study in rabbits with a NOAEL of 175 mg/kg/day and a corresponding LOAEL of 350 mg/kg/day (U.S. EPA, 1993a).

Several other reproductive studies are available on glyphosate which have noted no remarkable effects. In a 2-generation dietary study, no treatment-related effects on mating, fertility or reproductive parameters were observed at 2,000, 10,000, or 30,000 ppm exposure levels; the high exposure level resulted in decreased weight gain in parental animals and reduced pup weight gain, suggesting that the maximum tolerated dose was achieved (Reyna, 1990). This study also found no treatment-related effects on the morphology of the reproductive organs in male or female rats.

A 2-year dietary study, in which rats were exposed to 0, 2,000, 8,000 or 20,000 ppm glyphosate in diet, examined morphology of the reproductive organs, mammary glands, and all major endocrine glands, including the testis, ovary, pituitary, and thyroid (Stout and Ruecker, 1990). No treatment-related effects on reproductive organs or endocrine glands were observed at or below the maximally tolerated dose (20,000 ppm in diet) which resulted in decreased weight gain and histopathologic changes in liver, stomach, and eye lens. U.S. EPA (2001) summarized a study in which dogs were exposed to 0, 20, 100, and 500 mg/kg/day *"glyphosate in gelatin capsules"* for 1 year (Reyna and Ruecker, 1985). The summary notes that a decrease in absolute and relative pituitary weight was observed at the 100 and 500 mg/kg/day dose levels.

Subchronic studies, in which mice and rats were exposed to 3,125, 6,250, 12,500, 25,000, or 50,000 ppm glyphosate in the diet, examined morphology of all reproductive organs; mammary glands; and major endocrine glands, including adrenal, ovary, pancreas, parathyroid, pituitary, thymus and thyroid; the study also evaluated sperm counts and morphology and estrous cycle length (NTP, 1992). No treatment-related effects were observed on the morphology of reproductive organs or endocrine glands at or below the maximally tolerated dose (50,000 ppm in diet) which resulted in decreased weight gain in both rats and mice. A statistically significant decrease (20%) in sperm count was observed in male rats exposed to 25,000 or 50,000 ppm. NTP (1992) concluded that there was no evidence of adverse effects on the reproductive system of rats or mice, and summarized the findings as follows:

> *"Measures of sperm density, or the number of sperm/g caudal epididymal tissue, were reduced somewhat in male rats in the 2 highest dose groups (25,000, 50,000 ppm); other spermatozoal measurements were not different from controls in rats or mice. There was a slight lengthening of the estrous cycle in high dose female rats (50,000 ppm), but the biologic significance of these findings, if any, is not known."*

Several other subchronic and chronic studies of glyphosate are noted in Williams et al. (2000), with no mention of treatment-related effects on endocrine glands or reproductive organs; however, the specific tissues that were evaluated are not reported.

As also summarized by Williams et al. (2000), glyphosate has been evaluated for its effects on the developing fetus when administered during gestation. In rats, oral (gavage) exposures to 3,500 mg/kg/day (but not 200 or 1,000 mg/kg/day) during gestation resulted in decreased fetal weight and fetal viability, and skeletal abnormalities. Maternal toxicity was evident at 3,500 mg/kg/day (Tasker, 1980a). In rabbits, oral (gavage) exposures to 75, 175, or 350 mg/kg/day had no effect on fetal weight, viability or morphology. Maternal toxicity and mortality were evident at the 350 mg/kg/day dose level (Tasker, 1980b). As detailed in Section 2.1, the Tasker (1980b) study cited in Williams et al. (2000) appears to be identical to the Rodwell et al. (1980) study cited in U.S. EPA (1993a) and is the basis for the U.S. EPA's Office of Pesticides RfD.

Yousef et al. (1995) has reported substantial decreases in libido, ejaculate volume, sperm concentrations, semen initial fructose and semen osmolality as well as increases in abnormal and dead sperm in rabbits after acute exposures to glyphosate. The authors report that all of the effects were statistically significant at $p<0.05$. A serious limitation of this study is that the authors report the doses as proportions of 0.1 and 0.01 of the LD_{50} but do not specify the actual doses. Using a reported rabbit LD_{50} of 3,800 mg/kg (SERA 1996), the doses would correspond to 38 and 380 mg/kg.

The toxicological significance of the observed effects described by Yousef et al. (1995) is clear. As noted above, however, a 3-generation study in rats found no treatment-related effects of glyphosate on mating, fertility, or reproductive parameters at doses of 3, 10, or 30 mg/kg body weight, although changes in kidney morphology were noted at the 30 mg/kg/day dose level (Schroeder, 1981). In addition and as also summarized above, very high dietary concentrations of glyphosate have not been associated with impaired reproductive performance or signs of damage in testicular tissue.

The basis for the inconsistency between the Yousef et al. (1995) study and all other studies that have assessed the reproductive effects of glyphosate cannot be identified unequivocally. As discussed by Williams et al. (2000), the Yousef et al. (1995) study can be criticized for a number of reporting and experimental design limitations or deficiencies. In addition, it should be noted that the rabbits in the Yousef et al. (1995) study were dosed by gelatin capsules whereas the Schroeder (1981) multigeneration study involved dietary exposures. The use of gelatin capsules is a reasonable mode of administration but, like gavage exposures, it results in a high spike in body burden that is not typical or particularly relevant to potential human exposures – other than attempted suicides. On the other hand, dietary exposures, as used in the Schroeder (1981) study, result in more gradual and steady exposures over the course of the day that are more comparable and relevant to potential human exposures.

While there may be some uncertainties in the interpretation of the Yousef et al. (1995) study, these do not have a significant impact on the Forest Service risk assessments on glyphosate. The SERA (1996) risk assessment on glyphosate did consider the Yousef et al. (1995) study as well as the Schroeder (1981) study. Both of these studies report adverse effects – although very different adverse effects – at doses of about 30 mg/kg/day. The use of the lower U.S. EPA (1993b) RfD of 0.1 mg/kg/day specifically encompasses the LOAEL of 30 mg/kg/day in that it is based on the NOAEL of 10 mg/kg/day from Schroeder (1981).

Wildlife – The developmental and reproductive toxicity of glyphosate has been studied in various amphibians, birds, and fish. Mann and Bidwell (1999) compared the lethality of various glyphosate formulations on tadpoles of four species of frogs. Glyphosate (isopropylamine salt, 48 hr LC_{50} = 340-680 mg/L) was at least 50-100 times less toxic than either Roundup or Touchdown (commercial herbicides containing glyphosate isopropylamine salt). Batt et al. (1980) examined the effect of exposure to Roundup on hatchability of domestic chicken eggs. Immersion of eggs (on days 0, 6, 12, or 18 days of development) for 5 seconds in 1 or 5% solution of Roundup had no effect on hatchability or time to hatching. Hoffman and Albers (1984) examined the effect of Roundup on growth and morphology of mallard embryos. Eggs (day 2 of development) were either immersed in an aqueous emulsion of Roundup for 30 seconds, or Roundup in an oil vehicle was injected into the egg air space. Embryo growth was not decreased and no morphologic abnormalities in the embryos (up to day 18) were observed at exposure levels near the LC_{50}. Folmar et al. (1979) examined the effect of glyphosate exposure on fecundity (eggs per female) or gonadosomatic index (gonad weight/body weight) in rainbow trout. Exposures to Roundup or the isopropylamine salt of glyphosate (12-hour exposures to 0, 0.02, 0.2, or 2.0 mg/L) had no effect on fecundity or gonadosomatic index.

Glyphosate, Roundup, and Rodeo have been tested in the frog embryo teratogenesis assay – i.e., a screening test for the development of birth defects. Rodeo appeared to be weakly fetotoxic (LC_{50}, 7,300 mg/L) compared to Roundup (LC_{50}, 9.3 mg/L) (exposures expressed as acid equivalents) (Perkins et al., 2000). Embryos were evaluated for morphological abnormalities; according to the investigators: *"Significant increases (analysis of variance, p 0.05) in the incidence of malformations were not observed at any concentration of the glyphosate, ... or surfactant treatments in this study that were also not lethal to the embryos at 96 h."*

5.2.2. Triclopyr

Overview – Epidemiological studies of health outcomes of triclopyr have not been reported, nor is there clinical case literature on human triclopyr intoxication. Several long-term experimental studies in dogs, rats, and mice have examined the effects of exposure to triclopyr on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects on these endpoints were not observed. Triclopyr did not produce morphological abnormalities in frog embryos at exposures below the LC_{50}.

Triclopyr has not undergone evaluation for its potential to interact or interfere with the estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding, or postreceptor processing). However, extensive testing in experimental animals provides reasonably strong evidence that triclopyr is not an endocrine disruptor.

Human Data – No studies involving humans and related to endocrine disruption have been reported in the literature. Thus, all inferences regarding the potential risks to humans must be based on studies in experimental mammals.

Experimental Mammals – Triclopyr has not been tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone). Thus, all inferences

concerning the potential effect of triclopyr on endocrine function must be based on inferences from standard toxicity studies.

The effects of toxicology on reproduction have been examined in experimental studies in rats and mice, including multigeneration studies in rats. In addition to the published studies summarized in SERA (1995), the U.S. EPA (1998, pp. 12-14) RED summarizes the results of several teratology and reproduction studies in rats and rabbits involving both the triethanolamine salt and butoxyethyl ester of triclopyr. None of these studies provide any information indicating that triclopyr interferes with or in any way disrupts normal endocrine function.

As noted in Section 2.2, the U.S. EPA has based the current RfD on a 2-generation reproduction toxicity study in rats (Vedula et al. 1995) with a NOEL of 5.0 mg/kg/day. It is worth noting, however, that this RfD is based on increases in the incidence of proximal tubular degeneration of the kidneys in parental rats – i.e., no specific developmental toxicity was noted in offspring. There is no indication in this or any other studies summarized by U.S. EPA that triclopyr caused any of the toxic effects through a mechanism involving endocrine disruption.

Wildlife – Garlon 4 has been tested for its toxicity to frog embryos and tadpoles. Exposure of frog embryos to 0.6, 1.2 , 2.4, or 4.8 mg/L for 8 days had no effect on hatching success, embryo morphology, tadpole growth, or tadpole avoidance behavior (movement away from prodding) (Berrill et al., 1994). Tadpoles died or became immobile when exposed to 1.2 or 4.6 mg/L. Garlon 4 (butoxyethyl ester) and Garlon 3a (triethylamine salt) were tested in the frog embryo teratogenesis assay. Garlon 3a appeared to be more fetotoxic (LC_{50}, 159 mg/L) compared to Garlon 4 (LC_{50}, 10.0 mg/L) (exposures expressed as acid equivalents, Perkins et al. 2000). Embryos were evaluated for morphological abnormalities and, according to the investigators:

> *"Significant increases (analysis of variance, p 0.05) in the incidence of malformations were not observed at any concentration of the ... triclopyr ... treatments in this study that were also not lethal to the embryos at 96 h."*

Garlon 4 decreased embryo growth relative to controls at doses at or above 6 mg/L.

Mayes et al. (1984) exposed fathead minnow embryo-larval stages to triclopyr (tetraethylamine salt) for 31 days and observed decreased larval survival (LC_{50}= 245 mg/L, static exposure; 120 mg/L, flow-through exposure); however, no treatment-related effects were observed on hatching time, hatchability of embryos, or on morphology or growth of larvae.

5.2.3. Hexazinone

Overview – As with triclopyr, hexazinone has not undergone evaluation for its potential to interact or interfere with the estrogen, androgen, or thyroid hormone systems (i.e., assessments on hormone availability, hormone receptor binding or postreceptor processing). Again, however, extensive testing in experimental animals provides reasonably strong evidence against hexazinone being an endocrine disruptor.

Epidemiological studies of health outcomes of hexazinone have not been reported, nor is there clinical case literature on human hexazinone intoxication. Nonetheless, several long-term experimental studies in dogs, mice, and rats have examined the effects of exposure to hexazinone on endocrine organ morphology, reproductive organ morphology, and reproductive function; treatment-related effects on these endpoints were not observed. In addition, hexazinone did not produce morphological abnormalities in frog embryos at exposures below the LC_{50}.

Human Data – As with triclopyr, no studies involving humans and related to endocrine disruption have been reported in the literature. Thus, all inferences regarding the potential risks to humans must based on studies in experimental mammals.

Experimental Mammals – Also as with triclopyr, hexazinone has not been tested for activity as an agonist or antagonist of the major hormone systems (e.g., estrogen, androgen, thyroid hormone) and all inferences concerning the potential effect of hexazinone on endocrine function must be based on inferences from standard toxicity studies.

The effects of hexazinone on reproduction have been examined in experimental studies in rats, including two multigeneration studies (Kennedy and Kaplan, 1984; SERA, 1997; U.S. EPA, 1994). In a 3-generation study in rats, dietary exposures (200, 1,000, or 2,500 ppm) to hexazinone had no effect on fertility or other reproductive parameters (Kennedy and Kaplan, 1984). Growth rates of pups from the F_2 and F_3 generations of the 2,500 ppm exposure group were lower than controls; no other treatment-related abnormalities in the pups or adults were observed. In a 2-generation dietary study in rats (0, 200, 2,000, or 5,000 ppm in diet), exposure to 2,000 or 5,000 ppm hexazinone decreased body weight gain in P_1 and F_1 females during gestation and growth; decreased pup weights of F_1 and F_2 generations; exposure to 5,000 ppm resulted in decreased pup survival of F_2 pups (Mebus, 1991).

Several studies have explored the effects of gestational exposures on fertility and fetal development in rodents. In one study conducted in rats, exposures to 400 or 900 ppm (but not 40 or 100 ppm) hexazinone during gestation resulted in kidney and bone abnormalities in pups. In a second rat study, dietary exposures to 200, 1,000 or 5,000 ppm had no effect on reproductive success, and no treatment-related fetal abnormalities were observed. In rabbits, exposures to 125 mg/kg/day (but not 20 or 50 mg/kg/day) resulted in decreased fetal weight and skeletal abnormalities in pups (delayed ossification).

Subchronic and chronic studies of hexazinone have been conducted in dogs, mice, rabbits, and rats (Kennedy and Kaplan, 1984). These studies included histopathological evaluations of the reproductive organs and major endocrine glands, including adrenal, pancreas, parathyroid, pituitary, and thyroid. No treatment-related abnormalities in these tissues were found.

Wildlife – Hexazinone has been tested for its toxicity to frog embryos and tadpoles (Berrill et al., 1994). Exposure of leopard frog embryos to 100 mg/L for 8 days had no effect on hatching success or embryo morphology and, after hatching, the exposed tadpoles were of similar size to that from control embryos, and exhibited normal avoidance behavior (movement away from prodding). As summarized in SERA (1997), comparable concentrations of hexazinone over

shorter periods of exposure have been associated with mortality in fish and aquatic invertebrates. Thus, based on the limited available data, amphibians do appear to be less sensitive than fish or aquatic invertebrates to hexazinone and the study by Berrill et al. (1994) provides no evidence that hexazinone interferes with endocrine function in amphibians.

6. REFERENCES

Acquavella JF; Weber JA; Cullen MR; Cruz OA; Marten MA; Holden LR; Riordan S; Thompson M; Farmer D. 1999. Human ocular effects from self-reported exposure to Roundup® herbicides. Human Exp. Toxicol. 18: 479-486.

Arbuckle TE; Sever LE. 1998. Pesticide exposure and fetal death: A review of the epidemiologic literature. Crit. Rev. Toxicol. 28: 229-270.

Arbuckle TE; Lin Z; Mery LS. 2001. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an Ontario farm population. Environ. Health Perspect. 109: 851-857.

ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Toxicological Profile for Fuel Oils. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA. Available from NTIS. PB95-264222.

Auletta CS. 1983. A dermal sensitization study in guinea pigs. Unpublished report. Bio/Dynamics Inc. East Millstone NJ. (Cited in Williams et al., 2000).

Barbosa ER; Leiros da Costa MD; Bacheschi LA; Scaff M; Leite CC. 2001. Parkinsonism after glycine-derivate exposure. Mov. Disord. 16: 565-585.

Barna-Lloyd, T; et al. 1992. Triclopyr butoxyethyl ester (Triclopyr BEE): Subchronic dietary toxicity study in Fisher 344 rats. MRID No. 42274901, HED Doc. No. 009533. (Cited in SERA, 1995)

Batt BDJ; Black JA; Cowan WF. 1980. The effects of glyphosate herbicide on chicken egg hatchability. Can. J. Zool. 58: 1940-1942.

Berrill M; Bertram S; McGillivray L; Kolohon M; Pauli B. 1994. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. Environ. Toxicol. Chem. 13: 657-664.

Bigazzi, PE. 1992. Lessons from animal models: The scope of mercury-induced autoimmunity. Clin. Immunol. Immunopathol. 65: 81-84.

Blakley BR. 1997. Effect of Roundup and Tordon 202C herbicides and antibody production in mice. Vet. Hum. Toxicol. 39: 204-206.

Blaszcak DL. 1987. A dermal sensitization study in guinea pigs. Unpublished report. Bio/Dynamics Inc. East Millstone NJ. (Cited in Williams et al., 2000).

Burgat V; Keck G; Guerre P; Bigorre V; Pineau X. 1998. Glyphosate toxicosis in domestic animals: A survey from the data of the Centre National d'Informations Toxicologiques Veterinaires (CNITV). Vet. Hum. Toxicol. 40: 363-367.

Burns LA; Meade BJ; Munson AE. 1996. Toxic response of the immune system. In: Klaassen CD, ed. Casarett and Doull's Toxicology. The Basic Science of Poisons. New York: McGraw-Hill. pp. 355-402.

Chang C-Y; Peng Y-C; Hu W-H; Yang D-Y; Lin T-J. 1999. Clinical impact of upper gastrointestinal tract injuries in glyphosate-surfactant oral intoxication. Human Exp. Toxicol. 18: 475-478.

Connor K, Ramamootrhy K, Moore M, Mustain, M, Chen, I, Safe, S, Zacharewski, T, Gillesby, B, Joyeux, A, Balaguer, P. 1997. Hydroxylated polychlorinated biphenyls (PCBs) as estrogens and antiestrogens: Structure-activity relationships. Toxicol Appl Pharmacol 145:111-123.

Connor K, Safe S, Jefcoate CR, Larsen, M. 1995. Structure-dependent induction of CYP2B by polychlorinated biphenyl congeners in female Sprague-Dawley rats. Biochem Pharmacol 50(11):1913-1920.

Cooper RL; Goldman JM; Rehnberg GL; McElroy WK; Hein JF. 1987. Effects of metal cations on pituitary hormone secretions in vivo. J. Biochem. Toxicol. 2: 241-249.

Coyle JT; Schwarcz R. 1976. Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. Nature 263: 244-246.

Curran PG; DeGroot LJ. 1991. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocrine Rev 12(2): 135-150

Curtis KM; Savitz DA; Weinberg CR; Arbuckle TE. 1999. The effect of pesticide exposure on time to pregnancy. Epidemiology. 10: 112-117.

Davies TF. 2000. Graves' disease. In: Braverman LE; Utiger RD, eds. Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text. Philadelphia, PA: Lippincott-Raven. pp. 518-531.

Dawson R Jr; Beal MF; Bondy SC; DuMonte DA. 1995. Excitoxins, aging and environmental neurotoxins: implications for understanding human neurodegenerative disease. Toxciol. Appl. Pharmacol. 134: 1-17.

Denman, A.M. (1983). Viruses and immunopathology. *In Immunology in Medicine*. Edited by E.J. Holborrow & W.G. Reeves. Grune and Stratton.

Diasio RB; LoBuglio AF. 1996. Immunomodulator: Immunospressive agnets and immunostimulants. In: Hardman JG; Limbird LE, eds. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Ninth Edition. New York: McGraw-Hill. pp. 1291-1308.

Dickson, SJ; Meinhold, RH; Beer, ID; Koelmeyer, TD. 1988. Rapid determination of glyphosate in postmortem specimens using 31P. J. Analyt. Toxicol. 12(5): 284-6.

Dourson ML; Stara JF. 1983. Regulatory history and experimental support of uncertainty (safety) factors. Reg. Toxicol. Pharmacol. 3: 224-238.

Dunn, FL; Hoover, WE; Keyes, DG. 1980. Sponsor validation of a two-year chronic oral toxicity study of DOWCO 233 in rats performed by Industrial Bio-Test laboratories, Inc. Unpublished report. Toxicology Research Laboratory, Health and Environmental Sciences Laboratory, Dow Chemical Company. (Cited in SERA, 1995)

Durkin, PR; Stiteler, WS; Dourson, ML; Knauf, L. 1992. Categorical Regression of Toxicity Data: A Case Study Using Aldicarb. Presented at the International Congress on the Health Effects of Hazardous Waste. Sponsored by U.S. Department of Health and Human Services, Public Health Service, May 3-6.

EDSTAC. 1998. Endocrine Disruptor Screening and Testing Advisory Committee Final Report. August, 1998. http://www.epa.gov/oscpmont/oscpendo/history/finalrpt.htm

Eisenbrandt, DL; et al. 1987. Triclopyr: 2-year dietary chronic toxicity-oncogenicity study in Fischer 344 rats. MRID No. 40107701, 41200302, 92189021, 921890221, HED Doc. No. 006683, 088378. (Cited in SERA, 1995)

El-Gendy KS; Aly, NM; El-Sebae AH. 1998. Effects of edifenphos and glyphosate on the immune response and protein biosynthesis of bolti fish (*Tilapia nilotica*). J. Environ. Sci. Health B. 33: 135-149.

Flaherty, DK; Gross, CJ; McGarity, KL; Winzenburger, PA; Wratten, SJ. 1991. The effect of agricultural herbicides on the function of human immunocompetent cells. II. Effect on natural killer cell and cytotoxic T cell function. In Vitr. Toxicol. 4(2): 145-160.

Folmar LC; Sanders HO; Julin AM. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch. Environ. Contam. Toxicol. 8: 269-278.

Fonnum F. 1997. Excitotoxicity in the brain. Arch. Toxicol. 20 (Suppl): 386-395.

Fonnum F. 1999. Neurotoxicology. In: Ballantyne G; Mars T; Syversen T, eds. General and Applied Toxicology. United Kingdom: MacMillan Reference Ltd. pp. 631-647.

Gilman AG. 1987. G proteins: transducers of receptor-generated signals. Ann. Rev. Biochem. 56: 615-649.

Goldman JM; Stoker TE; Cooper RL; McElroy WK; Hein JF. 1994. Blockade of ovulation in the rat by the fungicide sodium N-methyldithiocarbamate: relationship between effects on the

luteinizing hormone surge and alterations in hypothalamic catecholamines. Neurotoxicol. Teratol. 16: 257-268.

Goldstein DA; Johnson G; Farmer DR; Marten MA; Ford JE; Cullen MR. 1999. Pneumonitis and herbicide exposure. Chest. 116: 1139-1140.

Hanley, TR; et al. 1976. Three-generation study in rats; DOWCO 233. MRID No. 400057084, 00137618, HED Doc. No. 000000. (Cited in SERA, 1995).

Hanley, TR: Murray, JS; Cobel-Geard, SR; Hayes, WC; John, JA; Rao, KS. 1980. DOWCO 233: Dominant lethal study in mice. Unpublished report. Toxicology Research Laboratory, Health and Environmental Sciences, Dow Chemical Company. (Cited in SERA, 1995)

Hanley, TR Jr; Thompson, DJ; Palmer, AK; Beliles, RP; Schwetz, BA. 1984. Teratology and reproduction studies with triclopyr in the rat and rabbit. Fund. Appl. Toxicol. 4: 872-82.

Hardman JG; Limbird LE. 1996. Goodman & Gilman's The Pharmacological Basis of Therapeutics. Ninth Edition. McGraw-Hill, New York.

Hertzberg, RC. 1989. Extrapolation and scaling of animal data to humans: Fitting a model to categorical response data with application to species extrapolation of toxicity. Health Physics. 57(Sup 1): 405-409.

Hirsch KS; Weaver DE; Black LJ; Falcone JF; MacLusky NJ. 1987. Inhibition of central nervous system aromatase activity a mechanism for fenarimol-induced infertility in the male rat. Toxicol Appl. Pharmacol. 91: 235-245.

Hoffman DJ; Albers PH. 1984. Evaluation of potential embryotoxicity and teratogenicity of 42 herbicides, insecticides, and petroleum contaminants to mallard eggs. Arch. Environ. Contam. Toxicol. 13: 15-27.

Hollmann M; Heinemann S. 1994. Cloned glutamate receptors. Ann. Rev. Neurosci. 17: 31-108.

Humiston, CG; Schwetz, BA; Quast, JF. 1975. 3,5,6-trichloro-2-pyridyloxyaxetic acid (DOWCO 233 herbicide): 90-Day dietary feeding study in rats. Unpublished reports. Toxicology Research Laboratory, Dow Chemical Company. (Cited in SERA, 1995)

Hung D-Z; Deng J-F; Wu T-C. 1997. Laryngeal survey in glyphosate intoxication: a pathophysiological investigation. Human Exp. Toxicol. 16: 596-599.

Jauhiainen A; Räsänen K; Sarantila R; Nuutinen J; Kangas J. 1991. Occupational exposure of forest workers to glyphosate during brush saw spraying work. Am. Ind. Hyg. Assoc. J. 52: 61-64.

Johansen JA; Green GH. 1990. Sublethal and acute toxicity of the ethylene glycol butyl ether ester formulation of triclopyr to juvenile coho salmon (*Oncorhynchus kisutch*). Arch. Environ. Contam. Toxicol. 19: 610-616.

Johnson JW; Ascher P. 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature. 325: 529-531.

Jones EG. 1988. The nervous system. In: Weis L, ed. Cell and Tissue Biology, Baltimore: Urban & Schwarzenberg. p. 306.

Juntunen J; Linnoila I; Haltia M. 1977. Histochemical and electron microscopic observations on the myoneural junction of rats with carbon disulfide polyneuropathy. Scand. J. Work Environ. Health. 3: 36-42.

Kanthasamy AG; Kanthasamy A; Matsumoto RR; Vu TQ; Truong DD. 1997. Neuroprotective effects of the strychnine-insensitive glycine site NMDA antagonist (R)-HA-966 in an experimental model of Parkinson's disease. Brain Res. 759: 1-8.

Karcz KM; Lorenz B; Danysz W. 1999. Glycine B antagonists and partial agonists in rodent models of Parkinson's disease, comparison with uncompetitive N-methyl-D-aspartate receptor antagonist. Neuropharmacology 38:101-119.

Kavlock RJ; Daston GP; DeRosa C; Fenner-Crisp P; Gray LE Jr.; Kaattari A; Lucier G; Luster M; Mac MJ; Maczka C; Miller R; Moore J; Rolland R; Scott G; Sheehan DM; Sinks T; Tilson HA. 1996. Research needs for risk assessment of health and environmental effects of endocrine disruptors. A review of the U.S. EPA-sponsored workshop. Environ. Health Perspect. 104: 715-740.

Kelce WR; Monosson E; Gamcsik MP; Laws SC; Gray LE Jr. 1994. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antoandrogenic metabolites. Toxicol. Appl. Pharmacol. 126: 276-285.

Kelce WR; Stone CR; Laws SC; Gray LE Jr; Kemppainen JA; Wilson EM. 1995. Persistent DDT metabolite p,p'DDE is a potent androgen receptor antagonists. Nature 375: 581-585.

Kennedy GL. 1984. Acute and environmental toxicity studies with hexazinone. Fund. Appl. Toxicol. 4: 603-611.

Kennedy GL; Kaplan AM. 1984. Chronic toxicity, reproductive, and teratogenic studies of hexazinone. Fund. Appl. Toxicol. 4: 960-971.

Klaassen CD; Amdur MO; Doull J. 1996. Casarett and Doull's Toxicology: The Basic Science of Poisons. Fifth Edition. McGraw-Hill, New York, New York. 1111 pp.

Krasavage WJ; O'Donoghue JL; De Vencenzo GD; Terhaar CJ. 1980. The relative neurotoxicity of MEBK, n-hexane and their metabolites. Toxicol. Appl. Pharmacol. 52: 433-441.

Landry, T; Kastl, PE; Gushow, TS. 1984. Triclopyr: 13-week dietary toxicity study in Fisher 344 rats. MRID No. 0000150378, HED Doc. No. 005667. (Cited in SERA, 1995)

Larsen SB; Giwercman A; Spano M; Bonde JP; The ASCLEPIOS Study Group. 1998a. A longitudinal study of semen quality in pesticide spraying Danish farmers. Reprod. Toxicol. 12: 581-589.

Larsen SB; Joffe M; Bonde JP; The ASCLEPIOS Study Group. 1998b. Time to pregnancy and exposure to pesticides in Danish farmers. Occup. Environ. Med. 55: 278-283.

LeBel CP and Bondy SC. 1991. Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol. Teratol. 13: 341-346.

Lee H-L; Chen K-W; Chi C-H; Huang J-J; Tsai L-M. 2000. Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: A review of 131 cases. Academic Emergency Medicine. 7: 906-910.

Leveridge YR. 1998. Pesticide poisoning in Costa Rica during 1996. Vet. Hum. Toxicol. 40: 42-44.

Lin C-M; Lai C-P; Fang T-C; Lin C-L. 1999. Cardiogenic shock in a patient with glyphosate-surfactant poisoning. J. Formosan Med. Assoc. 98: 698-700.

Lin N; Garry VF. 2000. In vitro studies of cellular and molecular developmental toxicity of adjuvants, herbicides, and fungicides commonly used in Red River Valley, Minnesota. J. Toxicol. Environ. Health. Part A 60: 423-439.

Luster MI; Munson AE; Thomas PT; Holsapple MP; Fenters JD; White KL Jr; Lauer LD; Germolec DR; Rosenthal GJ; Dean JH. 1988. Development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. Fundam. Appl. Toxicol. 10: 2-19.

Luster MI; Portier C; Gayla Pait DG; White KL; Gennings C; Munson AE; Rosenthal GJ. 1992. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immun tests. Fund. Appl. Toxicol. 18:200-210.

Luster MI; Portier C; Gayla Pait DG; Rosenthal GJ; Germolec DR; Corsini E; Blaylock BL; Polloca P; Kouchi Y; Craig W; White KL; Munson AE; Comment CE. 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. Fund. Appl. Toxicol. 21:71-82.

Maibach HI. 1986. Irritation, sensitization, photoirritation and photosensitization with a glyphosate herbicide. Cont. Dermat. 15(3): 152-156.

Mann RM; Bidwell JR. 1999. The toxicity of glyphosate and several glyphosate formulations for four species of southwestern Australian frogs. Arch. Environ. Contam. Toxicol. 36: 193-199.

Mayes MA; Dill DC; Bodner KM; Mendoza CG. 1984. Triclopyr triethylamine salt toxicity to life stages of the fathead minnow (*Pimephales promelas* Rafinesque. Bull. Environ. Contam. Toxicol. 33: 339-347.

McCullagh, P. 1980. Regression models for ordinal data. J. Roy. Statist. Soc. B. 42:109-142.
Mebus C. 1991. Reproductive and fertility effects with IN A3674207 multigeneration reproduction study in rats: Lab Project No. 404-91: 8873-001. Unpublished study prepared by E.I. du Pont de Nemours & Co., Haskell Lab. MRID No. 42066501. 1218 pp. (Cited in SERA, 1997)

Menkes DB; Temple WA; Edwards IR. 1991. Intentional self-poisoning with glyphosate-containing herbicides. Human Exp. Toxicol. 10: 103-107.

Meirer CA; Burger AG. 2000. Effects of drugs and other substances on thyroid hormone synthesis and metabolism. In: Braverman LE; Utiger RD, eds. Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text. Philadelphia, PA: Lippincott-Raven. pp. 265-280.

Miller K; Meredith C. 1999. Immunotoxicology. In: Ballantyne G; Mars T; Syversen T, eds. General and Applied Toxicology. United Kingdom: MacMillan Reference Ltd. pp. 997-1015.

Molello, JA; Gerbig, CG; Barnard, SD. 1976. Results of 28-day test in rhesus monkeys treated daily via nasogastric intubation with DOWCO 233. Unpublished report. Department of Pathology and Toxicology, Dow Chemical Company. (Cited in SERA, 1995)

Molello, JA; Ayers, KM; Strebing, RJ; Starrett, MG; Ehalt, WL; Cheng, W. 1979. Results of a carcinogenic study in mice on dietary treatment with DOWCO 233 (AGR134832) for two years. Unpublished report. Department of Toxicology, Dow Chemical Company. (Cited in SERA, 1995)

Morgan JD; Vigers GA; Farrell AP; Jazz DM; Mandible JF. 1991. Acute avoidance reactions and behavioral responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to Garlon 4®, Garlon 3A® and Vision® herbicides. Environ. Toxicol. Chem. 10: 73-79.

Monsanto Co. 1982. Material safety data sheet, glyphosate. Unpublished report. Monsanto Company, St. Louis, MO. (Cited in SERA, 1996)

Narahashi T. 1992. Nerve membrane Na^+ channels as targets of insecticides. Trends Pharmacol. Sci. 13: 236-241.

NAS. 1999. Hormonally active agents in the environment. National Academy Press. Washington DC.

Newell DW; Barth A; Ricciardi TN; Malouf AT. 1997. Glycine causes increased excitability and neurotoxicity by activation of NMDA receptors in hippocampus. Exp. Neurol. 145:235-244.

NTP (National Toxicology Program). 1992. Technical report of toxicity studies of glyphosate. National Toxicology Program. Toxicity Reports Series No. 16.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. National Research Council, National Academy Press, Washington, DC.

O'Donoghue JL. 1994. Defining what is neurotoxic. In: Weiss B; O'Donoghue J, eds. Neurobehavioral Toxicity: Analysis and Interpretation. New York, NY: Raven Press. pp. 19-33.

O'Donoghue JL. 1996. Clinical neurologic indices of toxicity in animals. Environ. Health Perspect. 104: 323-330.

Olorunsogo OO; Bababunmi EA; Bassir, O. 1979a. Effect of glyphosate on rat liver mitochondria in vivo.. Bull. Environ. Contam. Toxicol. 22(3): 357-64.

Olorunsogo OO; Bababunmi EA; Bassir O. 1979b. The inhibitory effect of N-(phosphonomethyl)-glycine *in vivo* on energy-dependent, phosphate-induced swelling of isolated rat liver mitochondria. Toxicol. Lett. 4: 303-306.

Perkins PJ; Boermans HJ; Stephenson GR. 2000. Toxicity of glyphosate and triclopyr using the frog embryo teratogenesis assay - *Xenopus*. Environ. Toxicol. Chem. 19: 940-945.

Petit F; LeGoff P; Cravedi J-P; Valotaire Y; Pakdel F. 1997. Two complementary bioassays for screening the estrogenic potency of xenobiotics: recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. J. Molecular Endocrinology. 19: 321-335.

Pushnoy LA; Avnon LS; Carel RS. 1998. Herbicide (Roundup) pneumonitis. Chest. 114: 1769-1771.

Quast, JF; et al. 1976. Dowco 233 Herbicide: Subchronic dietary feeding study in beagle dogs. MRID No. 00071793, HED Doc. No. 001838. (Cited in SERA, 1995).

Quast, JF; et al. 1977. Dowco 233 Herbicide: Supplemental subchronic dietary feeding study in beagle dogs. MRID No. 00071794, HED Doc. No. 001838. (Cited in SERA, 1995).

Quast, JF; et al. 1988. Triclopyr: A one-year dietary toxicity study in beagle dogs MRID No. 41200301, 92189023, HED Doc. No. 008593. (Cited in SERA, 1995).

Reyna MS. 1990. Two generation reproduction feeding study with glyphosate in Sprague-Dawley rats. Unpublished report. Monsanto Environmental Health Laboratory, St. Louis MO. (Cited in Williams et al., 2000).

Reyna MS; Ruecker FA. 1985. Twelve month study of glyphosate administered by gelatin capsule to beagle dogs. Unpublished report. Monsanto Environmental Health Laboratory, St. Louis MO. (Cited in Williams et al., 2000).

Rodwell, D.E.; Tasker, E.J.; Blair, M.; et al. (1980) Teratology Study in Rabbits: IRDC No. 401-056. (Unpublished study received May 23, 1980 under 524-308; prepared by International Research and Development Corp., submitted by Monsanto Co., Washington, D.C.; CDL:242516-B). Summarized in U.S. EPA (1993a).

Sabri MI; Spencer PS. 1990. Acrylamide impairs fast and slow axonal transport in rat optic system. Neurochem. Res. 15: 603-608.

Safe S. 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-p-dioxins and dibenzofurans. Ann. Rev. Pharmacol. Toxicol. 36:371-399.

Safe S; Astroff B; Harris B; Zacharewski T; Dickerson R; Romkes M; Biegel L. 1991. 2,3,7,8,-Tetrachlorodibenzo-p-dioxin (TCDD) and related compounds as antiestrogens; characterization and mechanisms of action. Pharmacol. Toxicol. 69: 400-409.

Sarafian T and Verity MA. 1991. Oxidative mechanisms underlying methyl mercury neurotoxicity. Int. J. Dev. Neurosci. 9: 147-183.

Savitz DA; Arbuckle T; Kaczor D; Curtis KM. 1997. Male pesticide exposure and pregnancy outcome. Am. J. Epidemiol. 146: 1025-1036.

Sawada, Y; Nagai, Y; Ueyama, M; Yamamoto, I. 1988. Probable toxicity of surface-active agent in commercial herbicide glyphosate [letter]. Lancet. 1(8580): 299.

Schiffman SS; Suggs MS; Abou Donia MB; Erickson RP; Nagle HT. 1995. Environmental pollutants alter taste responses in the gerbil. Pharmacol. Biochem. Behav. 52: 189-194.

Schneider Jr PW; Kaplan AM. 1983. Toxicological Information on Hexazinone. DuPont, Haskell Laboratory. October 12. (Cited in SERA, 1997)

Schroeder CA. 1981. A Three-generation reproduction study with glyphosate in rats. Unpublished report. Unpublished report. Bio/Dynamics, Inc. East Millsonte NJ. (Cited in Williams et al., 2000)

SERA (Syracuse Environmental Research Associates). 1995. Selected commercial formulations of triclopyr - Garlon 3a and Garlon 4. Risk Assessment Final Report. Syracuse Environmental Research Associates. SERA TR 95-22-02-02a.

SERA (Syracuse Environmental Research Associates). 1996. Selected commercial formulations of glyphosate - Accord, Rodeo, Roundup and Roundup Pro. Risk Assessment Final Report. Syracuse Environmental Research Associates. SERA TR 96-22-02-01c

SERA (Syracuse Environmental Research Associates). 1997. Selected commercial formulations of hexazinone - Human Health and Ecological Risk Assessment. Final Draft. Syracuse Environmental Research Associates. SERA TR 95-21-04-01c

SERA (Syracuse Environmental Research Associates). 2001a. Glyphosate - Worksheets for Human Health and Ecological Risk Assessments. SERA WPWS 01-43-07-03a. November 23, 2001.

SERA (Syracuse Environmental Research Associates). 2001b. Triclopyr Acid (Garlon 3A) - Worksheets for Human Health and Ecological Risk Assessments. SERA WPWS 01-43-07-04a-1. November 23, 2001.

SERA (Syracuse Environmental Research Associates). 2001c. Triclopyr-BEE (Garlon 4) - Worksheets for Human Health and Ecological Risk Assessments. SERA WPWS 01-43-07-04a-2. November 23, 2001.

SERA (Syracuse Environmental Research Associates). 2001d. Hexazinone - Worksheets for Human Health and Ecological Risk Assessments. SERA WPWS 01-43-07-02b. November 27, 2001.

SERA (Syracuse Environmental Research Associates). 2001e. Documentation for Worksheets Version 2.03 - Human Health and Ecological Risk Assessments. SERA WSD 01-2.03. October 31, 2001.

Shelanski MV. 1973. Roundup herbicide: Repeated insult patch test in humans. Unpublished report, Shelanski Holding Company, Consbohocken, PA. (Cited in Williams, 2000).

Sieghart LL. 1992. GABA A receptors: ligand gated Cl^- channels modulated by multiple drug binding sites. TIPS 13: 446-480.

Sierra-Santoyo A; Hernandez M, Albores A; Cebrian ME. 2000. Sex-dependent regulation of hepatic cytochrome P-450 by DDT. Toxicological Sci. 54:81-87.

Simpson LL. 1990. The study of clostridial and related toxins -- the search for the common denominator. J. Physiol. (Paris). 84: 446-480.

Smith EA; Oehme FW. 1992. The biological activity of glyphosate to plants and animals: a literature review. Vet. Hum. Toxicol. 34(6): 531-543.

Soderlund DM. 1995. Mode of action of pyrethrins and pyrethroids. In: Casida JE; Quistad GB, eds. Pyrethrum flowers: Production, chemistry, toxicology, and uses. New York, NY: Oxford University Press. pp. 217-233.

Sorensen FW; Gregersen M. 1999. Rapid lethal intoxication caused by the herbicide glyphosate-trimesium (Touchdown). Human Exp. Toxicol. 18: 735-737.

Spencer PS; Ross SM; Nunn PB; Seelig M. 1987. Detection and characterization of plant-derived amino acid motor system toxins in mouse CNS cultures. Prog. Clin. Biol. Res. 253: 349-361.

Spencer JR; Edmiston S; Cowan C; Orr MK; Hernandez BZ; Schneider FA; Sanborn JR; Fredrickson S. 1996. Exposure of Hand Applicators to granular hexazinone in forest settings, 1993-1996. Health and Safety Report HS-1750, Draft Final Report dated 11/04/96, Worker Health and Safety Branch, California Environmental Protection Agency, Department of Pesticide Regulation, 1020 N Street, Room 200, Sacramento, CA 95814. Project 9303, 46 pp. (Summarized in SERA 1997).

Stewart BG; Zorumski CF; Price MT; Olney JW. 1990. Domic acid: a dementia-inducing excitotoxic food poison with kainic acid receptor specificity. Exp. Neurol. 110: 127-138.

Stout, LD; Ruecker, FA. 1990. Combined chronic toxicity/carcinogenicity-rats. MSL-10495. EPA MRID No: 416438-01 (Volumes 1-6). pp. 1-46. (Cited in SERA, 1996).

Talbot AR; Shiaw —H; Huang J-S; Yang S-F; Goo T-S; Wang S-H; Chen C-L; Sanford TR. 1991. Acute poisoning with a glyphosate-surfactant herbicide ('Roundup'): A review of 93 cases. Human Exp. Toxicol. 10: 1-8.

Tasker EJ. 1980a. Teratology study in rats. Unpublished report. International Research and Development Corporation. Mattawan, MI. (Cited in Williams et al., 2000).

Tasker EJ. 1980b. Teratology study in rabbits. Unpublished report. International research and Development Corporation. Mattawan, MI. (Cited in Williams et al., 2000).

Taurog A. 2000. Hormone synthesis: Thyroid iodine metabolism. In: Braverman LE; Utiger RD, eds. Werner and Ingbar's the thyroid: A fundamental and clinical text. 8th ed. Philadelphia, PA: Lippincott-Williams and Wilkins. pp. 61-84.

Temple WA; Smith NA. 1992. Glyphosate herbicide poisoning experience in New Zealand. N.Z. Med. J. 105: 173-174.

Tominack RL; Yang G-Y; Tsai W-J; Chung H-M; Deng J-F. 1991. Taiwan National Poison Center survey of glyphosate - surfactant herbicide ingestions. Clin. Toxicol. 29: 91-109.

Tryphonas H., Feeley M. (2001). Polychlorinated biphenyl-induced immunomodulation and human health effects. In: *PCBs Recent Advances in the Environmental Toxicology and Health Effects*. Edited by L. Robertson and L.G. Hansen. Lexington, KY: University Press of Kentucky, p.193-209.

Tsuda, S; et al. 1992. Triclopyr: 22-Month oral chronic toxicity and oncogenicity study in mice. MRID No. 40356601, 92189021, 92189022, HED Doc. No. 006683, 008378. Classification: Core-minimum data according to the data evaluation record. (Cited in SERA, 1995)

U.S. EPA. 1993a. Reregistration Eligibility Decision (RED): Glyphosate. EPA 738-R-93-014. September 1993. Available at: http:/www.epa.gov/pesticides/reregistration/status.htm.

U.S. EPA. 1993b. Glyphosate: Integrated Risk Information System (IRIS). Available at: www.epa.gov/iris.

U.S. EPA. 1994. Reregistration Eligibility Decision (RED): Hexazinone. EPA 738-R-94-022. September 1994. Available at: http:/www.epa.gov/pesticides/reregistration/status.htm.

U.S. EPA. 1997. Special Report on Environmental Endocrine Disruption: An Effects Assessment and Analysis. Risk Assessment Forum. U.S. Environmental Protection Agency. EPA/630/R-96/012.

U.S. EPA. 1998. Reregistration Eligibility Decision (RED): Triclopyr. EPA 738-R-98-011 October 1998. Available at: http:/www.epa.gov/pesticides/reregistration/status.htm.

U.S. EPA. 2000. Supplementary Guidance for Conduction Health Risk Assessment of Chemical Mixtures. EAP/630/R-00/002. August, 2000.

U.S. EPA. 2001. Integrated Risk Information System. U.S. Environmental Protection Agency. www.epa.gov/iris.

van den Berg CJ; van den Velden J. 1970. the effect of methionine sulfoxamine on the incorporation of labeled glucose, acetate, phenylalanine and proline into glutamate and related amino acids in the brains of mice. J. Neurochem. 17: 98-991.

Vedula, U.; Breslin, W.; Kropscott, B.; et al. (1995) Triclopyr: Two-Generation Dietary Reproduction Study in Sprague-Dawley Rats: Lab Project Number: K-042085-048: K-042085-048P1: K-042085-048G0. Unpublished study prepared by Dow Chemical Co. 1065 p. Summarized in U.S. EPA (1998).

Visser TJ. 1990. Importance of deiodination and conjugation in the hepatic metabolism of thyroid hormone. In: Greer MA, ed. The thyroid gland. New York, NY: Raven Press, Ltd. pp. 255-283.

Walsh LP; McCormick C; Martin C; Stocco DM. 2000. Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. Environ. Health Perspect. 108: 769-776.

Weiss B. 1999. The assessment of behavioral toxicity. In: Ballantyne G; Mars T; Syversen T, eds. General and Applied Toxicology. United Kingdom: MacMillan Reference Ltd. pp. 649-673.

White R; Jobling S; Hoare SA; Sumpter JP; Parker MG. 1994. Environmentally persistent alkylphenols are estrogenic. Endocrinology 135: 175-182.

Williams GM; Kroes R; Munro IC. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul. Toxicol. Pharmacol. 31: 117-165.

Wrenn J. 1980. Dominant lethal study in mice. Unpublished report. International research and Development Corporation. Mattawan, MI. (Cited in Williams et al., 2000).

Yousef, MI; Salem, MH; Ibrahim, HZ; Helmi, S; Seehy, MA; Bertheussen, K. 1995. Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. J. Environ. Sci. Health. B30(4): 513-534.