

## **87. DELAYED TOXIC EFFECTS: EMERGING NEW CHALLENGES TO CHEMICAL BIOLOGICAL RISK ANALYSIS AND MEDICAL TREATMENT STRATEGIES**

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### **ABSTRACT**

To date, predictive toxicology of delayed effects associated with exposure to chemical and biological risk factors remains mostly in the realm of what is referred to as the "expert judgement." Noteworthy accomplishments during the past five decades, particularly in the areas of chemical carcinogenesis, neuro- and developmental toxicology have contributed to risk management decisions on chronic effects grounded on improved scientific principles. Despite progress in these areas, our understanding of chronic health and environmental consequences of exposure to toxic agents is limited, and shrouded with uncertainties. Research priorities in the crucial areas of long-term toxic effects and hazard assessment approaches are not in tandem with risk management decision process involving chemical and biological medical treatment and mitigation. New challenges such as terrorist threats and extended commitments with peace-keeping operations worldwide requires a coordinated assessment of the tools currently available for delayed toxic effects resulting from exposure to chemical and biological agents. This paper will present a conceptual chemical class-specific approach to delayed toxic effects and draw example data sets from organophosphate compounds in civilian and military uses.

### **INTRODUCTION**

The scope of delayed toxic effects of chemical and biological toxic agents of anthropogenic origin is an area of increasing focus both for the scientific community and government agencies involved in health and environmental regulation. A new dimension covering national security was introduced to the delayed toxicity analysis, which until recently was perceived as a public health and environmental problem. Although literature is replete with information on the acute and sub-chronic toxicities of chemical agents, information is scant from published human and experimental animal studies on the delayed effects of military-unique chemicals (SIPRI, 1975). Recently, the U.S. Army requested the National Research Council to perform a review and suggest methods to develop sub-chronic toxicity criteria for chemical weapon agents and degraded constituents (NRC, 2000).

Chemical-induced residual lesions, caused by acute or sub-acute exposures may result in essentially irreversible clinical manifestations (Munro, 1994). There is considerable literature both from human and experimental animal studies on the chemical-induced carcinogenesis and developmental toxicities with dose-response relationships spanning the life span following a period of exposure (Watson et al. 1989; Rao et al. 1989). Additionally, in the "real world", simultaneous or sequential exposure to low levels of multiple chemicals and biological risk factors presents additional risks due to interactive effects and leading to potentiation of delayed adverse impact.

A key distinction is that delayed toxic response is not the same as chronic adverse effects. For instance, published literature on Organophosphorus (OP) pesticides and chemical weapons (CW) agents has revealed that the lesions associated with delayed effects are dissimilar to those attributed to chronic poisoning (SIPRI, 1975). In contrast to chronic exposure-which involves continuous or intermittent exposure to low levels of a toxic agent-delayed toxicity may result from a single dose or a brief exposure producing an irreversible effect. As a result, dose, duration and exposure considerations for delayed toxicity are not comparable to those for chronic exposure.

Thus, delayed toxicity evaluation and hazard assessments in cases involving exposure to low-levels of OP-based chemical toxicants is a major challenge to design and development of rationale medical treatment strategies. As dual-use agents, OP compounds are ideal both for delayed toxicity analysis and to assess potentials for interactive effects. A collective hazard analysis of OP-induced delayed effects was considered necessary because of overlapping military and civilian applications of this class of compounds. Environmental releases due to widespread civilian uses of OP compounds and releases in the military operational environment may introduce levels hazardous to public health and general environment.

Over the past 15 years we have been involved in the analysis of interactive effects of large classes of chemical carcinogens including OP chemicals with a goal to develop better tools for hazard and risk analysis (Rao, 1991a, 199b, 1992). We have compiled large databases on the binary combination effects of chemicals and chemical groups on delayed effects such as carcinogenesis (Arcos et al. 1988, Rao et al. 1989), developed mechanistic-based tools for toxicity and hazard assessment of chemical mixtures (Rao and Unger, 1995; Rao 1991a), expert systems to elucidate

patterns in the interactive effects of chemical mixtures and delayed toxic effects such as tumor promotion (Polanksky et al. 1989; Rao et al. 1989).

This paper will outline: (a) approach in the analysis of select data sets from our studies involving a combination both of delayed toxic effects and opportunities for interactive effects, (b) development of a mechanistic-based toxicity parameters for delayed effects for OP compounds, (c) derive preliminary environmental concentrations protective of delayed toxicity endpoints, and (d) draw general conclusion on the role of chemical-class specific decision points on medical management issues.

## **MATERIALS & METHODS**

**Conceptual Premise:** Published studies have reported specific cellular or molecular lesions as the underlying mechanism for delayed toxicity demonstrated by anthropogenic chemical agents. For instance, OP-induced cellular lesions associated with delayed neurotoxicity involves, (a) inhibition of the neuropathy target esterase (NTE), a membrane-bound protein with high esterase catalytic activity, and (b) progressive aging of the inhibited NTE less amenable to catalytic reactivation (Johnson, 1990; Jokanovic and Hohnson, 1993).

OP induced delayed polyneuropathy (OPIP) is linked to the irreversible inhibition of NTE (Johnson, 1993). These effects are unrelated to the wide array of adverse acute toxicities associated with the inhibition of acetylcholinesterase. The potentials for delayed effects associated with exposure to low levels of OP are unclear, although limited epidemiological and controlled clinical trial studies have revealed transient neurological and neurobehavioral effects (Ray, 1998).

The striking similarity in the acute and delayed toxicity profiles of OP chemicals is attributed to the overlapping chemical structural and biochemical functional characteristics of this class of compounds. Notably, the toxicity profiles, including NTE-dependent delayed effects of divergent categories of OP chemicals such as insecticides, pesticides and rodenticides, and an entire class of CW agents is mediated via common molecular mechanism(s) of action. A central requirement is the need to develop toxicity parameters for hazard assessment.

*Selection of Data Sets:* Published data on OP chemicals from the pesticide groups and CW agents were compiled for a comparative analysis of the dose-duration-response relationships. OP compounds are a divergent category of chemicals with closely similar chemical structural and molecular mechanisms producing delayed toxic effects both in humans and experimental animals. Interestingly, the environmental transport and fate characteristics of OP compounds are similar. Structure-activity relationship studies have revealed interactive effects leading to potentiation of delayed toxic effects (Jokanovic and Johnson, 1993; Lotti and Moretto, 1999). Hence occupational or environmental exposures to OP compounds of divergent uses may posed risks for delayed long-term effects. .

Our comprehensive literature survey and analysis covered short-term and long-term studies on representative categories of OP pesticides and CW agents. A database on the reported short and delayed adverse effect together with dose and duration of exposure was compiled to prepare matrix of dose-duration-response relationships of various categories of OP compounds. Data both from epidemiological studies and animal experimentation were compiled and consolidated. Data on the analytical detection limits in the environmental media were collected for select OP compounds.

Dichlorvos (DV), a OP pesticide, was selected for a comparative dose-response analysis with OP CW agents because (a) DV is a halogenated OP compound like some OP CW agents, (b) have comparable physicochemical and environmental properties, (c) causes NTE-mediated delayed neurotoxicity (Sarin and Gill, 2000), and (d) DV has a well-defined reference dose for hazard and risk assessment (IRIS).

*Relative Hazard Assessment:* Short- and delayed toxicity parameters including macromolecular effects were identified, sorted and compiled for inclusion in the database. The database comprised of: No Observed adverse effects levels (NOAEL), Low observed adverse effects level (LOAEL), maximum tolerated dose (MTD), US EPA suggested reference dose (RfD) for risk analysis, and lethal doses (LD<sub>50</sub>). Effective dose (ED<sub>50</sub>) reported for biochemical, toxicological and physiological endpoints. Time course for development of toxicity was obtained either directly from the published studies or based on the experimental design (acute, sub-chronic, and chronic). By design, epidemiologic studies report sub-chronic and delayed effects, with limited information on the dose-duration and exposure relationships.

Standard exposure and risk analysis algorithms were used to calculate an estimated reference dose and environmental concentrations for OP CW agents (EPA, 1989). Based on an assumption involving exposure via ingestion and dermal contact and varying exposure duration, soil concentrations were estimated that represent intake doses for delayed effects. Note that these values may change markedly if exposure assessment is performed for

combined exposures involving OP compounds in civilian uses. Legend under Table 1 lists the exposure factors used in the algorithms.

## RESULTS AND DISCUSSION

Figure 1 reveals a considerable convergence in the post exposure developmental profile for toxic effects for the representative OP pesticides and CW compounds. The matrix for the toxicity profile indicated, (a) casual relationships between macromolecular effects and immediate and mid-term toxicities in experimental animal studies, (b) weak casual relationships for delayed toxicity both in human and experimental animal data, (c) existence of possible mechanistic linkages between macromolecular effects and toxicity, (d) irreversible nature of adverse effects, particularly with delayed responses, and (e) delayed effects manifested as a syndrome involving multiple physiological systems.

Published analyses on the elucidation of toxicity development of OP CW compounds were largely based on OP pesticide and derivatives. Likewise, delayed toxicity data such as carcinogenicity, mutagenicity and developmental toxicity are almost solely on OP pesticides and derivatives. Nevertheless, structure-activity relationship considerations and similarity in molecular mechanism of action form the basis for a meaningful comparison of delayed toxicity development between classes of OP compounds.

Figure 2 illustrates the relative magnitude of dose-response relationships between classes of OP compounds. Evidently, dose-response relationships between the classes of compounds differ by several orders of magnitude for comparable toxicity parameters. For instance, the reported estimated human lethal dose ( $LD_{50}$ ) of 0.01 mg/kg for OP CW compounds was less than the NOAEL dose of 0.05 mg/kg-day for DV in experimental animals. All published sub-lethal and lethal doses of VX in the published literature were well below the 0.05 mg/kg-day NOAEL dose of DV. In contrast, published  $ED_{50}$  -  $LD_{50}$  range for halogenated OP CW compounds such as Sarin (0.04-0.16 mg/kg) and Soman (0.004-0.13 mg/kg) were in the NOAEL-LOAEL-MTD range (0.05-16.3 mg/kg) of DV in experimental animals.

In the absence of a more scientifically defensible criteria for delayed toxic effects, RfD may be the nearest surrogate toxicity parameter to assess the relative delayed impact of very low concentrations of OP compounds on human health. Given the general lacunae of sub-chronic endpoints for OP CW agents, an alternative approach would involve extrapolation of the toxicology and medical literature of OP based compounds for delayed toxicity among populations potentially exposed to these classes of compounds either during military operations or at low levels in the general environment. A linear extrapolation of the relative differences in the scale and magnitude of threshold doses for adverse effects provided an estimate of NOAEL/LOAEL doses associated with delayed effects. However, this approach applies only for assessment of impact from exposure to individual compounds but not mixtures of OP compounds of differing origin and uses.

EPA has estimated a reference dose (RfD) of 0.0005 mg/kg-day for DV based on the study reporting decreased rat brain cholinesterase activity; NOAEL of 0.05 mg/m<sup>3</sup> based on the estimated inhalation dose (Blair et al. 1976, AMVAC, 1990). Based on a comprehensive review of the database, the overall in the RfD was rated as medium (EPA, 1993). Published LOAEL (oral) dose of 0.1 mg/kg-day for DV is 200 times the RfD (0.0005 mg/kg-day). Derivation of RfD value was based on the strengths and weakness in the principal study identified for this purpose (Blair et al 1976; AMVAC 1990).

Adopting a similar approach a tentative RfD of 0.00002 mg/kg-day for OP CW agents was estimated that is equivalent to 200 times the LOAEL (0.004 mg/kg) adjusted for averaged daily exposure. Based on a review of over a dozen studies for sub-chronic toxic effects, a recent report by Sirocco et al. (1990) was used to derive a tentative LOAEL. In this study, researchers reported neurobehavioral effects in male Wistar rats to single intra-peritoneal subtoxic doses of Soman even at the lowest single dose of 0.004 mg/kg. For the purposes of deriving a tentative RfD this value was treated as a LOAEL dose. Uncertainty factors were higher due to lack of doses below the LOAEL. Using a linear extrapolation approach, RfD values of 0.000008 mg/kg-day and 0.000004 mg/kg-day were derived that are equivalent of 500 and 1000 times the LOAEL doses are respectively.

Using standard risk analysis tools, hazard analysis was performed for combinations of intake doses in the LOAEL and RfD dose range. Table 1 is a summary of the estimated soil concentrations for different intake rates and exposure duration. Standard EPA-recommended algorithms were used to derive estimated soil concentrations for the ingestion (oral route) and topical (dermal) routes of exposure. Evidently, soil concentrations (mg/kg) are inversely related to estimated intake doses (mg/kg-day) and exposure duration. Thus, screening-level soil concentration for delayed effects was higher when exposure duration and intake rates were lower and decreased as exposure duration and/or intake rate increased.

Differences in the route of exposure on estimated soil concentration for delayed effects were significant with oral route being the most sensitive route (Table 1). Ratio of dermal/oral soil concentrations corresponding to dermal

exposure was anywhere from 1000 to 8000 with the highest ratio at the maximal exposure duration of 24 years. Strikingly, ratio of dermal/oral estimated soil concentrations were similar for all other exposure durations included in the assessment. Higher soil concentrations corresponding to dermal route reflects the uncertainties associated with this exposure pathway and assumptions used in the derivation of intake doses (Table 1, footnote).

With a growing threat of terrorism and release of chemical residues to general environment from chemical demilitarization programs, there is an increasing awareness of the far reaching environmental and legal liabilities among governmental agencies and the private sector.

The approach outlined in this paper may form the basis for a more formal assessment of the literature on delayed toxicity of OP compounds based on structure activity relationships and dose-duration-response relationships. This approach may help devise and develop medical and public health intervention strategies for OP CW compounds and breakdown products in the environment. The conceptual premise for the derivation of an RfD for oral route was based on the comprehensive database on OP CW from human and animal studies and follows the standard linearized assumption on the LOAEL and RfD dose-relationships.

However, there are several shortcomings with the approach outline here: (a) Data gaps on dose-response relationships of OP CW compound and use of a simplified assumption of linearity in the extrapolation of LOAEL doses to derive a tentative reference dose, (b) Exposure assumptions are based on exposure to single chemicals and not chemical mixtures, (c) LOAEL dose not constitute delayed toxic effect, (d) It is unclear as to how the outcome this analysis would help devise and develop the nature of medical intervention measures.

Additional studies on the available data together with a focus to consolidate the available data on OP compounds of all uses is necessary in order to develop framework for a more structured analysis.

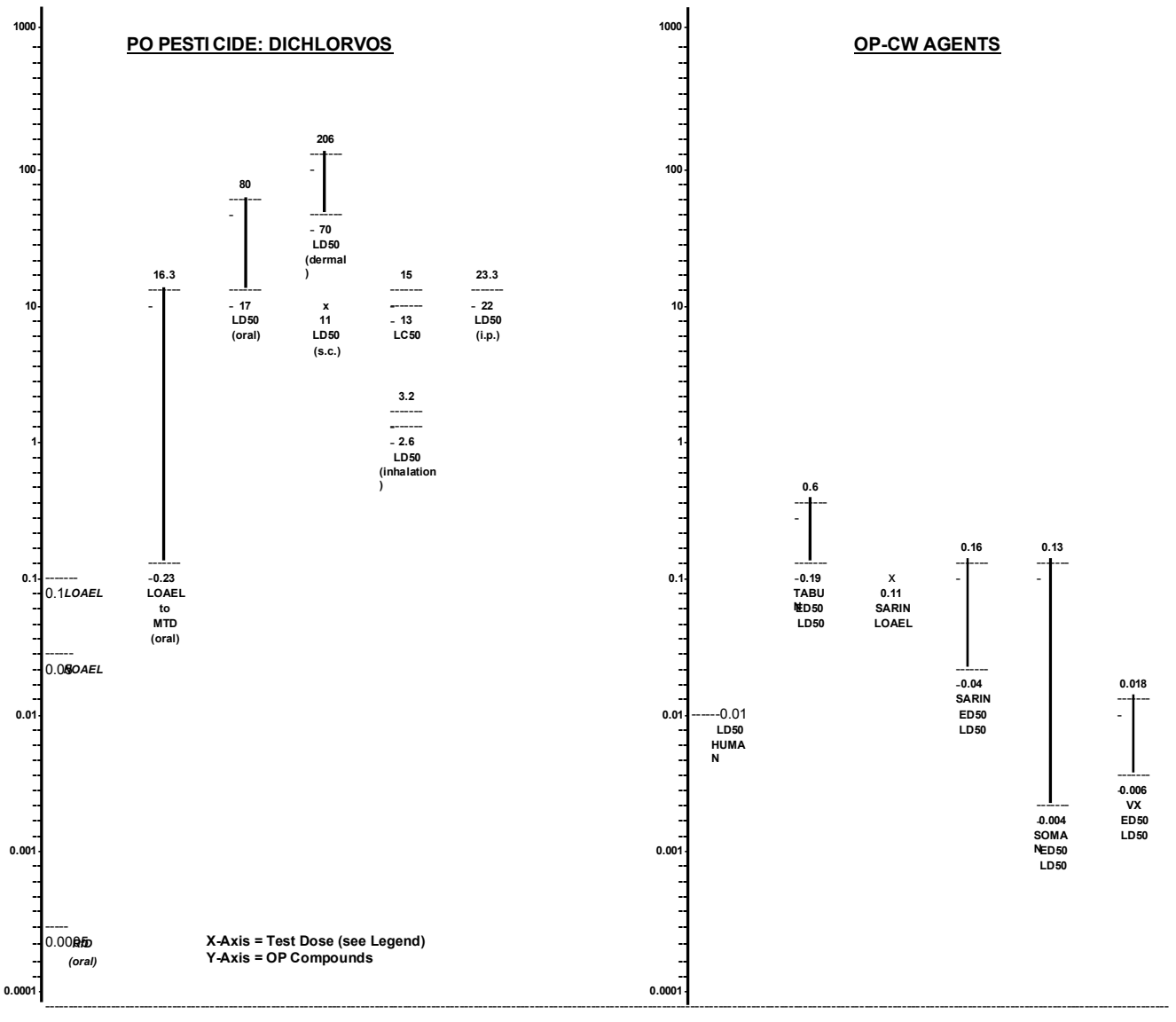
## REFERENCES

1. AMVAC Chemical Corporation. 1990. MRID No. 41593101; HED Doc. No.008178. Available from EPA. Write to FOI, EPA, Washington, DC 20460.
2. Arcos, J.C., Woo, Y.T. and Lai, D. 1988. *Env.Carcinogenesis Reviews*, (J. Environmental Sci. Health Part C), C6(1): 1-150.
3. Blair, D., Dix, K.M., Hunt, P.F., Thorpe, E., Stevenson, D.E. and Walker, A.I. 1976. *Arch. Toxicol.* 35(4):281-294.
4. Johnson, M.K. 1993. *Chem.Biol.Interact.* 87:339-346.
5. Jokanovic, M. and Johnson, M.K. 1993. *J. Biochem. Toxicol.* 8:19-31.
6. Lotti M. and Moretto, A. 1999. *Chem. Biol. Interact.* 119-120:519-524.
7. Munro, N. 1994. *Environ. Health Perspect.*, 102:18-37.
8. National Research Council (NRC). 2000. *J. Toxicol. Environ. Health*, 59:281-526.
9. Polansky G. and Woo. Y-T. 1989. *Env.Carcinogenesis Reviews*, (J. Environmental Sci. Health Part C), C7(1): 109-127, 1989.
10. Rao, V.R. and A. Unger. 1995. *Env.Carcinogenesis Reviews*, (J. Environmental Sci. Health Part C), C13:53-74.
11. Rao, V. R. 1992. *J. Pharmaceutical Sci.* 81(5): 403-407.
12. Rao, V. R. 1991a. *Toxic Sub.Journal*, 8:335-351.
13. Rao, V. R. 1991b. *J. Tox. Environ. Health*, 33: 237-248.
14. Rao, V. R., Y-t. Woo, D. Lai, and J. C. Arcos. 1989. *Env.Carcinogenesis Reviews*, (J. Environmental Sci. Health Part C), C7(2): 145-386.
15. Ray, D.E. 1998. *Toxicol. Lett.* 102-103:527-533.
16. Sarin, S. and Gill, K.D. 2000. *IUBMB Life*, 20:125-30.
17. Sirkka, U., Nieminen S.A. and Ylitalo P (1990). *Methods Find. Exp. Clin. Pharmacol.* 12:245-50.
18. U.S. EPA. 1993. *Integrated Risk Information System: Record on Dichlorvos (CASRN: 62-73-7)*, Updated: Oral RfD Assessment (11/01/1993) and Inhalation RfC Assessment (06/01/1994).
19. U.S. EPA. 1989. *Risk Assessment Guidance for Superfund. Volume 1. Human Health Evaluation Manual (Part A)*. EPA/540/1-89/002. OERR, Washington, DC.
20. Watson, A.P., Jones, T.D. and Griffin, G.D. 1989. *Reg. Toxicol. Pharmacol.*,10:1-25.

## FIGURES AND TABLES

**Figure 1:** Post Exposure Duration in the Development of OP CW Compounds-Induced Delayed Toxicity\*

Reversible (Except DNA Effects)	Partly Irreversible	Irreversible	Irreversible
Immediate Toxic Symptoms	Gradual development of symptoms	Delayed Toxicity	Delayed Toxicity
Medical Intervention Most Effective	Nongenotoxic	Genotoxic	Poor causal relationship
Better Causal Relationship	Limited causal relationship	Pronounced epigenetic effects	Complex syndromes
		Limited causal relationship	Medical intervention minimally effective
		Medical intervention minimally effective	
Inhibition of Cholinesterase (Acylation)–Acute toxicity	Hepatotoxic effects	Mutagenicity	Medical syndromes (paralysis, impotence)
Alkylation of DNA–Initiation of Delayed Toxicity	Hematologic (CVS) Effects	Carcinogenicity	Polyneuropathy (general)
Phosphorylation of biogenic amines	Central Nervous System (CNS) Effects	Developmental Effects	OPIDP
		Immunotoxicity	Cognitive Effects (neuro-behavioral)
<i>Immediate Toxicity</i>	<i>Intermediate Effects</i>	<i>Delayed Effects</i>	
<i>(1-30 days)</i>	<i>(30-300 days)</i>	<i>(&gt; 1 year)</i>	



**Figure 2:** Comparative Dose-Response Analysis for Short and Delayed Toxic Effects of OP Pesticides and CW Agents. Dose Response Pattern from Studies on Experimental Animals  
 Legend: RfD = Reference Dose; NOAEL = No Observed Adverse Effects Level; LOAEL = Lowest Observed Adverse Effects Level; LD<sub>50</sub> = Lethal Dose for 50% test population; ED<sub>50</sub> = Effective Dose for responses in 50% population; LC<sub>50</sub> = Lethal Concentration (ambient levels) for response in 50% population; MTD = Maximum Tolerated Dose; i.p. = Intraparetoneal route; s.c. = subcutaneous route.  
 Units: RfD, NOAEL, LOAEL and MTD = mg/kg-day; LD<sub>50</sub> and ED<sub>50</sub> = mg/kg; LC<sub>50</sub> = mg/m<sup>3</sup> (ambient)

**Table 1.** Intake Dose and Environmental Concentrations in the Health Hazard Assessment for Delayed Toxicities of OP-Based CW Compounds

<i>INTAKE DOSE</i> (mg/kg-day)	<i>ENVIRONMENTAL CONCENTRATIONS (mg/kg)</i>							
	<i>Ingestion Exposure Duration (years)</i>				<i>Ingestion Exposure Duration (years)</i>			
	24	5	1	0.5	24	5	1	0.5
0.0005	182	876	4,380	8,760	1.80E+06	9.00E+06	4.50E+07	9.00E+07
0.00004	15	70	350	701	15,051	72,247	3.60E+06	7.20E+06
0.00002	7	35	175	350	7,525	36,123	1.80E+06	3.60E+06
<b>0.000008</b>	<b>3</b>	<b>14</b>	<b>70</b>	<b>140</b>	<b>3,010</b>	<b>14,449</b>	<b>72,247</b>	<b>1.40E+06</b>
8E-07	<u>0</u>	<u>0</u>	<u>7</u>	<u>14</u>	30	1,444	7,224	14,449
8.00E-10	0.0003	0.001	0.007	0.014	0	0	0	0

Assumptions in the ingestion pathway: Ingestion rate = 200 mg of soil per event; Exposure frequency = 50 days per year;

Exposure duration = 24-0.5 years; Body weight = 70 kg; Averaging factor = 8,760.

Assumptions in the dermal exposure pathway: Skin surface area available for contact = 1.94 cm<sup>2</sup>/event; Soil to skin adherence factor = 0.1 (mg/m<sup>3</sup>);

Absorption factor = 100 percent; Exposure frequency = 350 days per year;

Exposure duration = 24 to 0.5 years; Body weight = 70 kg; and Averaging factor 8,760.

Exposure assumptions in the intake equations are based on conservative values used (EPA, 1989).