Chapter 5. PESTICIDE REGULATIONS: Exposure-dose modeling from FIFRA to FQPA

James B. Knaak[1], Cecilla Tan[2] and Curt C. Dary[3]

J.B. Knaak
[1]Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, SUNY at Buffalo, 3435 Main Street, Buffalo, NY 14214, USA.

C. Tan
[2]U.S. Environmental Protection Agency, National Exposure Research Laboratory, Mail Code: E205-0, Research Triangle Park, NC 27711, USA.

C.C. Dary
[3]U.S. Environmental Protection Agency, Human Exposure and Atmospheric Sciences Division, 944 E. Harmon Ave., Las Vegas, NV 89193-3478, USA.

References

Abstract

The federal laws and regulations governing the registration and use of pesticides in the United States under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) are published in the Federal Register, while state laws such as California are published in the California Food and Agricultural Code, Division 6, 7 and 13. Up until the passage of the FQPA (Food Quality Protection Act of 1996), federal and state regulations pertaining to the registration and use of pesticides were in most cases identical except for the fact that food tolerances were enforced but not set at the state level. The California Department of Pesticide Regulation's (CDPR's) Worker Health and Safety Program continues to monitor worker exposure to pesticides and report illnesses among workers associated with pesticide exposure. Under FQPA, ~~U.S.EPA~~the U.S. Environmental Protection Agency has taken a leadership role in the development of probabilistic pesticide exposure models, (i.e., DEEM, SHEDS, etc.) using pesticide application, human activity and exposure databases (i.e. CPPAES, CHAD, CSFII, FCID, NHANES and NHEXAS-~~)data bases~~. A physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling framework has been established by EPA to assess cumulative risk of dose ~~of~~ and injury ~~of~~ to infants and children to organophosphorus (OP), carbamate (NMC) and pyrethroid insecticides from aggregate sources and routes. Probabilistic models are being linked to PBPK/PD models to improve risk assessments.

Comment [VD1]: Do you use injury to imply that there are effects other than health effects, i.e. a broader concept of "harm"?

Key Words Organophosphorus insecticides, carbamate insecticides, pyrethroid insecticide, probabilistic models, physiological pharmacokinetic models, FIFRA,

FFDCA, FQPA, pesticide mixtures, parathion, chlorpyrifos, paraoxon, chlorpyrifos-oxon, acetylcholinesterase, V_{max}, K_m, urinary metabolites and risk assessment.

I. Introduction

In the United States, under the federal requirements of FIFRA, FFDCA and FQPA (1, 2, 3) agrochemical companies desiring to sell pesticides (~~i.e.,~~ e.g., active ingredients, formulated products) to agricultural users (~~i.e.,~~ e.g.,formulators, farmers, ranchers, horticulturalists~~, etc~~) for use on food crops or for non food uses must register active ingredients and formulated products with the Office of Pesticide Programs, US Environmental Protection Agency (USEPA)~~, Washington, DC~~.

In addition, following federal registration, s~~S~~tate agencies such as the California Department of Pesticide Regulation, California Environmental Protection Agency (CalEPA), ~~Sacramento, CA,~~ require companies to register products intended for sale in the state, after federal registration (4). In order to register active ingredients and formulated products, registrants must submit the results of studies according to test categories published in Pesticide Guidelines, National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161. The California and federal test requirements were published by Knaak et al. (5) as part of ~~a~~ an American Chemical Society Symposium on Effective and Safe Waste Management ~~presented at a Meeting of the American Chemical Society~~. The broad test categories are product chemistry, environmental chemistry, special chemistry, fish and wildlife toxicology, acute toxicology, chronic toxicology, special toxicology, half-life determination, and efficacy. In addition to these tests, CalEPA requires tests under SB950's (6) birth defects

Comment [VD2]: All, some, most? Don't all states now have FIFRA delegation?

prevention law. The requirements listed under special chemistry are ~~Animal animal~~ metabolism, residue tolerance clearance, residue; plant, soil and water, residue methods; plant, soil and water, and animal residue methods ~~are the requirements listed under special chemistry~~. Acute animal toxicity studies include oral, dermal, inhalation, neurotoxicity, primary eye and skin irritation, and dermal sensitization, while subchronic toxicity studies include oral, ~~21 and 90 day repeat~~ dermal ~~(21 and 90 day repeat),~~ and inhalation. Chronic studies involve oncogenicity/carcinogenicity, teratogenicity, reproductive and fertility studies. Genotoxicity studies involve mutagenicity protocols, structural chromosome aberration, gene mutation, and genotoxic effects.

Under the provisions of FIFRA and FFDCA, ~~U.S. EPA~~the USEPA is required to establish food tolerances for each pesticide residue on raw agricultural commodities intended for human consumption. The U.S. Food Drug Agency (FDA) under FFDCA (Federal Food Drug and Cosmetic Act) is required to monitor pesticide residues based on the tolerances set by ~~U.S. EPA~~the USEPA.

The environmental studies required by FIFRA include, but are not limited to, studies involving physicochemical degradation, metabolism, mobility, dissipation, and accumulation in plants, soil and water. Local municipal water companies supply water to residents after treating, purifying and testing water prior to its distribution. Water is tested for a variety of contaminants ranging from microbiological, radioactive, inorganic, organic (pesticides and herbicides) and disinfectants such as the end products of treating water with chlorine.

Comment [VD3]: This seems out of place. Are you saying this because FIFRA requires that local water suppliers conduct additional texts or that EPA's SDWA requires testing for MCLs, a large number of which are pesticides?

Efficacy studies involving the intended use of pesticides (i.e., insecticide, fungicide, nematocide, herbicide, disinfectant, sanitizer, etc) are required to support registration. These studies are reviewed by ~~U.S. EPA~~the USEPA, U.S. Department of ~~Food and~~ Agriculture, the California Department of Food and Agriculture, and other state departments of agriculture. Exposure studies involving the application of pesticides and contact with post application residues are required by the Agency.

The international marketing of food commodities has resulted in global pesticide standards being set up in the U.S. and overseas for the sale of food between countries. ~~The earliest attempt being carried out by WHO (~~The World Health Organization (WHO) and Food and Agricultural Organization ~~FAO (Food and Agricultural Organization~~FAO) ~~was~~ were the first to attempt to develop standards through the Codex Alimentarius Commission (CODEX). ~~Harmonization efforts have increased s~~Since 1993, ~~with~~ the European Union (EU) ~~have~~has increased harmonizing data requirements among member states through committees of the ~~OECD (~~Organization for Economic Cooperation and Development (OECD).

II. FIFRA

A. USDA

The U.S. Congress passed the Federal Insecticide Fungicide and Rodenticide Act (FIFRA) in 1947. The pesticide registration program under FIFRA (1947) was initially the responsibility of the United States Department of Agriculture, (USDA) ~~Washington, D.C~~. The USDA performed all the activities that are currently carried out by ~~U.S. EPA~~the USEPA, i.e., registration, tolerance setting, and reviewing the efficacy of pesticides and

approving their use in agriculture. U.S. FDA was charged with the duties of enforcing tolerances by sampling and analyzing raw agricultural products going to market under the Federal, Food, Drug and Cosmetic Act (FFDCA). All activities (i.e., tolerance setting, etc.) associated with this work were published in the Federal Register.

B. USEPA

In 1970-1971, Congress formed the USEPA and the registration and tolerance setting duties of USDA under FIFRA were transferred to the Office of Pesticide Programs (OPP), USEPA. In 1972, FIFRA was amended by the Federal Environmental Pesticide Control Act of 1972. The basic purpose of FIFRA as amended was to ensure that pesticides used in the United States perform their intended functions without causing unreasonable adverse effects on man or the environment.

FIFRA requires ~~EPA~~the USEPA to balance the risks and benefits of a pesticide before granting registration. In a typical year ~~EPA~~the USEPA reviews over 5000 registration submission (new products and new use applications for old products containing registered actives). About 20 applications for registrations of new active products are received each year. Currently, there are over 13,000 registered products containing over 400+ registered active ingredients. Data development for a major agricultural chemical may cost as much as 10 million dollars and take as long as 10 years to complete (7). Good Laboratory Practice (GLP) and quality assurance (QA) standards were implemented in a 1988 amendment to FIFRA (8).

Reference doses (RfDs) and reference concentrations (RfCs) are routinely established from NOELs determined in 30-d, 90-d or 2 yr feeding or drinking studies involving the laboratory rat. These values (RfDs and RfCs) are used to establish food tolerances and safe levels in drinking water for these pesticides along with ~~ADIs (~~acceptable daily intakes (ADIs). Pesticide residues in raw agricultural commodities (RACs) are routinely measured by the Food and Drug Administration and by agricultural states such as California. RACs with over tolerance residues are either held until pesticide residues dissipate or they are destroyed or used for non food purposes.

C. Exposure to Pesticide Residues in Food: Tolerance Assessment System

The USEPA used the Tolerance Assessment System (TAS) dietary program on a main frame computer to estimate dietary exposure to a pesticide and compare that estimate to a previously determined ~~acceptable dietary intake (~~ADI~~)~~ (9). TAS was composed of (1) data files (food consumption, toxicological endpoints (NOELS), and residue data) and (2) software to access these data files and to track Agency decisions regarding pesticide tolerances. TAS was able to estimate dietary exposures for the U.S. population and for 22 subgroups of the population, expressed as mg chemical intake kg^{-1} d~~ay~~$^{-1}$. TAS was similar to the old USEPA system of "food factors" in that a Theoretical Maximum Residue Contribution (TMRC) was compared to an ADI. TAS calculated the TMRC by using the average consumption for each food multiplied by the tolerance for that food and summed over foods to produce the TMRC. TAS did not evaluate toxicology or residue data. Food consumption estimates were based on the 1977-78 USDA Survey involving 30,770 persons for 3 days.

The Dietary Analysis Program from Technical Assessment Systems, Inc., Washington, D.C. Exposure 1™ (Chronic Dietary exposure) and 2™ (Acute Dietary exposure) was the first available dietary exposure software to run on desk top computers. Exposure 1 estimated the chronic dietary exposure by using "annualized" consumption of each food. Exposure 1 estimates may be compared to the ~~Acceptable Daily Intake (~~ADI~~)~~ generated from experimental chronic toxicology or from the risk probability estimates from oncogenicity studies. Exposure 2 calculated the theoretical acute (daily) intake of a pesticide for the U.S. population and the following subgroups:

Females > 13 years
Males > 13 years
Infants < 1 year
Children 1-6 years
Children 7-12 years

In the late 1990s, ~~EPA~~the USEPA used DEEM™ (Dietary Exposure Evaluation Model) developed by Durango Software, LLC, to assist ~~them~~ in regulating and setting food tolerances. DEEM™ incorporated the 1994-1996, 1998 CSFII (Continuing Survey of Food Intakes by Individuals) with USDA-USEPA FCID (Food Consumption Intake Database) recipes to translate the foods as eaten to ~~RACs (Raw~~raw a~~A~~gricultural c~~C~~ommodities (RAC) and food forms (FF). DEEM-FCID consists of four software modules: the main DEEM-FCID module, the acute analysis module, the chronic analysis module, and the RDFdoc utility for validating and documenting residue distribution files (RDFs). DEEM is a Windows™ based model used with desk top computers. Gammon et al. (10) used TAS (11) and DEEM (12) to calculate dietary margins-of-safety (MOSs) for methamidophos (CAS no.10265-92-6)— on cotton, potato and tomato. No adverse

~~Observed~~ ~~observed~~ ~~Effect~~ ~~effect~~ ~~Level~~level(s) (NOAELs) of 0.3 mg kg^{-1} d^{-1} (rat acute) and 0.02 mg $kg^{-1}d^{-1}$ (dog) for brain acetylcholinesterase (AChE) inhibition were used.

Comment [VD4]: Should this be NOAEL?

The LifeLine Group developed the Customized Dietary Assessment Software (CDAS™), which allows the user to calculate dietary exposure and risks from unique diets (13). The software consists of four modules: 1) Food Residue Translator; 2) Active Ingredient Module; 3) Exposure Analysis Module; and the 4) Report Generator.

D. Worker Exposure: Reentry, Mixing, Loading and Application

Poisoning incidences were first reported among workers who reentered pesticide-treated orchards and vineyards in California in 1949 shortly after the registration of parathion [CAS no. 56-38-2] (14). Seventy-nine incidences were reported from 1949--1958 with an additional 87 reports of injury during the years 1961-1969. Two incidents involved azinphosmethyl (CAS no. 86-50-0) and ethion (CAS no. 563-12-2) with the remaining associated with parathion ~~(CAS no. 56-38-2)~~. ~~During the years~~Nine episodes occurred from 1970~~-~~ to 1972~~-~~, ~~there were nine episodes~~ involving 86 persons. Poisoning incidences were also linked to the foliar application of carbofuran (CAS no. 1563-66-2) to corn in 1974 and methomyl (CAS no.16752-77-5) to grapes in 1981.

In 1972, legislation in California established the Worker Health and Safety (WHS) group in the Department of Food and Agriculture and brought about the adoption of regulations allowing for the establishment of reentry intervals (14). Studies by researchers at the University of California~~-~~ (Davis, Riverside, Berkeley and San Francisco), California

Department of Health Services and, the California Department of Food & Agriculture (CFDA), U.S. EPAthe USEPA, and pesticide manufacturers divided partitioned the reentry problem up into three distinct parts- (i) dissipation of the foliar residue, (ii) transfer of the residue to the skin and clothing of workers, and (iii) percutaneous absorption/dermal dose-cholinesterase (ChE) response (14). Reentry intervals were established by CDFA on the recognition that, i) safe pesticide levels exist for each pesticide based on their ii) dermal dose-ChE response and iii) foliar dissipation rates (14).

On March 11, 1974, EPAthe USEPA published 48-h reentry standards for 11 organophosphorus pesticides, endrin (CAS no. 72-20-8), and endosulfan (CAS no. 115-29-7) in the Federal Register (15). The regulations recognized state responsibility and authority to set additional restrictions to meet local problems. Subdivision K – reentry guidelines were published by EPAthe USEPA to cover post-application exposure to workers (16).

The Agency developed the Rebuttable Presumption Against Registration (RPAR) process under the 1972 Act and published criteria in 1975 which triggered the determination of unreasonable adverse effects associated with pesticide use. Under the RPAR process, it became apparent that the risks to individuals applying pesticides are distinct from those in the general population. A 1978 amendment to FIFRA emphasized the importance of evaluating direct exposure (i.e., mixing, loading and application) in OPP's regulatory decisions. As a result of Congressional deliberations and FIFRA amendments, applicator exposure monitoring guidelines involving passive dosimetry (measure of chemical on skin or available for inhalation) and biological monitoring (measure of internal dose) were published in Subdivision U of the Pesticide Assessment Guidelines (17). These

guidelines also include indoor testing procedures to measure post-application pesticide concentrations into exposed individuals.

A considerable number of exposure monitoring studies were carried out after the passage of California's worker health and safety regulations (14) and EPAthe USEPA's guidelines on reentry and worker exposure (16, 17). In California, one of the most dangerous activities was found to be the transfer of concentrated toxic pesticides ([i.e., organophosphorus and carbamate insecticides (NMC)]) from 5 gallon containers to mix tanks was found to be one of the most dangerous jobs. The pour spouts on these containers made it difficult to cleanly dispense the desired amount of pesticide cleanly to measuring devices or directly to mix tanks without spilling a portion of the liquid down the side of the container to hands, shoes and clothing of a worker performing the transfer. This observation resulted inled to the design and manufacture of closed-transfer systems for removing known quantities of the concentrated liquids from these containers and transferring them to mix tanks without contaminating the outside of the containers, the soil, or the hands, shoes and clothes of the mixer-loader. The closed-transfer systems were also designed to rinse the containers with water when empty and to transfer the washes to mix tanks. Starting in 1976 by Knaak et al. (18, 19) tThe safety effectiveness of these devices (i.e., reduction in blood ChE activity, airborne residues, and urinary alkyl phosphates) was monitored starting in 1976 by Knaak et al. (18, 19). Early prototypes with untrained workers did not substantially decrease exposure, as measured by a decrease in blood ChE activity and no decrease in airborne residues. However, when measurements were made over a period of 18 weeks, involving five trained mixer-loaders

and four mixer-loader applicators ~~and~~ using new closed-transfer equipment, a majority of the workers ~~,~~showed increased blood ChE activity, with ~~increased and~~ urinary dialkyl phosphate levels ~~were~~ at 0.02 ppm. ~~for a majority of the workers.~~ During the study, blood ChE activity of two mixer-loaders decreased and dialkyl phosphate levels was at 2.4 ppm for one of the workers. Airborne residues averaged 5.6 µg m^{-3}, while dusty powders averaged 153 µg
~~-~~m^{-3}.

Worker monitoring programs following the activity of blood esterases ~~(~~[AChE and butyrylcholinesterase (BuChE)] before and after exposure are required in California and often carried out in other states where large quantities of these materials are applied on a commercial basis. Nigg and Knaak (20) recommended a blood esterase monitoring program for workers coming in contact with OPs in the work place.

Induction bowls are currently being used in Europe to load undiluted product ~~(~~[~~PPP,~~known as ~~Plant~~ plant ~~Protection~~ protection ~~Products~~products (PPP)] into the main spray tank. The devices are fitted to the sprayer in a position that the operator can safely reach from the ground to avoid spillage (21). Sprayers and spraying technology ~~has~~ have been greatly improved over the last few years. The operators are supported with personal computer applications on CDs, active flow charts, moving images and devices that help to insure proper adjustment of equipment and delivery of pesticide product to crops.

The results of exposure studies were presented at several ACS sponsored ~~Symposia~~ symposia and published by ACS Books in their Symposium Series (273, 382, 542, and 643) (22, 23, 24, 25). Symposium Series 273 (22), integrates dermal absorption, field exposure and risk assessment in the book. Symposium Series 382 (23) assesses worker exposure to pesticides through biological monitoring. Symposium Series 542 (24) covers the importance of biomarker data in evaluating the impact of human environmental and occupational exposure to pesticides. Symposium Series 643 (25) introduced PBPK/PD modeling into the process of predicting the fate of pesticides and their action on enzyme systems such as AChE and BuChE.

Of interest are several re-entry studies involving azinphosmethyl (CAS no. 86-50-0) on peaches carried out by investigators in California (26, 27). In the harvesting study by McCurdy et al. (26) dislodgeable foliar residues on peach leaves ranged from 0.32 to 0.96 μg cm^{-2}. Median reduction in red blood cell (RBC)-AChE activity was 7% over an initial 3-day exposure period and 19% median depression over the 6 week monitoring period. Urinary alkyl phosphate levels [~~(~~dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP)] ~~DMP, DMTP and DMDTP) started~~ ~~out~~began at >1.0 μmoles d^{-1} and increased to as high as 20 μmoles d^{-1} on day 3 for individual workers. At the end of the monitoring period urinary dialkyl phosphate (DAPs) ~~DAPs~~ were at baseline levels.

In the peach orchard reentry study conducted by Schneider et al. (27) dislodgeable foliar residues ranged from 0.82 to 1.72 μg cm^{-2}. Blood AChE values decreased 10-20% over the 3-week exposure period. Urinary metabolites, DMP and DMTP increased with

continuous exposure from 1.5-3.1 and from 1.8-3.1 mg g^{-1} creatinine, respectively. Mean creatinine values were 1.4 g L^{-1} with 90% of the 24-hr urines having volumes of 700 ml. Knaak et al.(28) proposed a safe level for azinphos-methyl on foliage of 1.6 µg cm^{-2} using dermal dose-response curves for cholinesterase inhibition, developed from rat models and field exposure data. No recommendations were made by either McCurdy et al. (26) or Schneider et al. (27) regarding safe foliar levels.

In an azinphosmethyl reentry study (DAP metabolites) by Doran et al.(29), the results of a traditional model, Eq 1, was compared to that of a time-integrated model proposed by Kissel and Fenske (30).

$$ADD = (DFR \cdot TF \cdot t_1 \cdot DA) / BW \quad (1)$$

Where, ADD is the absorbed daily dose (µg kg^{-1}d~~ay~~$^{-1}$), DFR is dislodgeable foliar residue (µg cm^{-2}), TF is a transfer factor (cm^2 hr^{-1}), t1 is the length of the workshift (hr d~~ay~~$^{-1}$), DA is unitless dermal absorption factor that varies between 0 and 1, and BW is the worker's body weight (kg).

The time-integrated model is similar in some respect to code in ~~EPA~~the USEPA's ERDEM model where chemical uptake from skin during the workshift and uptake during the interval between workshift and washoff is considered. The Kissel and Fenske model (30) utilizes absorption constant, k_{abs} (hr^{-1}) where absorption is first order with respect to the residual mass on skin. The model calculates total chemical uptake (mg) from skin exposure as the sum of uptake during the work shift and uptake during the interval between the work shift and decontamination. Absorbed daily dose may be calculated by

dividing the total uptake by the worker's body weight. The absorbed daily doses predicted by the time-integrated model was 24 µg kg^{-1} d~~ay~~$^{-1}$ (geometric mean) with a range of 1.6-370. The traditional model predicted an absorbed dose of 79 µg kg^{-1} d~~ay~~$^{-1}$.

~~In a book chapter published by~~ Knaak et al. (31) ~~, the authors~~ used PBPK/PD models for parathion and isofenphos to examine previously determined reentry levels of 0.09 µg cm^{-2} for parathion on citrus and 0.6 µg cm^{-2} for isofenphos on turf. According to the PBPK/PD models approximately 3 percent of the transferred foliar residues were absorbed during reentry. In the models, the foliar dose rate k_dR was determined as follows:

Constant R = 0.1, 1.0, 5.0 and 10, foliar pesticide concentration in µg cm^{-2}
Constant k_d = 10,000, slope factor in cm^2 h^{-1}
EXPOS = k_d x R
RP = (EXPOS/MW)
k_dR = RP x 1.0 x 10^6, pmol h^{-1}

Material balance for the parathion model: foliar residues of 0.1 µg cm^{-2} resulted in the transfer of 8.0 mg of parathion per worker (8 hr work day), with 2.12% lost to air, 95.2% retained on skin, 0.415% in urine and feces and 2.7% in body tissues. Transfer coefficients, k_d, vary according to crop and may be calculated by Eq 2 according to Exposure and Risk Assessment Calculations (Guideline Series 875 – Part D), page D2-50.

$$\frac{cm^2}{hr} = \frac{ug}{hr} / \frac{ug}{cm^2} \cdot \frac{mg}{1000ug} \quad (2)$$

To our knowledge PBPK/PD models have not been used to analyze the data collected in field reentry studies. The Pesticide Assessment Guidelines Subdivision K were revised in 1997 by ~~U.S. EPA~~the USEPA, OPP, Health Effects Division and published as Series 875

– Part B: Post application Exposure Monitoring Guidelines to assist the regulated community in designing and conducting studies (32). The use of PBPK/PD models was not included in these guidelines.

The parathion PBPK/PD model used by Knaak et al. (31) was converted to a chlorpyrifos model by Ellison et al. (33) to study the transfer of chlorpyrifos in spray to skin and clothing of cotton workers, dermal absorption, distribution, metabolism to 3, 5, 6-trichloro-2-pyridinol [(TCP) (3, 5, 6 trichloro 2 pyridinol, CAS no. 6515-38-4),], inhibition of AChE and BuChE by chlorpyrifos-oxon and the elimination of TCP in urine. Equations depicting evaporation losses and losses from showering were included in the model. This model was used to further examine chlorpyrifos urinary biomarker data from farm families reported by Alexander et al. (34). Five day maximum cumulative TCP urinary elimination profiles were modeled for applicators (2.22×10^5, 5.94×10^5, 1.48×10^6, 1.53×10^6 and 9.01×10^5 pmoles, total = 4.72×10^6 pmoles) and their spouses (1.21×10^5, 1.75×10^5, 1.75×10^5, 4.76×10^5 and 2.91×10^5 pmoles, total = 1.24×10^6 pmoles). The total pmoles of TCP predicted by the PBPK/PD model for applicators and spouses were 4.84×10^6 and 1.55×10^6. The TCP in urine amounted to approximately 1.7% of the dermal dose. Wash off removed 95% of the dermal dose. Plasma BuChE inhibition in applicators and their spouses were predicted to be 92 and 73% of preexposure values, respectively, at the end of the 5 day exposure period, while whereas red cell inhibition was predicted to be 3.0 and 1.0%, respectively. On the basis of these inhibition values, the NOAELs for red cell inhibition were predicted to be 0.02 mg kg^{-1} for applicators and 0.01 mg kg^{-1} for spouses.

Lu et al. (35) used a PBPK/PD model [Exposure Related Dose Estimating Model (ERDEM)] ~~(PBPK/PD model)~~ to predict the urinary elimination of TCP by children exposed to chlorpyrifos in three meals, by inhalation and ingestion by hand-to-mouth activity. Overall, ERDEM ~~under~~ under-predicted absorbed chlorpyrifos doses. The two highest predicted doses (2.3 and 0.44 µg $kg^{-1}d$~~ay~~$^{-1}$) were associated with two 24-hr duplicate food samplings containing 350 and 12 ng g^{-1} of chlorpyrifos. None of the predicted or calculated daily dose estimates exceeded the oral RfD of 3 µg $kg^{-1}d$~~ay~~$^{-1}$.

E. U.S. FDA enforcement of pesticide residues in food under FFDCA.

The Center for Food Safety and Applied Nutrition (CFSAN) is the branch of the U.S. FDA responsible for regulating food, dietary supplements, and cosmetics. The US Population (i.e., adults, infants and children) is exposed to pesticide residues in foods grown in the US and outside the US. The level of these residues are controlled by "Use Instructions on Pesticide Labels", pesticide residue tolerances (in ppm) set by US-EPA (i.e., published in the Federal Register), and by periodic sampling and testing of raw agricultural commodities by FDA, state and federal agricultural programs, and by food processors (i.e., companies canning and freezing foods) and distributors (i.e., raw agricultural commodities-fruits and vegetables). The results (i.e., pesticide residues, in ppm) of these programs are used to regulate pesticide use and insure that pesticide levels in foods meet published tolerances. The consumption of residues in foods (i.e., meals) containing one or more pesticides is routinely followed by FDA, by the sampling and testing of prepared meals. The results of these programs are often compared to default studies, where meals are made from randomly selected raw and processed commodities.

Importers of food products intended for introduction into U.S. commerce are responsible for ensuring that the products are safe, sanitary, and labeled according to U.S. requirements under the provision of the U.S. Federal Food, Drug and Cosmetic Act. The Bioterrorism Preparedness Act of 2002 also requires importers to provide prior notice to FDA for each import shipment of food products. Information is available on the following FDA link: http://www.cfscan.fda.gov/~pn/pnoview.html/. Import shipments of a food commodity containing pesticides for which tolerances have not been established for the commodity may be refused entry or detained. The USEPA may be contacted through the agency's website at this link: http://www.epa.gov/ about what pesticides are allowed.

III. USDA, status after 1971

A. Section 3 and 24c Registrations

After the transfer of pesticide regulation to the USEPA in 1971, the USDA continued to be active in a number of areas involving pesticide use. The USDA Animal and Plant Health Inspection Service (APHIS) maintains approximately 30 Section 3 (federal) or Section 24c (state) vertebrate pesticide registrations for the Wildlife Services (WS) programs to control wild mammals and birds that damage crops, impact endangered species, or pose human health risks (8).

B. Pesticide Data Program

The Pesticide Data Program had its origins following the 1989 "Alar in Apple" crisis in the Pacific northwest where Alar (butanedioic acid, 1-(2, 2-dimethylhydrazide)(CAS no. 1596-84-5) and/or metabolites exceeded federal tolerance levels (36, 37).

Insert Figure 1.

The lack of residue data combined with inadequate toxicological data and high tolerance levels prompted USDA to cooperate with ~~EPA~~the USEPA and FDA to develop a PDP to provide additional data on fresh fruits, vegetables, grain products, and fluid milk (38). The PDP, a federally funded-State cooperative program includes 10 participating states: California, Colorado, Florida, Maryland, Michigan, New York, Ohio, Texas, Washington and Wisconsin (39). The authorities under which PDP operates are the Agricultural Marketing Act of 1946 and the more recent Food Quality Protection Act (FQPA) of 1996. The role of PDP has significantly increased as a result of the provisions of FQPA which requires data to evaluate cumulative exposure on a common toxicological effect and data on endocrine disruptors such as aldicarb (CAS no. 116-06-3), benomyl (CAS no. 17804-35-2), DDT (CAS no. 50-29-3), endosulfan and parathion. The purpose of PDP is not to enforce federal food tolerances, but to provide the most statistically-reliable set of residue data (38). PDP's objectives include 1) addressing the recommendations of the 1993 National Academy of Sciences report, "Pesticides in the Diets of Infants and Children"; 2) supporting the Foreign Agricultural Service's (FAS) international marketing of U.S. commodities and 3) addressing USDA's responsibility under the Food Quality Protection Act.

The number of users of PDP's data has increased over the years, with data being used by ~~EPA~~the USEPA, FAS, the economic Research Service of USDA, academia, private companies, the agricultural community, environmentalists, international organizations

using the Codex Alimentarius, and global traders. California's Department of Food and Agriculture use PDP data to support California's trade with Pacific Rim countries.

Trade is also enhanced by the U.S. Sanitary and Phytosanitary (SPS) Enquiry Point at USDA. The SPS Enquiry Point is responsible for notifying the World Trade Organization (WTO) of any potential risks that could arise from disease, pests, food additives, pesticide treatment, toxins, and other contaminants and coordinate this work with Codex Activities (38).

Comment [VD5]: Interesting choice of words….

IV. The Food Quality Protection Act (FQPA) of 1996.

A. Provisions of FQPA

The publication of the National Academy of Sciences study (40), "Pesticides in the Diets of Infants and Children" brought to the attention of Congress the long held concerns of health officials that pesticide tolerances were inadequate to protect the health of infants and children. The act requires ~~EPA~~the USEPA to incorporate a 10-fold safety factor into the tolerances unless reliable information is available to say otherwise; establishes a single safety standard for setting tolerances under FFDCA and a reassessment program. The Agency is also required to consider cumulative effects from aggregate exposure (e.g., multiple routes of exposure-dietary, drinking water, and non-occupational exposures) to pesticides with a common mechanism of toxicity, and the potential for endocrine disruption effects. The Act did not require the development of new methods (i.e., exposure and risk assessments), interpretation/clarification of the various provisions of the Act, or data before implementation.

FFDCA was amended by FQPA by repealing the Delaney "zero tolerance" clause for pesticides and was replaced with reasonable certainty that no harm to humans comes from aggregate exposure. A special finding for the protection of infants and children were included in the law.

Comment [VD6]: Is this the same as the zero cancer risk of the original language? Either way, it could use some explanation…..

The key to implementing FQPA science provision was the development of science policy papers by OPP in the areas of:

1.) 10-fold safety factor:
2.) Dietary Exposure and Risk Assessment:
3.) Drinking water exposure:
4.) Residential exposure:
5.) Aggregate exposure and risk assessment and
6.) Cumulative risk assessment for pesticides with a common mechanism of toxicity.

With the USEPA, OPP ~~agreed to work~~collaborates with ~~ORD (~~Office of Research and Development (ORD) to collect and disseminate dietary information about subgroups and carry out research to address these gaps. In addition to subgroups based on dietary information, OPP is required to address risks associated with exposure to pesticides registered for residential use. The subgroups are:

1.) Applicator (adult)
2.) Post-application adult
3.) Post-application youth
4.) Post-application toddler

A memorandum of understanding (MOU) was drawn up between OPP and ORD addressing these issues. These subgroups take into account that a young child's ~~post~~ ~~post~~ exposure to pesticides may be quite different from those of an adult's.

B. DEEM, LifeLine, SHEDS Dietary and Nondietary Models.

The federal requirement under FQPA to assess human risks to aggregate and cumulative pesticide exposures resulted in the development of a number of probabilistic exposure models [(e.g., Stochastic Human Exposure and Dose Simulation (SHEDS) and, LifeLine], ~~etc)~~ involving exposure to pesticide residues in food, home, work place and the general environment. To be realistic these models require actual measurements of pesticide residues in food, home and work place environments and the elimination of their biomarkers (metabolites) in human urine. FQPA monitoring and human risk assessment requirements are significantly different from the type of data (e.g., toxicity, food residue tolerances, application rates, insect control, residue dissipation data and NOAEL data) required under FIFRA for single pesticides. Consequently the health and regulatory community has struggled to monitor the results of exposure preventative tools (label use instructions, food tolerances, preharvest intervals, reentry intervals, closed system mixing and loading, etc) developed under FIFRA to determine if they meet or exceed FQPA requirements. The requirements under FQPA are more difficult to fulfill than those under FIFRA, because they involve exposure to multiple pesticides in a regulated, but somewhat semi controlled environment as opposed to well controlled animal toxicity, field use and dissipation studies required under FIFRA.

The US-EPA is replacing DEEM™ with their Stochastic Human Exposure and Dose Simulation (SHEDS)-Dietary module in which food consumption patterns are linked to pesticide residue files to give dietary exposure estimates (41, 42, and 43). The SHEDS-Multimedia 3 model is a single chemical aggregate model which includes only dietary

and residential modules. Version 4 of SHEDS, now under development, will include the ability to do both aggregate (single chemical) as well as cumulative (multi-chemical) exposures. SHEDS version 4, Calendex, the Cumulative and Aggregate Risk Evaluation System (CARES), and REX™ LifeLine include tools that allow the assessor to estimate dietary, aggregate and cumulative exposures. Durango Software, LLC introduced Calendex to meet ~~EPA~~the USEPA's requirements for a model capable of carrying out aggregate and cumulative exposure analysis in conjunction with DEEM. CARES (Cumulative and Aggregate Residue Evaluation System) was originally developed under the auspices of CropLife American (CLA) and is designed to conduct complex exposure and risk assessments of pesticides.

The Lifeline Group developed LifeLine™ Version 5 (44). This software uses probabilistic techniques to model exposure, risks and benefits for the general population or selected subpopulations, such as children, woman of child bearing age and the elderly. Sources of exposure include the diet, home environments and products, drinking and tap water, consumer products, pesticide users or an aggregate of all these sources. Routes of exposure include inhalation, dermal, oral, and child's mouthing behaviors. The exposure estimates may be linked to PBPK/PD models to obtain risk estimates. Figure 2 provides a schematic overview of the relationship between dietary and nondietary exposure, aggregate exposure and cumulative risk assessment (45).

Insert Figure 2.

C. Conceptual Framework for modeling aggregate and cumulative exposures.

Since the passage of the 1996 Food Qualtiy Protection Act (FQPA) there has been a need to develop software to assess exposures to single chemicals via different routes and multiple chemicals having the same mode of action through multiple routes (cumulative exposure). ~~The paper by~~ Price and Chaisson (46) proposed a conceptual framework for achieving these goals. The framework is based on placing the individual or person at the center of the design in Figure 3 as opposed to modeling a single source as shown in Figure 4.

The source-to-dose modeling moves the chemical through the environment and models the rate and amount of chemical absorbed by an individual via multiple sources. This type of model creates a need for consistency in the model in that the individual or person must logically be exposed to the various sources of chemical in the environment over a sufficiently short period of time so that 1) the doses from each source may be treated as a constant dose for the duration of the time period; 2) the levels of each chemical in the microenvironment may be treated as constants, and 3) the person's parameters (physiological, biochemical, etc) may be treated as constants.

Exposure Event Loop. Once the data for a person are determined, the program enters the exposure event loop shown in Figure 5. In this loop the probability of being exposed to each of the sources is determined based on the characteristics of the person. The decision of whether a person is exposed is made independently for each source of each chemical. Exposure to a source may result in doses that occur by a single or multiple routes (oral, dermal, and inhalation) of exposure. The estimates from each of the routes are not

combined, but are saved at this time. The program continues until all of the sources for all of the chemicals have been evaluated. The doses from each source can be summed to give total route-specific doses

Insert Figure 3.

Insert Figure 4.

for each chemical in the mixture to which the person is exposed. In addition, the doses can be segregated by source to give the source-specific doses. The route-specific doses can be used as:

1.) inputs to route-specific risk characterization models such as toxicological benchmarks for oral, inhalation and dermal doses;
2.) used to estimate total dose and used in non-route-specific models of risk;
3.) used in models of cumulative risk for exposure to mixtures; or
4.) used as inputs to PBPK/PD models of organ specific doses in the person.

Once the determination of the first person's exposures is complete, the program exits ~~leaves~~ the exposure event loop, returns to the beginning of the program, and selects another person.

Insert Figure 5.

Individual Loop. This return creates the second type of loop in the framework, the individual loop. In this loop the characteristics of the new person are selected. Once these values are assigned, the exposure event loop is reentered. The program continues to cycle through the individual loop until the desired number of individuals has been simulated.

The outputs of this process are set of route- and source-specific doses for each chemical for each of the simulated persons in the model run. This set of doses characterizes the interindividual variation in the dose(s) of a chemical or a mixture of chemicals across the population for a specific duration at a specific point in time.

A number of software programs use this approach to estimate daily doses of pesticides and chemicals from dietary and air exposures (i.e., DEEM™, LifeLine™ and CARES™). When assessing a daily dietary dose, these programs pull a dietary record for one person from the USDA's Continuing Survey of Food Intakes by Individuals (CSFII). At the end of the list, the total oral dose of pesticide from all foods is determined and given as an output of the daily dose.

Insert Figure 6.

This process is repeated with other records (the individual loop). Environmental software programs track persons through a series of environments and determine the total air exposure. Examples of such programs include pNEM (47); SHAPE (48); CPIEM (49) and SHEDS (50).

Time ~~step~~ Step Loop. Figure 6 presents a flow chart for a longitudinal software program that determines how individual's exposures change overtime. Modeling longitudinal exposures is achieved with an additional loop called the "time step loop". The time step loop occurs between the exposure event and the individual loops. The program begins with a definition of the person's characteristics that apply to the first time step. The program then enters the exposure event loop and the exposure from each exposure source

is modeled. Here again the exposure is considered to be short and that all inputs can be viewed as constants. The output from a longitudinal model is an "exposure history" for each of the modeled persons. This approach has been used in a number of longitudinal models LifeLine™, (51), CARES™ (52), SHEDS (53), APEX (54), and Calendex™ (55).

The output of the time step loop can be used as 1) estimates of the average dose over longer period of time; 2) the highest dose in a given year; and 3) exposure histories as inputs to PBPK models.

Models built around Figure 6 involving an uncertainty loop may be used to model an uncertainty distribution around each of the outputs (confidence limits).

Price et al. (56) used LifeLine™ Version 1.0 (57) to model the risks associated with aggregate (single chemical, multiple routes) and cumulative exposures (multiple chemicals, multiple routes). Assessments of cumulative (multiple chemicals) risk involves the use of toxicity equivalents where toxicity is normalized to one 'standard' or 'index' pesticide (58). Under this approach, the pesticides modeled are assumed to have additive effects and the effect of each pesticide can be defined in terms of a toxicologically equivalent dose (TEQs) of a single index pesticide.

Comment [VD7]: Does the model calculate these or are they those published by EPA or WHO?

V. Dietary and nondietary exposure monitoring studies, children and adults.

In addition to authorizations by the Food Quality Protection Act of 1996 (e.g., food tolerances, susceptibility of infants and children to pesticides, etc), the Children's Health

Act of 2000 authorized the National Institute of Child Health and Human Development (NICHD) to conduct a national longitudinal study (National Children's Study) of environmental influences (including physical, chemical, biologic and psychological) on children's health and development (59, 60). Exposure was defined as contact between an agent and a target; contact takes place at an exposure surface over an exposure period (61, 62). The strategy for exposure monitoring depends on the study design. If the study is a long-term longitudinal cohort study of 100,000 children, fewer direct exposure measurements maybe made for each child. If a series of smaller direct exposure measurements are made, more exposure measurements maybe made for each child. Procedures for collecting samples range from those that are invasive, such as drawing blood to noninvasive such as collecting urine samples. Metabolites measured in urine maybe problematic because multiple chemicals may form the same metabolite (DAPs) in urines. To gain specificity, the parent chemical must be measured in blood or a specific leaving group, such as TCP from chlorpyrifos in urine. For a single dermal episode, Furtaw (63) recommends biological monitoring be continued for about 4 days in order to observe decay of peak blood concentration to 12%-of-peak level. Total urine volumes should be collected at each urination, the date and time recorded, and each urine sample preserved, stored, and analyzed separately. If spot urine samples are taken, an effort should be made to collect the total volume and to record the date and time of sample collection and the duration since the last urination. If this cannot be done, the next best alternative would be to analyze creatinine in each sample, and then report the creatinine-adjusted DAP concentration in urine (in units of mass of DAP per unit mass of

creatinine). If possible, a pre-exposure "background" urine sample should be collected and analyzed.

In cases where the exposure scenario is completely unknown, as in non-occupational studies, Furtaw (63) recommended sampling urine twice a day, morning and evening, record the volumes of urine eliminated and the times of sample collection and previous elimination time.

A. Organophosphorus Pesticides (OPs).

In Figure 7, Barr et al. (64) reported population-based urinary concentrations, stratified by age, sex, and racial/ethnic groups, of urinary dialkyl phosphate (DAPs) metabolites of multiple organophosphorus pesticides. The authors measured dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) concentrations in 1,949 urine samples collected in U.S. residents 6-59 years of age during 1999 and 2000 as part of the ongoing National Health and Nutrition Examination Survey (NHANES) (65). DAP metabolites were detected in more than 50% of the samples, with DEP being detected most frequently (71%). The limit of detection (LOD) was 0.2 $\mu g\ L^{-1}$ of urine. DMTP (1.85 $\mu g\ L^{-1}$) and DEP (1.04 $\mu g\ L^{-1}$) were detected in 60% of the samples with DMP (13 $\mu g\ L^{-1}$), DMTP (46 $\mu g\ L^{-1}$), DMDTP (19 $\mu g\ L^{-1}$), DEP (13 $\mu g\ L^{-1}$), DETP (2.2 $\mu g\ L^{-1}$), and DEDTP (0.87 $\mu g\ L^{-1}$) in the 95^{th} percentile. Multivariate analyses showed concentrations of DAPs in children 6-11 years of age that were significantly higher than in adults and often higher than in adolescents.

Insert Figure 7.

Insert Figure 8.

In Figure 8, DAP urinary concentrations in the U.S. population were much lower than those of other reference populations in the literature (Aprea et al. (66, 67)); Hardt and Angerer (68); Heudorf and Angerer (69).

B. Pyrethoids and OPs.

As part of planning efforts for the National Children's Study, Bradman et al. (70) conducted a children's study to test multimedia sampling methods in the Salinas Valley of California (Table 1). Pesticide exposures to 20 farmworker children aged 5-27 months were studied. Environmental (house dust, indoor/outdoor air, surface wipes, and C_{18} surface press disks) and clothing samples (union suits and socks) were analyzed for 12 OP pesticides, 13 pyrethroids, two fungicides, two OCs, and one herbicide.

Insert Table 1.

Food samples were taken and analyzed for a range of OP, OC, and pyrethroid pesticides and fungicides using gas chromatography. Two urine samples were collected from each child during a 24-h sampling period: one spot sample and one overnight diaper sample. Urines specimens were freeze-dried and the residue dissolved in acetonitrile:diethyl ether (1:1). The DAPs were derivatized to their chloropropyl phosphate esters and analyzed by GC-MS/MS (Bravo et al. (71)). The DAP analytic results for spot and overnight samples were expressed in nmol L^{-1}. Table 2 gives the result for all children. Urines were not analyzed for the presents of pyrethroid metabolites.

Insert Table 2.

In a study carried out by Naeher et al. (72) in Jacksonville, Fl, urine samples, one per child, were collected from 203 children (43% females and 57% males) ages 4 to 6 yrs old. The urines were analyzed for the six common DAPs, 3-PBA, 4F-3-PBA, cis/trans DCCA. The DAPs in urine (μg L^{-1}, creatinine corrected) were 14.4, 27.4, 4.4, 8.1, 1.8, and 0.4 respectively, for DMP, DMTP, DMDTP, DEP, DETP and DEDTP. The concentration of urinary DAPs were less than those found by Barr et al. (64) in U.S. residents 6-59 yrs of age. Urinary concentrations (μg L^{-1}, creatinine corrected) were 6.6, 2.9, 4.6, and 0.3, respectively for 3-PBA (CAS No. 3739-38-6), cis/trans-DCCA (3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane-1-carboxylic acid) and 4F-3-PBA (4-fluoro-3-(2, 2-dichlorovinyl)-2, 2-dimethylcyclopropane-1-carboxylic acid). 3-PBA (3-phenoxybenzoic acid)(CAS No. 3739-38-6) is a metabolite of pyrethroids possessing the 3-phenoxyphenyl group. Cis- and trans-DCCA are metabolites of cyfluthrin (CAS No. 68359-37-5), cypermethrin (CAS No. 52315-07-8) and permethrin (CAS No 52645-53-1).

Table 3 shown below was developed by Bravo et al. (71).

Insert Table 3.

C. Carbamates

Hill et al. (73) collected urine samples from approximately 1000 adults as part of the NHANES III study conducted by the National Center for Health Statistics and analyzed them for carbofuranphenol (CFP, 2,3-dihydro-2,2-dimethyl-7-hydroxybenzofuran), 1-naphthol (carbaryl metabolite), 2-isopropoxyphenol (IPP, propoxur metabolite), their parents and a series of eight other analytes such as TCP (3,5,6-trichloro-2-pyrindinol) a metabolite of chlorpyrifos. Pesticide residues or metabolites were reported in ug L^{-1} (ppb)

and in creatinine-corrected concentrations in ug g^{-1} of creatinine. The analytes, 1-naphthol, TCP and four others were detected in more than 50% of the population. The frequency of detection was low (6.8 and 1.5%), respectively for IPP, CFP and 86% for 1-naphthol. The presence of 1-naphthol in urines maybe associated with exposure to carbaryl or to naphthalene. Population exposure to carbaryl was considered unlikely.

Shealy et al. (74) collected urines from six farm families as part of a pilot Agricultural Health Study conducted by the National Cancer Institute (NCI), the USEPA, and the National Institute of Environmental Health Science (NIEHS) to correlate environmental carbaryl measurements with serum and urinary 1-naphthol measurements. Of the six farmers, only one was actively spraying carbaryl on crops during the monitoring period. Carbaryl was detected in air (µg m^{-3}), on a dermal patch (11 µg cm^{-2}), on a handwipe (20100 µg), and in serum (0.12 µg L^{-1}) during application day. 1-naphthol was present in evening urine to the extent of 22000 (µg g^{-1} of creatinine).

D. Pesticide Mixtures

Biomonitoring studies provide clear and unequivocal evidence of combined exposures (NHANES)(65). In addition to the co-occurrence of biomarkers, combinations of pesticide residues were found in certain raw commodities and processed foods (USDA, PDP and FDA/CFSAN). The direct attribution of combinations of biomarkers in excreta, primarily urine, to the ingestion of pesticide residues at tolerance levels is speculative. One way to reduce the uncertainty in this attribution is to examine the co-occurrence of combinations of urinary metabolites with combinations of residues in raw commodities

and processed food. The analytical detection or quantification of urinary metabolites corresponding to a combination of active ingredients labeled for use on raw commodities and stored products or detected as residues in raw commodities and processed foods would offer prima facie evidence of exposure.

The CDC Fourth National Report on Human Exposure to Environmental Chemicals, 2009 (the Report. http://www.cdc.gov/exposurereport) provides tables of pesticide urinary biomarker concentrations (in ug g^{-1} of creatinine) for the U.S. population by geometric mean and selected percentiles (50^{th}, 75^{th}, 90^{th}, and 95^{th}). The summary statistics provide a glimpse of possible combinations that might be inferred with the caveat of a lack of direct individual attribution. For example, we might infer the possibility of generalized exposure to a single diethyl organophosphorus insecticide or several in the survey year 1999-2000 that coincides with possible concomitant exposure to cypermethrin, deltamethrin, or permethrin (3-PBA) or a combination of all three. Greater specificity may be systematically gleaned from comparisons with TCP, the specific urinary metabolite of chlorpyrifos and chlorpyrifos-methyl. Exposure of the 1999-2000 cohorts appears to most likely involve chlorpyrifos and chlorpyrifos-methyl with permethrin. This supposition is made with the understanding that the NHANES survey (1999-2000) and the FDA/CFAN total diet study (1997-1998) are out of phase by at least two years. The appearance of these pesticides in food is not serendipitous but more a consequence of several sequential events in agriculture and food production. The greater question is whether these single or mixed residues are toxicologically meaningful or simply trivial curiosities. Reffstrup et al. (75) outlined current approaches (flow charts and eight risk assessment methods; hazard index, relative potency factor, etc.) to

assessing risks to single compounds and to whole mixtures involving carcinogenic and noncarcinogenic chemicals. The Agency for Toxic Substances and Disease Registry (ATSDR) recommends using PBPK/PD models, if available, to predict the effects of mixtures (76, 77).

VI. PBPK/PD Models, risk prediction

Parameter selection has played a major role in the development of physiological-pharmacokinetic/pharmacodynamic models. Arms and Travis (78) and ILSI (79) published a reference manual for the physiological parameters (i.e., cardiac output, pulmonary ventilation, tissue weights or volumes and tissue blood flows). Gargas et al. (80) developed a method for tissue/blood partition coefficients for volatile chemicals, while Jepson et al. (81) published a procedure for nonvolatile chemicals (i.e., pesticides). Mechanistic approaches for predicting partition coefficients were developed by Poulin and Theil (82, 83) and are currently being used by modelers in PBPK/PD models. Knaak et al. (84, 85 and 86) reviewed the parameters for organophosphorus, carbamate and pyrethroid insecticide quantitative structure activity relationships (QSAR) and PBPK/PD models for human risk assessment. The reviews emphasized the development of partition coefficients; liver CYP based metabolic rate constants (i.e., V_{max}, K_m, and k_{cat}) and bimolecular inhibition rate constants for the acetylcholinesterase inhibiting insecticides.

A. Organophosphorus Pesticides

Organophosphorus (OPs) PBPK/PD models involving: di-isopropyl-fluorophosphate (DFP, Gearhart et al. (87)), O-O-diethyl-O-4-nitrophenylthiophosphate (parathion; Sultatos, (88)), O-ethyl O-2-isopropoxylcarbonyl-phenyl isopropyl phosphoramido-

thioate (isofenphos; Knaak et al. (89)), O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothiolate (chlorpyrifos, Timchalk et al. (90)), O,O-diethyl-O-(2-isopropyl-6-methyl-pyrimidine-4-yl) phosphorothioate (diazinon; Poet et al. (91)), O,O-dimethyl S-[1,2-bis-(ethoxycarbonyl)ethyl phosphorodithioate (malathion: Power et al.(92)), O,O-diethyl-S-(p-nitrophenyl) phosphorothoate (parathion; Foxenberg et al.(93)) and O,O-diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate (chlorpyrifos; Timchalk et al. (90)), Mosquin et al.(94), Foxenberg et al.(93) are available. Except for Mosquin et al. (94), the models listed above include code for tracking cholinesterase inhibition.

The metabolic pathways for parathion and chlorpyrifos are given below (Foxenberg et al. (93) designating the individual enzymes, liver CYP450s and PON1 A-esterase involved in the individual reactions.

Foxenberg et al. (93) converted the microsomal based chlorpyrifos model developed by Timchalk et al. (90**)** into two models; a human chlorpyrifos CYP based/age-specific model using recombinant human CYP based V_{max}, K_m values and a parathion CYP based/age specific model (Foxenberg et al.(93)). The CYP based models used the V_{max}, K_m values of CYP 1A2, 2B6, 2C9, 2C19, 3A4, 35A and 3A7 against chlorpyrifos and parathion (45). See Figures 9 and 10 for the metabolic pathways. In vitroV_{max} values (pmol $min^{-1}nmol^{-1}$) were converted to in vivo values (μmol $hr^{-1}kg^{-1}$ of bw) using Eq 3.

$$V_{\max(invitro)} = \frac{CYPcontent \cdot V_{\max(invitro)} \cdot 60 \cdot micpro \cdot liverwt}{1.0E6} \qquad (3)$$

where; CYP content = (pmol mg^{-1} microsomal protein), V_{max} = (in vitro, pmol $min^{-1}nmol^{-1}$), 60 = (minutes hr^{-1}), mic pro = (amount of microsomal protein in human liver, mg protein g^{-1} liver) and liver weight = (weight of liver in g kg^{-1} bw).

Insert Figure 9.

The equations for the two microsomal metabolic rate constants (RAM, rate of metabolism: hydrolysis and oxidation) were replaced by two of the following RAM statements, Eq 4:

$$RAM = \left[\frac{V_{max1}(S)}{K_{m1}+S}\right] + \left[\frac{V_{max2}(S)}{K_{m2}+S}\right] + \left[\frac{V_{max3}(S)}{K_{m3}+S}\right] + \left[\frac{V_{max4}(S)}{K_{m4}+S}\right] + \left[\frac{V_{max5}(S)}{K_{m5}+S}\right] + \left[\frac{V_{max6}(S)}{K_{m6}+S}\right] \quad (4)$$

CYP2B6 and CYP2C19 were the most active CYPs involved in the metabolism of chlorpyrifos and parathion (95).

This is the first example of the use of CYP generated V_{max}, K_m values in an OP PBPK/PD model. The OP models of Knaak et al. (89), Timchalk et al. (90), Poet et al. (91), Power et al. (92), and Mosquin et al. (94) used values from microsomal studies.

Insert Figure 10.

B. Carbamates

Two carbamate PBPK/PD models were published. One model by Zhang et al. (96) involves carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl-N-methylcarbamate) and

another by Nong et al. (97) involves carbaryl (1-naphthyl-N-methyl carbamate, CAS no. 63-25-2).

The metabolic pathways for carbofuran were taken from Knaak et al. (85) and are displayed in graphic form in Figure 11. The abbreviations for each metabolite are given in Table 4.

Insert Figure 11.

Insert Table 4.

The tissue: blood partition coefficients for carbofuran were obtained using the mechanistic models of Poulin and Theil (82, 83) published in Knaak et al. (85). An initial set of V_{max}; K_m values used by Knaak et al. (85) to run an ACSL carbofuran model were used to test their ERDEM model. Optimization (i.e., V_{max}, K_m) was carried out by visual examination of the output of the model with an experimental data set with V_{max} being the value changed. The CYP values (i.e., CYP3A4, 1A2, and 2C19) of Usmani et al.(98) for the conversion of carbofuran to 3-OH carbofuran were not used by Zhang et al. (96) as ERDEM model code similar to that used by Foxenberg et al. (93) in ACSL was not available. Monte Carlo simulations of 109 sensitive parameters were carried out to evaluate the impact of their variability on model predictions. The model predicted the inhibition of blood AChE and BuChE.

Nong et al. (97) developed a carbaryl PBPK/PD model from experimental measurements in the rat. The model describes the tissue dosimetry of carbaryl and its metabolites (1-naphthol and hydroxylated metabolites) and predicts the inhibition of cholinesterase activity in the brain and blood from dosages ranging from 0.8 to 9.2 mg/kg/kg of body

weight. Radioactive tracer studies were undertaken to determine total tissue levels of carbaryl and metabolites. Markov Chain Monte Carlo (MCMC) calibration of the rat model parameters was implemented using prior information from the literature for parameter distributions. The posterior estimates of the parameters were not greater than a twofold deviation from their means. The model emphasized the formation of 1-naphthol and conjugates over that of metabolites of hydroxylated carbaryl. According to Dorough et al. (99) carbaryl is extensively hydroxylated by the rat and eliminated as the glucuronide or sulfate conjugates of 4-, 5-OH carbaryl and 5,6-dihydrodihydroxy carbaryl with lesser amounts of the hydrolysis product, 1-naphthol. This finding was confirmed by the work of Knaak et al. (100, 101), Leeling and Casida (102) Sullivan et al. (103), and Chin et al. (104, 105). Nong et al. (97) cited the V_{max}, K_m values for the metabolism of carbaryl by carboxylesterases to 1-naphthol by McCracken et al. (106) and Tang et al. (107) in their development of a PBPK/PD model for carbaryl.

~~According to the studies of~~ Tang et al. (107~~),~~) report that human rCYP metabolizes carbaryl to 4-OH, 5-OH and hydroxymethyl carbaryl. This work is in agreement with the metabolites of carbaryl found in human urines being 4-OH and 5-OH carbaryl glucuronide and an unknown glucuronide, possibly that of hydroxymethyl carbaryl (Knaak et al. (100, 101)). No evidence was found in human urine for the presents of a major rat metabolite, 5, 6-dihydrodihydroxy carbaryl.

C. Pyrethroids

Two types of pyrethroids are used as insecticides, Type I compounds (i.e., permethrin, etc) that do not contain a cyano group, and Type II compounds (i.e., deltamethrin) that

contain a cyano group. Type I compounds cause tremors and skin parathesias, while Type II compounds produce salivation, hyperexcitability, tremors, and choreoathetosis (Soderlund et al.(108); Wolansky et al. (109)).

PBPK/PD models were developed for deltamethrin (Mirfazaelian et al. (110); Godin et al. (111); Tornero-Velez et al. (112)). Mirfazaelian et al. (110) used the metabolic rate constants developed by Anand et al. (113). Deltamethrin is metabolized in rats by liver CYPs and carboxylesterases (CaEs) and by plasma CaEs. Anand et al. (113) determined the relative rates of each in vitro by monitoring the rates of disappearance of the parent compound. The K_m and V_{max} values for each metabolic pathway were used in the Mirfazaelian et al. (110) PBPK model without any modifications. IsoOMPA (tetra isopropyl pyrophosphoramide) was used to inhibit microsomal carboxylesterases, while obtaining Michaelis-Menten rate constants for the hydroxylation of deltamethrin by liver microsomes with NADPH as a co-factor. Liver microsomal carboxylesterase activity was obtained in the absence of IsoOMPA and NADPH. In the PBPK model, V_{max} and K_m values were expressed in mg $h^{-1}kg^{-1}$ bw and mg L^{-1}, respectively. Delivery of deltamethrin to the brain, liver, GI tract and rapidly perfused tissues were flow limited, while delivery to RBCs, fat and slowly perfused tissues were diffusion limited. Of interest was low partition coefficients used for liver/plasma, brain/plasma and rapidly perfused/plasma. The model was considered by the authors to be a preliminary model. In a later PBPK diffusion-limited model published by Godin et al. (111), all tissue compartments were described with diffusion-limited kinetics and a single blood compartment. The rat model was extrapolated to humans using physiological parameters

from Brown et al. (114) and metabolic parameters from Godin et al. (115) using the parent depletion approach. The results (fitting of the output of the model to experimental values) were significantly improved over the results obtained by Mirfazaelian et al. (110).

Comment [VD8]: At what statistical level?

D. Pesticide Mixtures

The chlorpyrifos PBPK model of Timchalk et al. (90) was the template for individual models of parathion and chlorpyrifos by Foxenberg et al. (93) and the binary mixture of chlorpyrifos and diazinon by Timchalk and Poet (116). The individual models of Foxenberg et al. (93) incorporated human CYP V_{max} and K_m values into the individual models, while the binary mixture model of Timchalk and Poet (116) used rat microsomal V_{max} and K_m values. The model assumed that chlorpyrifos (CDF) was a substrate and diazinon (DZN) (CAS no. 333-41-5) was the metabolic inhibitor or vice versa, that diazinon was the substrate and chlorpyrifos the metabolic inhibitor. The measured inhibition Ki_s ($\mu mol\ L^{-1}$) are shown in Table 5 and were mathematically described as non-competitive (CDF or DZN to oxon, and CPF to TCP), or competitive ~~(~~[DZN to ~~IMHP (~~2-isopropyl-6-methyl-4-pyrimidinol~~))~~. (IMHP)].

Insert Table 5.

According to the model runs the binary interactions between CPF and DZN at environmentally relevant exposure levels are negligible. CPF has greater impact than

DZN as a binary mixture. Foxenberg et al. (93) did not determine the dosimetry and cholinesterase inhibition produced by a binary model of parathion and chlorpyrifos.

VII. Source-To-Outcome Models for Dietary Exposures

The combining of probabilistic exposure models to PBPK/PD models results in the formation of a source-to-outcome models, where exposure models (i.e., source-to-dose models) are linked to PBPK/PD to quantify pharmacokinetic (i.e., tissue dose or urinary excretion) or pharmacodynamic (i.e., AChE, BuChE inhibition, etc) outcomes. Although this procedure appears to be a natural thing to do, there hasn't been sufficient interest in constructing these models as exposure and PBPK/PD models have not always been available in the same laboratory.

A. Organophosphorus Insecticides.

Hinderliter et al. (117) were among the first to publish a dietary source-to-outcome model for chlorpyrifos using the published PBPK/PD model of Timchalk et al. (90**)** and the CARES dietary exposure model (Cumulative and Aggregate Risk Evaluation System; CARES version 3.0 (42)).

The scaled organ volume and cardiac output as a function of body weight equations used by Timchalk et al. (90) were replaced by equations for calculating a body mass index (BMI) from age, body height, body mass, and gender to calculate compartment volumes. Compartmental perfusion rates were estimated for each tissue volume. Blood flows for each tissue were calculated by multiplying the perfusion rate (L blood $h^{-1}L^{-1}$ tissue) by

the corresponding volume. Blood flows (QC, in L h^{-1}) were adjusted based on activity levels.

Variation in metabolic clearance (liver CYP450: chlorpyrifos to oxon or TCP; liver and blood PON1: chlorpyrifos oxon to TCP) were described based on enzyme distributions and body surface areas.

Linking the output of an exposure model to that of ~~a~~ PBPK/PD models requires careful consideration of how each model handles, exposure, dose and uptake (13). The CARES dietary exposure model was used to determine a single daily dose of chlorpyrifos on 5 consecutive days. The linked model (CARES-PBPK/PD) was run for 1000 adults and 1000 children using the outputs from the dietary exposure model (9 ng^{-1} kg^{-1} ~~bw^{-1}~~ d^{-1}~~ay~~, median dose). For each individual the concentration of chlorpyrifos in blood, AChE inhibition in the RBCs and brain, and urinary elimination of TCP were simulated and recorded at 15-min intervals over a period of 5 days. Peak RBC AChE inhibition in the most sensitive individuals (3 years olds) was less than 0.1% of basal levels.

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Hinderliter et al. (117) predicted that multiple oral dosages of 300 µg $kg^{-1}d^{-1}$ would produce 50% RBC AChE inhibition in individuals with the lowest 5% of liver PON1 activity. The model did not take into consideration differences in the rate of conversion of chlorpyrifos by liver CYP2B6 to chlorpyrifos-oxon prior to its hydrolysis by PON1. This inhibition is greater than the 75% AChE inhibition at 1000 µg kg^{-1} predicted by Foxenberg et al. (93) using a CYP-specific chlorpyrifos human PBPK/PD model.

Price et al. (118) linked exposure values from two chlorpyrifos dietary exposure models; LifeLine model estimates of 11 (2.7-47) and 3.4 (0.8-15) ng $kg^{-1}d^{-1}$ for children and adults, respectively, and CARES model estimates of 20 (4.7-67) and 5.7 (1.2-24) ng $kg^{-1}d^{-1}$ for children and adults, to the revised Timchalk et al. (90) model described by Hinderliter et al. (117). Simulations (5-day) were performed for each age group to estimate chlorpyrifos and its oxon in blood; the amount of TCP eliminated in urine; inhibition of AChE activity in blood and blood as a percentage of pre-exposure baseline levels.

Unfortunately in these two studies, the exposure levels (9 to 20 ng $kg^{-1}bw^{-1}d^{-1}$) were below those required to produce RBC AChE in exposed individuals receiving steady state dietary concentrations. Threshold exposure levels (i.e., dosages producing minimum changes in AChE levels, evaluation of model parameters) need to be established for adults and children using PBPK/PD models prior to linking them to exposure models.

B. Carbamate Insecticides.

No full length published source-to-outcome models were found in the literature involving carbamate insecticides, however, several abstracts/posters were presented at the Annual Meetings of the Society of Toxicology and ToxExpo™ (119, 120). In a poster by Zhang et al. (119), ~~EPA~~the USEPA's ERDEM ~~(Exposure Related Dose Estimating Model)~~ was used to develop a PBPK/PD model for the estimation of cumulative risk from exposure to three N-methyl carbamates (NMC) (i.e., carbaryl, aldicarb, and carbofuran). ERDEM with its powerful graphical interfaces allows modelers to enter model parameters for

more than one insecticide into the front end of the model without doing tedious computer programming. Exposure to the three insecticides was based on outputs from the SHEDS model. PBPK/PD model simulations (e.g., urinary biomarkers) were compared with urinary biomarker data from CDC's Third National Health and Nutrition Examination Survey, NHANES III. The cumulative model was also used to predict the distribution on red blood cell AChE activity.

In ~~an~~ ongoing research on a dietary/drinking water source-to-outcome model for carbaryl, Tan et al. (120) linked a refined PBPK model from Nong et al. (97) and the CARES dietary/drinking water exposure model (42). CARES was used to generate time-concentration profiles of 500 virtual individuals exposed to carbaryl in food and drinking water for 365 days. Using these time-concentration profiles as inputs to the refined PBPK model, time-course biomarker concentrations (i.e., 1-naphthol in urine) and other dose metrics (e.g., AChE in red blood cell) were simulated. The simulated biomarker data were then used to reconstruct an average daily carbaryl intake under various exposure conditions to evaluate several sources of uncertainty. Some examples of these sources are time between the last meal eaten and urine sample collection, frequency of food consumption, and urine volume and time of voids.

C. Pyrethroid Insecticides.

The linking of SHEDS version 4 model to pyrethroid PBPK/PD models (permethrin, cypermethrin and cyfluthrin.) was proposed by Tornero-Velez et al. (1~~1~~21) at a ~~U.S. EPA~~USEPA FIFRA Science Advisory Panel Meeting. A PBPK/PD model published by Godin et al. (111) (involving deltamethrin may be used as a template for pyrethroids. An

extensive review of pyrethroid metabolism by Kaneko (1221) (maybe found in Hayes Handbook of PesticidesToxicology, 3rd Edition, 2010. Urinary metabolite data may be used as a means of correcting exposure values obtained from SHEDS version 4 using dose reconstruction procedures with PBPK/PD models.

D. Pesticide Mixtures.

The binary mixture model of Timchalk et al. (116) could be readily used in constructing dietary/environmental source-to-outcome models for mixtures of chlorpyrifos and diazinon.

VIII. Conclusionsding Remarks

The U.S. Environmental Protection Agency (USEPA) under the provisions of FIFRA, the Federal Insecticide, Fungicide and Rodenticide Act, regulates the development, registration, sales and use of pesticides in the United States. The Act requires the development of extensive toxicological data (acute and chronic studies), field efficacy and dissipation of crop and animal residues, and the fate of pesticides in the environment (i.e., water, soil and air) prior to registration and use. USEPA is required to set pesticide food tolerances while the U.S. Food and Drug Administration under the provisions of FFDCE (Federal Food, Drug and Cosmetics Act (FFDCE) enforce pesticide tolerances.

The provisions of FIFRA and FFDCE were managedable at the state and federal levels until the passage of FQPA (Food Quality Protection Act of 1996 (FQPA) which added a ten fold safety factor to food tolerances to protect the health of children and requirements to assess human exposure (i.e., children and adults) to food and environmental pesticide

residues. Since the 1972, 1975 and 1978 amendments to FIFRA resulted in the development of application and post application exposure monitoring test guidelines 875 (32), FQPA did not require the development of new methods or procedures for carrying out these assessments ~~as the 1972, 1975 and 1978 amendments to FIFRA resulted in the development of application and post application exposure monitoring test guidelines 875 (32)~~. The passage of FQPA led to the development of exposure data bases (i.e., CAPS, CHAD, CPPAES, CSFII, CTEPP, NHANES, NHAPS, NHEXAS-Maryland and NHGPUS) and the development of stochastic/probabilistic exposure models (i.e., DEEM, SHEDS, LifeLine, etc.). ~~The total cost of developing these exposure data bases and models is unknown,~~ The exposure databases and models ~~but they~~ are designed to simulate exposure. As such they compliment ~~have largely replaced the use of~~ predictive models (i.e., PBPK/PD and QSAR models) in estimating exposure and human risks to pesticides using margins of exposure (MOEs), ADIs, and NOAELs. Recent use of the probabilistic exposure models suggest they need to be linked to single or mixed pesticide PBPK/PD models to predict risks (121). This chapter and recent reviews of the availability of PBPK/PD models and parameters (84, 85, and 86) indicate that there are an insufficient number of models/parameters to meet the needs of FQPA. Development of ~~We highly recommend~~ PBPK/PD models ~~these models be developed~~ under FIFRA using data gained as part of the registration process and adoption of probabilistic exposure linked PBPK/PD models would address issues important for FQPA ~~as part of the registration process.~~

Comment [VD9]: Can you say this? Or would you rather say something like, "…development of more rigorous models would….?

Insert Table 6

Acronyms and Abbreviations

ADI	Acceptable daily intake

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CAPS	Children's Activity Pattern Study in California
CFSAN	Center for Food Safety and Applied Nutrition
CHAD	Consolidated Human Activity Database
CPPAES	Children's-Post-Pesticide-Application-Exposure-Study
CSFII	USDA Continuing Survey of Food Intakes by Individuals
CTEPP	Children's Total Exposure to Persistent Pollutants study
DEEM	Dietary Exposure Evaluation Model
ERDEM	Exposure Related Dose Estimating Model
FCID	Food Commodity Intake Database
FDA	Food and Drug Administration
FF	Food form
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FFDCA	Federal Food, Drug and Cosmetics Act
FQPA	Food Quality Protection Act
LOD	Limit of detection
MOE	Margin of Exposure
NCI	National Cancer Institute
NHANES	National Health and Nutrition Survey
NHAPS	National Human Activity Pattern Survey
NHEXAS	National Human Exposure Assessment Survey
NHGPUS	National Home and Garden Pesticide Use Survey
NMC	N-methyl carbamate insecticide
NOAEL	No observable adverse effect level
OP	Organophosphorous insecticide
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
PDP	USDA Pesticide Data Program
QSAR	Quantitative structure activity relationships
RAC	Raw agricultural commodity
SAP	Science Advisory Panel
SHEDS	Stochastic Human Exposure and Dose Simulation
US EPA	United States Environmental Protection Agency
USDA	United States Department of Agriculture

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