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Exposure, Risk Assessment and Predictive Exposure Model Development for Agricultural Operator in Representative Crop Fields

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Exposure, Risk Assessment and Predictive Exposure Model Development for Agricultural Operator in Representative Crop Fields

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Abstract

Korea predictive model for the estimation of agricultural operator exposure has been developed on the basis of new exposure data to improve the current agricultural operator exposure and risk assessment in the Korea. The new operator exposure model represents current application techniques (speed sprayer and power sprayer) and practices in representative crop fields (apple orchard and rice field). 30 replicate exposure studies conducted between 2010 and 2012 were evaluated for the new model. Exposure and risk assessment were conducted for agricultural applicators during preparation of spray suspension and application with speed sprayer and power sprayer on crop fields. Several exposure matrices, including patches, cotton gloves, socks, masks and XAD-2 resin were used to measure the potential exposure for workers. The analytical methods were fully validated to guarantee the precision and accuracy of analysis. As a major factor contributing to the exposure of operators, the amount of active ingredient used per day was identified. Other parameters such as formulation type, density of the canopy were selected as factors for sub-scenarios. Accordingly, 75 percentile of exposure dose was calculated for mixing / loading and application according to scenario, and it was derived as exposure factor of Korean model. *In vitro* metabolism of kresoxim-methyl was conducted with human liver microsome. Two metabolites were identified. The screening test for identifying which recombinant CYP involved with metabolism of kresoxim-methyl was conducted with 10 human cDNA-expressed CYP isoforms. Eight rCYPs (except 2A6, 2E1) contributed to metabolism of kresoxim-methyl.

Key Words: dermal exposure, exposure model, human liver microsome, inhalation exposure, metabolism, risk assessment

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Introduction

Occupational exposure study

Pesticides have been used for the management of many insect pests, diseases, and weeds in agriculture for a long time. Agricultural workers would be exposed occupationally to pesticides during manufacture, mixing/loading, spraying, and harvest, while consumers could be exposed routinely by consumption of contaminated foods and drinks. In agriculture, the representative routes of exposure of pesticide applicators are dermal deposition and inhalation during pesticide application activities (Choi et al., 2006).

From recent studies that assessed occupational exposure to pesticides, it was consistently demonstrated that 99% or more of total exposure occurred by the dermal route and 1% or less occurred via the respiratory route (Machado-Neto, 2001). The most important variables that may affect occupational dermal exposure are the type of formulation and packaging, application technique, working method, and agricultural and climatic conditions (van Hemmen, 1992). Because pesticides are inherently toxic to living organisms, they are more likely to affect the health of humans than other agricultural chemicals (Tahir and Anwar, 2012). Therefore, it is positively necessary to quantify the occupational exposure to pesticides for safety evaluation of workers.

Many exposure studies have been conducted for a workers, such as mixer/loader and applicator in a greenhouse, paddy field, and orchard, including apple, mandarin, and mango (Calumpang and Medina, 1996; Capri et al., 1999; Liu et al., 2003; Byoun et al., 2005a; Ramos et al., 2010; Baharuddin et al., 2011; Kim et al., 2012a; Kim et al., 2012b; Moon et al., 2013), estimating the deterministic exposure of individual workers to pesticide.

Table 1. Some exposure studies of pesticide on crop (2000-2016)

Pesticides	Substance	Dermal exposure	Inhalation exposure	Risk assessment	Reference
Spinosad	Grape	Whole body	-	-	(Thouvenin et al., 2016)
Flonicamid	Apple	Patches	PAM XAD-2	-	(Zhao et al., 2015)
Deltamethrin Procymidone	Tomato*	Whole body	-	MOS	(Ramos et al., 2010)
Chlorpyrifos	Cotton	Patches	PAM XAD-2 OVS tubes	MOE	(Farahat et al., 2010)
	Pepper	Patches	-	-	(Nuyttens et al., 2009)
Malathion	Pepper	Whole body	-	-	(Machera et al., 2009)
Deltamethrin	Maize/Broccoli	Whole body	-	MOS	(Hughes et al., 2008b)

Pesticides	Substance	Dermal exposure	Inhalation exposure	Risk assessment	Reference
Endosulfan	Cotton	Patches	-	another	(Kuye et al., 2007)
Captan	Maize	Whole body	-	MOS	(Hughes et al., 2006)
Cypermethrin	Mandarin	Patches	PAM-XAD-2	MOS	(Choi et al., 2006)
Methidation	Cucumber	Patches	PAM-XAD-2	MOS	(Byoun et al., 2005b)
Malathion	Tomato	Whole body	PAM-XAD-2	-	(MACHERA et al., 2003)
Mancozeb	Mandarin	Patches	-	MOS	(Liu et al., 2003)
Lindane,.etc	Tomato ,.etc	Whole body	-	-	(Vidal et al., 2002)

Pesticides	Substance	Dermal exposure	Inhalation exposure	Risk assessment	Reference
Visible tracers Sunset Yellow	Tomato Cucumber	Whole body	IOM samplers-GFA	-	(Machera et al., 2002a)
Lindane,.etc	Tomato ,.etc	Whole body	-	-	(Frenich et al., 2002)
Chlopyrifos		Patches Whole body	OVS Sampling pump	-	(Cattani et al., 2001)
Chlorpyrifos-methyl Fenitrothion	Green been	Whole body	-	-	(Castro Cano et al., 2000)

Methodology of agricultural worker exposure to pesticides

Agricultural workers who mix/loads and spray pesticide in fields expose to pesticide through dermal and inhalation routes. In such situation, exposed amount should be measured quantitatively for reasonable risk assessment. Patch, gloves, socks and mask will be good materials for monitoring for dermal exposure while personal air monitor equipped with solid adsorbent and air pump will be a tool for inhalation exposure. For extrapolation of absorbed amount in dermal exposure matrices and of trapped amount in solid sorbent to total dermal or inhalation exposure, Korean standard body surface area and respiration rate were proposed in substitution of EPA data. Important exposure factors such as clothing and skin penetration ratio of dermal and inhalation exposure were suggested based on Spraying time for exposure monitoring must be long enough that the amount of pesticide to get absorbed/trapped in exposure matrices results in reasonable analytical value. In domestic case for the both of speed sprayer and power spray machine, spraying time of 20~40 minutes (0.1~0.2ha) will be reasonable per single replicate before extrapolating to 4 hours a day with triplicates experiment.

Dermal exposure

Skin is an important route of exposure for materials at workplaces. It is clear that the behavioral component of exposure is very important, and that uptake of chemicals through the skin can exhibit high variability. Since the primary route of exposure in pesticide handlers is through the skin, most work in this field has focused on characterizing deposition of chemicals on the skin and their subsequent absorption into the body (Fenske and Day, 2005)

Dermal exposure is often divided into potential dermal exposure and actual dermal exposure. The definitions of these concepts vary in different sources. Whole body dosimetry, patch were mainly used to measure dermal exposure. In patch method, the potential contamination of the clothing is measured using a variable number of absorbent cloth or paper patches, attached to body regions inside or outside clothing (Durham and Wolfe, 1962). The surface area covered by the patches represents less than 10% of the total body surface area. After a defined or measured period of exposure, the patches are removed and analyzed for pesticide content. Body part surface areas can be obtained from standard reference texts and exposure guidance documents. The potential dermal exposure to pesticide sprays can be measured with the patch method (Durham and Wolfe, 1962; Davis, 1984; Gold and Holcslaw, 1985; Kurtz D and Bode W, 1985; Nigg H and Stamper J, 1985; Grover et al., 1986; Fenske et al., 1990; Fenske, 1993; Soutar et al., 2000; Liu et al., 2003; Kuye et al., 2007; Nuyttens et al., 2009; Farahat et al., 2010). The whole body method is an alternative to the patch method and allows measuring the body exposure over its whole surface area using dedicated cloth layers. The cloth layers mimic as well as possible the clothes people would normally wear during their work. Using this method, dermal exposure of the body and head (with a hood or a separate head cover or face/neck wipes) can be measured accurately.

Risk assessment

PDE, measured by analyzing the quantity of a pesticide that deposited on body regions, provides the information on the amount of exposure. But PDE data by itself cannot be used as a risk indicator because it must be related to acceptable

exposure limit. For this purpose, the MOS has been proposed as a useful risk indication (Machado-Neto, 2001) that relates the acceptable exposure to a pesticide with the mass absorbed by the body, which can be estimated from PDE. MOS value higher than 1 are considered as an indication of safe working condition. Durham and Wolfe (1962) derived the first equation to calculate the risk of intoxication from pesticides considering the toxicity and the exposure; residue/unit area of exposure pad surface is multiplied by a time factor, which is determined by the length of time the worker is exposed. In this equation, the risk was expressed in terms of percentage of toxic dose per hour (Davis, 1980). The MOS formula, proposed by Severn (1984), was the first equation to calculate the safety of work conditions for the agricultural handling of pesticides. Franklin (1985) proposed the following steps for risk assessment in the use of pesticides: obtain work exposure data and determine dermal/inhalation absorption correction, dosage estimation, NOEL from toxicological data, MOS or quantitative risk assessment, and acceptability of MOS. Work conditions, according to those risks, can be classified as safe or unsafe, considering the estimated MOS. In addition to the level of exposure incurred per hour of work, the hazard of pesticide exposure to workers was also related to the amount of time spent under these specified working conditions (Wolfe, 1976). However, risk characterization may have to proceed in the absence of an adequate or complete data set in many cases. In such circumstances, and when the adverse effect is considered to show a threshold in the dose-response relationship, additional uncertainty factors may be applied to the NOAEL (mg/kg body weight per day). The MOE is the ratio between the NOAEL and the estimated amount of human exposure. These approaches are

normally used when limited toxicological data or human data exist but the hazard identification and hazard characterization data are insufficient to set a health-based guidance value. Therefore, MOE is the ratio between the NOAEL in experimental or epidemiological studies, and the estimated amount of human exposure, while MOS is the ratio between the NOEL and the actual amount of human exposure (Renwick et al., 2003). Risk management / reduction is a major measurement if risk possibility was identified through risk assessment procedure. The determination of SWT and exposure control need ECN for unsafe work conditions (MOS < 1) can also be used in management strategies dealing with pesticide risks. The calculation of SWT can be used as a safety measure to control occupational exposure in pesticide use. The calculation of ECN permits the selection of a more appropriate safety measure, in regard to the needs of each set of working conditions (Machado-Neto, 2001).

Predictive model

Exposure estimates that are required for risk assessment may be obtained from chemical specific field studies, or from extrapolations from other field studies. This requires high quality exposure data that have been obtained under conditions relevant for the exposure and use scenarios under consideration. For risk assessment purposes, the exposure data obtained for relevant use scenarios can be compared with an appropriate accepted exposure level (Acceptable operator exposure level (AOEL)) based on the toxicological profiled of the compound. The generic approach to exposure assessment based on the grouping of uses into scenarios where activities are similar was presented by Franklin et al. (1982). This approach was further advanced by Hackathorn and Eberhart (1985), who discussed the development of a database for predictive

exposure modelling. The basic idea behind the development of such databases is that the level of exposure, when in a suitable format, can be extrapolated for similar-use scenarios. Currently, it is assumed that for mixing/loading, the main differentiation is for formulation type (i.e. between powders, granules, and liquids) used for hand-held application equipment, vehicle-mounted equipment. For application, the main differentiations used are upward or downward spraying and hand-held or vehicle-mounted spraying scenarios. Such databases contain field studies and exposure data extracted from those field studies in several formats (Kangas and Sihvonen, 1996). These studies are all different and most of them are not publicly available or critically reviewed in respect to documented criteria. The most important independent databases in use for risk assessment purposes in formal pesticide registration processes are PHED, the German model, UK-POEM, the Dutch model and EUROPOEM. These databases, also called predictive exposure models.

The UK-POEM database is based on a review of the data available on the exposure of pesticide spray operators (in the UK). The review indicated that several factors determined the dose absorbed by a spray operator. These included the following: the volume of external contamination, the extent to which this external contamination penetrated clothing to reach the skin and the rate at which the chemical come into direct contact with the skin surface and was absorbed. These various independent factors were assumed, with the exception of dermal absorption, to be of a sufficient generic nature to be suitable for extrapolation purposes. Two major work activities were differentiated: mixing/loading and application. An updated of the default values in UK-POEM has been presented (POEM, 1992) or mixing/loading, two formulation types are considered, namely liquids and powders. The database is largest for liquids and the level of exposure is shown to be largely dependent

on the container size and the neck aperture. A default value for the 75th percentile was based on test data on pouring. The current risk assessment for pesticide operators is based on the comparison of a reliable exposure estimate with the respective AOEL normally derived from subacute or subchronic toxicological studies. The use of a plant protection product is considered safe when the exposure estimate calculated for daily systemic exposure is below the AOEL. UK POEM is deterministic models that rely on empirical data from exposure studies conducted before 1990 and allow exposure predictions for mixing/loading and application. Exposure in the models largely depends on the total amount of active substance used, the duration of exposure or the container size and number of mixing/loading tasks. Moreover, the formulation type and the spray equipment are important factors, too. In the UK-POEM professional operators are assumed to wear work clothes that cover the whole body and are permeable for a certain fraction of the contaminating pesticides. Additional protective equipment for the operators (e.g. protective gloves) can be chosen in model in order to reduce the exposure prediction (Großkopf et al., 2013).

The models are based on a limited number of studies and are not validated. Thus, the models may not always be sufficiently representative for Korea conditions. The limitations of model estimates of exposure are taken into consideration when the calculated level of exposure is close to the threshold limit for AOEL.

Korea Predictive Operator Exposure Model

A new model for prediction of exposure of professional operators applying plant protection products outdoors has been developed using previously unpublished field data collected between 2011 and 2012. It provides calculations for estimating the exposure for typical scenarios including the mixing/

loading and the application of plant protection products. The underlying equations are based on log linear models for prediction of the 75th percentile and consist of exposure factors that were selected after a statistical analysis. The exposure mainly depends on the total amount of active substance used per day and is further described by additional factors or particular sub-scenarios. The model allows a tiered approach starting with estimating exposure for an operator wearing at least one layer of clothing; risk mitigation by using personal protective equipment can be considered if the AOEL is exceeded. The model reflects current application techniques and typical work conditions in Korea. It is, therefore, applicable for registrations of plant protection products in Korea. Updated versions of the model will be periodically created if new data become available.

Part 1

Probabilistic Exposure Assessment for Applicators during Treatment of the Fungicide Kresoxim-methyl on Apple Orchard by Speed Sprayer

Introduction

The deterministic approach (point estimation) is very simple method to estimate the exposure and completely lacking in uncertainty quantification (WHO, 2008). The deterministic exposure assessment could not be sufficient for risk management decision because the levels of pesticide exposure for applicator could be fluctuated widely by different agricultural conditions, even if same person apply the pesticide suspension on the same kinds of crops. For substances requiring further refinement beyond point estimates of exposure, a probabilistic (stochastic) analysis of exposure variability can be conducted (WHO, 2008). Probabilistic approach is conducted by combining frequency distributions that the variable will take any specific value in the range of values and can quantify uncertainties (Gilsenan et al., 2003). Therefore, worker exposure must be thought of a range of values, rather than a single value, because individual of worker population have different levels of exposure. Kresoxim-methyl is a strobilurin fungicide which has been used for the control of scab (Venturia spp.) and powdery mildew (Podosphaera leucotricha) in apples, mandarin, grape and pears. It has an inhibition activity against spore germination with long residual effect. It is poorly soluble in water (solubility: 2 mg/L), and is relatively stable at pH 5. Toxicity to mammalian is low as acute oral LD50 for rat is >5,000 mg/kg, and acceptable daily intake (ADI) value is 0.4 mg/kg b.w./day (Tomlin, 2009). To the best of our knowledge, there are no previously published studies on probabilistic exposure assessment for workers during application of kresoxim-methyl. The present study was carried out to estimate probabilistic exposure by Monte-Carlo simulation when kresoxim-methyl was applied in apple orchard using speed sprayer by thirty replicates.

Major exposure characteristics were identified and related risk assessment was conducted using acceptable operator exposure level (AOEL) of kresoxim-metyl.

Materials and Methods

Reagents and materials

Kresoxim-methyl water-dispersible granule formulation (Haevichi, WG, 50%, Sungbo Chemical, Seoul, Korea) was obtained through the local vendor. Analytical standard of kresoxim-methyl (purity, 97.8%) was purchased from the ChemService Inc. (West chester, PA). All solvents were HPLC grade, and purchased from Burdick & Jackson (SK Chemical, Ulsan, Korea).

Dermal exposure matrices

Patches made by putting cellulose TLC (Thin-layer chromatography) paper (17CHR, 1 mm thickness, Whatman International Ltd., UK) in the patch pocket (10 cm × 10 cm). The area of the circular exposure part on the patch was 50 cm^2. The back of the TLC paper in a patch was covered with aluminum foil to prevent contamination (Kim et al., 2012b). Cotton gloves, socks and square cotton mask were used for hand, feet and face exposure measurement, respectively (Kim et al., 2011). The area of square cotton mask was 200 cm^2.

Inhalation exposure matrices

For inhalation exposure measurement, a personal air monitor equipped with air pump (GilAir-3, Sensidyne, Clearwater, FL), solid sorbent tube (ORBOTM 609 Amberlite XAD-2 400/200 mg, Supelco, Bellefonte, PA) and glass fiber filter (Type AE, SKC, Eighty four, PA) in 37 mm open-faced cassettes were used (Kim et al., 2012b; Kim et al., 2013; Moon et al., 2013). The solid sorbent tube consisted of one larger bed of absorbent (400 mg) followed by a smaller back-up bed (200 mg) to capture any sample breakthrough.

Experimental sites and field trials

The field studies were conducted in apple field (Gunwi-myeon, Gyeongbuk, Korea). The temperature and relative humidity were monitored with a thermometer and a hygrometer at the start and end of the mixing / loading or spraying activity, respectively. Wind speed was measured using a pocket weather meters (Kestrel 3000, Nielsen-Kellerman, Boothwyn, PA) (Table 2). Before mixing/loading or application, all workers wore protective garments (SP protective, KleenGuard, Yuhan-Kimberly Korea Ltd, Seoul, Korea). Workers made spray suspension for 9~18 min by mixing Haevichi WG 50% (167 g, 1 pack) with 500 L water in mixing tank and sprayed two tanks of 500 L spray suspension with speed sprayer (Model TLD ASS-555, Asia Motors, Daegu, Korea) for 24~48 min on 0.2 ha. Each replicate consisted of two 500L mixing/loadings or applications and 30 replicates were made (60 spray of 500L tank).

Table 2. Field characteristics, application and meteorological conditions in apple orchard

Field		Application	
application area (ha)	0.20–0.33	application method	speed sprayer
age of plants (years)	3–35	number of nozzle	25–29
plant growth stage	fruiting stage	boom length (m)	4–12
planting density	dense	climate	
plant height (cm)	350–500	temperature (°C)	15–31
inner row distance (cm)	100–500	relative humidity (%)	22–82
row distance (cm)	100–400	wind speed (m/sec)	0.3–3.0

Exposure matrices sampling

During mixing/loading, workers wore only gloves. In application, dermal patches were placed on the outer protective garment, over forehead, front of neck, back of neck, chest/abdomen, back, upper arms, forearms, thighs and shins of workers. Applicators wore cotton gloves, cotton socks and masks. A glass fiber filter cassette and a XAD-2 resin tube were attached with clips on breathing zone of worker, and air pump was fastened on the belt. The air flow rate was 1 L/min. After mixing/loading or spraying, exposure samples were collected by preventing contamination.

Extraction of kresoxim-methyl from exposure matrices

Kresoxim-methyl on patches, gloves, socks and mask were extracted with 50 or 300 mL of methanol in a 100 or 500 mL glass bottle by shaking at 200 rpm for 1 h in a shaker (Wooju Scientific, Gimpo, Korea). After 1–2 mL of the extract were filtered through a 0.2 μm pore syringe filter (4 mm, Milipore, Billerica, MA), aliquots were analyzed with high performance liquid chromatography (HPLC). The pesticide trapped on XAD-2 resin and filter was extracted using 10 mL of methanol in a 20 mL vial and concentrated 10 times by nitrogen evaporator (Reacti-VapTM, PIERCE, Rockford, IL) before HPLC analysis.

Instrumental conditions

Kresoxim-methyl residues were determined with HPLC (Agilent 1100 series, Agilent Technologies Inc., Santa Clara, CA) with an automatic injector. Patch extract was analyzed with a Luna C18 column (5 μm particle size, 4.6×250 mm,

Phenomenex, Torrance, CA) using the mixture of acetonitrile : water = 80 : 20 (v/v) as mobile phase. Meanwhile, for analysis of gloves, socks, mask, XAD-2 and filter samples, gradient elution and a ZORBAX Eclipse XDB-C18 column (5 μm particle size, 4.6 x 250 mm, Agilent Technologies Inc., Santa Clara, CA) was used due to more complex matrix. The mobile phase in a gradient system was programmed as follows: 20% acetonitrile at initial time, increased to 50% acetonitrile for 10 min, held for 20 min, increased to 80% acetonitrile for 1 min, and held for 4 min. The flow rate was 1.0 mL/min and injection volume was 20 μL. A diode array detector (Agilent Technologies Inc., Santa Clara, CA) was used for detection at 220 nm.

Method validation

Aliquots of standard solutions from 0.001 to 1 mg/L were analyzed for determination of instrumental limits of detection (ILODs) and instrumental limits of quantitation (ILOQs). The method limits of quantitation (MLOQs) were calculated from ILOQs, injection volume and extract solvent volume in analytical method. After injecting three levels (MLOQ, 10MLOQ and 100 MLOQ) of standard solution 7 times, C.V. values of the integrated peak area was determined to validate instrumental repeatability. Various standard solutions (LOQ level) were analyzed and the linearity of the curve was investigated after one day and three days of preparation. For recovery test, control exposure samples were fortified in three levels (MLOQ, 10 MLOQ and 100 MLOQ) of standard solution. The trapping efficiency was tested by spiking two levels of standard solution (10 MLOQ and 100 MLOQ) on the bottom of U-shape glass tube (Daejung Chemical, Daejeon, Korea) connected with solid

sorbent, and passing air through the system at 1 L/min for 4 h. U-shape glass tube was heated to 70ºC to help volatilization of compounds. The residue in U-shape glass tube and the amount trapped in XAD-2 resin were analyzed for mass balance. Breakthrough test was conducted by spiking two levels of standard solution (10 MLOQ and 100 MLOQ) in the 1º-resin part of the solid sorbent tube and passing air through the tube at 1 L/min for 4 h. Subsequently, 1º- and 2º-part of resin were analyzed separately. All analysis and test were repeated 3 times.

Exposure assessment

Dermal exposure amount (μg) per body part was determined by extrapolating kresoxim-methyl residue (μg) on a dermal exposure matrix to the ratio of the area of the matrix to the body surface area (cm2) for adult Korean (Kim et al., 2011). Inhalation exposure amount (ng) was obtained by extrapolating pesticide residue (ng) in an inhalation exposure matrix to the ratio of the air volume collected to the respiration rate of working situation (1,270 L/h) for adult male Korean (Kim et al., 2011). The PDE and PIE per day were determined by multiplying the corresponding exposure amount with ten working activities (mix/loading and application using speed sprayer). The ADE for mixer/loader and applicator was calculated from PDE based on 1% and 10% the penetration rate through clothes (Kim et al., 2011) and 0.3% (neat formulation) and 13% (spray mix) skin absorption (European Food Safety, 2010), respectively. The AQE (mg/day) value was determined by adding up of ADE and PIE on the assumption of 100% absorption through inhalation (Oliveira and Machado-

Neto, 2003; Fenske and Day, 2005; Kim et al., 2012b). The AQE per unit body weight was obtained by dividing AQE by body weight.

Exposure estimation using Monte Carlo simulation

Pesticide exposure (AQE per body weight) was modeled as:

AQE per body weight =

$$\frac{\{\sum_i (dermal\ exposure)_i \times (penetration\ rate)_i \times (skin\ absorption\ rate)_i\} + inhalation\ exposure}{Body\ weight} \times 10$$

Where, dermal exposure for each body part i, inhalation exposure and body weight are randomly selected from input distributions. Dermal and inhalation exposure were fitted with the best distribution to describe the distribution of exposure per body part. Akaike Information Criterion (AIC) was used to choose the best fitted distribution. The quality and stability of the reported parameters and statistics for the best fitted distribution was proved by the method of parametric bootstrap. We made an a priori determination to represent body weight for adult male Korean (mean 70.5 kg b.w., 95%CI 51.2–94.7 kg b.w.) as empirical cumulative distribution, because this distribution was considered the most representative description of the data of the large sample size. The forecasts, including PDE, PIE, ADE, ADE and AQE were determined by performing Monte Carlo simulations using probability distribution functions of body parts exposure. Monte Carlo simulations were conducted with Latin Hypercube sampling. After exposure estimation using Monte Carlo simulations, sensitivity analysis was conducted to look at which input distributions have the greatest effect on the eventual distribution. For checking the output reproducibility by simulations, the variance of forecasts for AQE per unit body weight was calculated by repeating each simulation three times with different

number of iterations. Fitting distributions to data and Monte Carlo simulations were conducted using @RISK (Palisade Corporation, Ithaca, NY, USA).

Risk assessment

The risk assessment was carried out by comparing the pesticide exposure forecasted to the relevant risk value, AOEL. For this, the margin of safety (MOS) for workers was calculated using following modified formula: MOS = AOEL / AQE per unit body weight. AOEL of kresoxim-methyl is 0.9 mg/kg b.w./day. If MOS $\geq$ 1, the working condition is considered to be safe.

Results and discussion

Selection of crops and pesticide

Apple, one of the most produced and consumed fruits in Korea, was selected to represent orchard because the cultivated area and yield of apple was 30,449 ha (18.9% of fruits) and 493,701 tons (19.6% of fruits) in 2013, respectively. In orchard, 64.9% of farmers prefer to use speed sprayers than power sprayers for the application of pesticides (Hong et al., 2007). Kresoxim-methyl was selected for this study because this pesticide was applied on more than 60% of apple farms in Korea.

Method validation

ILOQ was defined as 1.0 ng, 5 times greater than ILOD, 0.2 ng (signal-to-ratio of >3). MLOQs of exposure matrices were determined as 2.5, 15, 15, 15 and 0.05 μg for patches, gloves, socks, mask and XAD-2 resin, respectively. These values were low enough to quantify the trace level of kresoxim-methyl in exposure matrices. Good instrumental repeatability (C.V. < 8%) and consistent linearity of calibration curves for 3 days (R^2 >0.999) showed the analytical instrument was precise and stable (Table 3). Recovery from various matrices were ranged from 71.2 to 119.5% (C.V. = 1.3–10.7%) (Fig. 1), providing the extraction efficiencies are reliable. In trapping efficiency test, mass balance (> 90%) was good and the most of kresoxim-methyl remained in XAD-2 resin (Table 4). Kresoxim-methyl was retained more than 90% on the first part of the XAD-2 resin in the breakthrough test, indicating that the first resin part possessed a suitable holding capacity (Table 4). Therefore, XAD-2 resin was useful for trapping and holding kresoxim-methyl in air, as successfully applied

for other pesticides (Kim et al., 2012b). In short, all analytical and sampling methods were well validated according to above results.

Determination of the number of iterations

The output stability from simulation to simulation was an especially important for the precision of forecasts. The variance of the mean and high-end 95th percentile forecasts of AQE per unit body weight was calculated by repeating each simulation three times with 100, 500, 1,000, 5,000 and 10,000 iterations. Because the high-end forecasts consistently stabilized at <0.5% variance when the number of iterations was more than 10,000 iterations, 10,000 iterations were used in all subsequent analysis (Fig. 2).

Table 3. Instrumental repeatability and linearity of calibration curve

Instrumental repeatability		
Levels	Average (area)	C.V. (%)
ILOQ	4.7	7.7
10 ILOQ	37.6	1.3
100 ILOQ	374.3	0.4
Linearity (R^2)		
Day of preparation		0.999
After 1 day		0.999
After 3 days		0.999

Figure 1. Recovery of kresoxim-methyl from exposure matrices

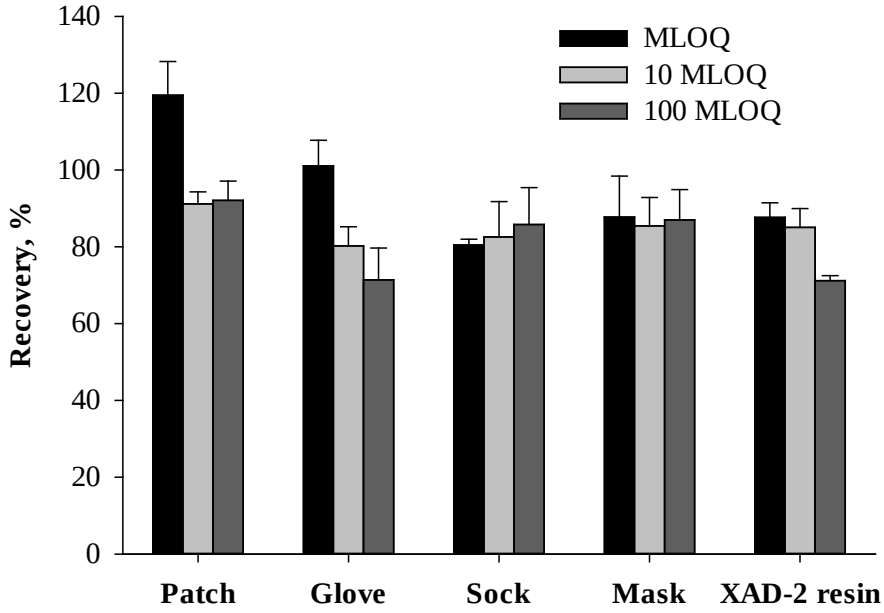

140
120
100
80
60
40
20
0
Recovery, %
MLOQ
10 MLOQ
100 MLOQ
Patch
Glove
Sock
Mask
XAD-2 resin

Figure 2. Variability in AQE per unit body weight as simulation estimates by a function of iteration number

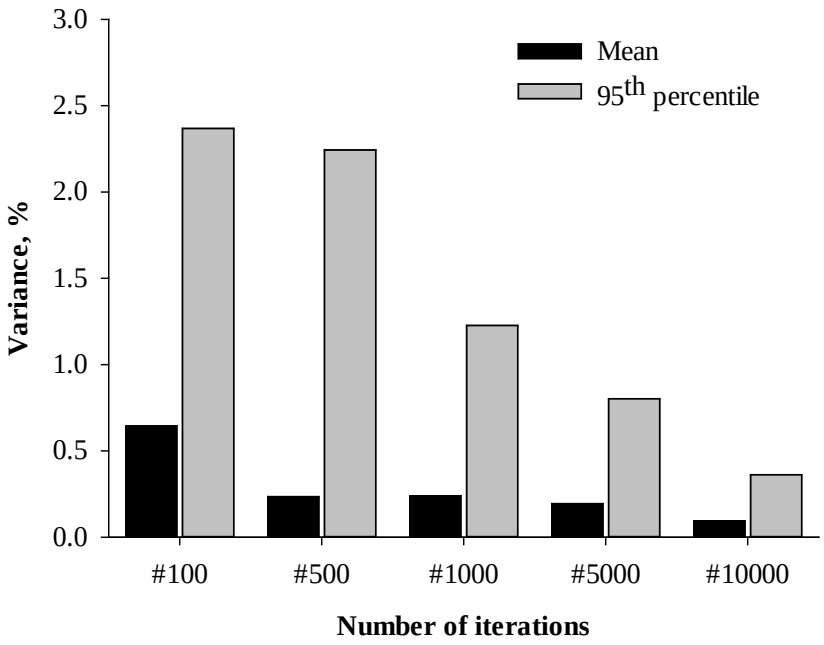

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Variance, %
Mean
95th percentile
#100
#500
#1000
#5000
#10000
Number of iterations

Table 4. Breakthrough and trapping efficiency of XAD-2 resin

Tests	treated level	recovery (%, mean±standard deviation)		
		1º-XAD		2º-XAD
breakthrough	10 MLOQ	94.4±6.5		13.4±10.8
	100 MLOQ	110.4±5.7		23.6±14.0
		residue	XAD	Total
trapping efficiency	10 MLOQ	11.0±0.3	84.5±1.8	95.5±1.6
	100 MLOQ	12.6.0±0.2	88.0±0.8	100.6±1.0

Dermal exposure assessment

The dermal exposure during mixing/loading was measured on only hands as suggested by national guidelines. Because the hands were reported to be significantly more exposed than other body parts and occupied 19~100% of the total dermal exposure for mixer/loader (Machera et al., 2002b; Kim et al., 2012b; Choi et al., 2013; Moon et al., 2013; Choi and Kim, 2014). The hand exposure to kresoxim-methyl for mixer/loader was 0.7 mg (95%CI 0.02–2.4) (Table 8), taking 0.0005% (95%CI 1.2×10^{-5}–0.001) of total prepared active ingredient (a.i.). This ratio of dermal exposure was less than 0.0007~0.59% reported in the previous studies (Kim et al., 2012a; Kim et al., 2012b; Choi et al., 2013; Kim et al., 2013; Moon et al., 2013; Choi and Kim, 2014), because WG formulation used in this study is larger and heavier than WP and is less absorbed into cotton glove than EC or SL.

During application of kresoxim-methyl, the amount of dermal exposure was 17.5 mg (95%CI 9.3–28.9), corresponding to 0.010% (95%CI 0.006–0.017) of total applied a.i. (Table 8), being within the range of 0.003~0.048% on apple orchard as reported in previous studies (Kim et al., 2012b; Kim et al., 2013; Moon et al., 2013). The results of sensitive analysis showed that thighs and shins, each with correlation coefficients of 0.53 and 0.43, respectively, had the greatest influence on the dermal exposure during application, followed by chest/abdomen and upper arms showed correlation coefficient 0.33 and 0.30, respectively (Fig. 3). Similar tendency was also observed during application of acetamiprid, fenvalerate and methomyl with SS (Kim et al., 2012b; Kim et al., 2013; Moon et al., 2013). Whereas the hands was reported to be the major exposed parts if the power sprayer was used on apple orchard (Machera et al.,

2002b; Choi et al., 2013; Choi and Kim, 2014), hands for applicator using SS was exposed less than chest and arms.

Inhalation exposure assessment

The inhalation exposure during application was estimated as 6.8 ng (95%CI 0.4–17.0) from Triang distribution. The ratio of inhalation exposure to dermal exposure was 0.04%, being less than 0.1% of dermal exposure as observed in other studies (Kim et al., 2012b; Moon et al., 2013).

Exposure database for predictive model

The data for the model were calculated as mL/h and is shown in Table 5.

Table 5. Dermal exposure per hour of kresoxim-methyl (mL/h)

Trials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Head	24.7	9.1	3.6	7.2	11.8	4.4	8.1	19.4	7.6	4.5	4.3	17.3	12.2	3.4	20.3
Neck of front	5.4	0.5	0.3	0.6	0.4	0.2	1.1	1.0	1.8	0.7	0.6	2.1	1.3	0.4	2.3
Neck of back	4.0	1.4	1.4	1.0	1.7	0.7	1.3	2.8	0.9	0.8	0.5	4.6	1.5	3.1	2.3
Chest	31.0	9.0	2.2	6.4	10.9	0.2	3.9	9.9	9.9	2.2	5.0	38.1	27.7	27.3	47.2
Back	14.4	5.8	1.2	3.7	2.9	0.7	2.7	3.8	2.2	3.5	4.0	16.5	16.9	19.7	10.8
Upper arm(L)	10.8	2.3	0.8	1.3	2.1	0.5	2.2	13.3	9.0	2.2	2.2	9.2	10.6	16.2	8.9
Upper arm(R)	51.8	10.2	10.0	12.3	12.5	2.0	11.1	28.8	8.4	4.8	1.3	58.0	9.1	10.9	14.4
Forearm(L)	6.9	1.8	0.9	0.8	1.6	0.5	1.1	6.3	1.8	0.3	1.1	8.9	11.1	9.6	7.0
Forearm(R)	8.6	0.2	0.6	2.8	9.3	0.3	6.9	14.1	3.8	0.8	0.3	4.9	3.8	6.0	5.2
Thigh(L)	20.6	8.2	7.7	10.1	12.0	2.3	7.7	20.2	9.3	6.7	4.7	18.3	20.6	41.9	22.2
Thigh(R)	59.1	17.4	18.2	18.0	20.8	5.0	20.7	43.6	16.9	8.5	3.0	18.8	21.5	44.0	34.4
Shin(L)	24.5	13.6	6.2	3.7	8.8	4.4	7.4	10.6	10.1	3.2	5.7	27.9	25.9	19.0	14.6

Shin(R)	36.3	10.6	8.9	7.4	8.1	4.4	11.1	24.8	6.5	5.0	2.9	18.1	8.3	11.1	11.2
Face	5.5	0.7	0.6	1.4	0.9	0.8	1.3	2.6	2.8	2.7	0.5	1.0	0.7	1.3	1.0
Hands	14.8	2.9	2.4	3.2	4.1	1.5	1.4	0.8	14.2	1.3	14.6	1.0	28.6	3.8	2.8
Feet	6.2	1.0	0.5	1.2	1.5	0.6	2.5	2.8	2.7	8.9	1.3	4.9	2.6	5.2	3.9
Sum	324.6	94.7	65.5	81.1	109.4	28.5	90.5	204.8	107.9	56.1	52	249.6	202.4	222.9	208.5

Table 5. Continued

Trials	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Head	54.5	7.0	29.2	13.2	21.3	19.4	28.9	14.0	29.2	15.7	21.9	2.6	2.7	38.2	23.8
Neck of front	8.8	5.9	6.8	3.6	3.4	0.7	5.4	0.7	1.5	1.8	2.3	0.8	0.6	4.1	2.4
Neck of back	8.4	4.8	3.5	2.1	1.5	1.3	4.1	2.2	2.7	1.9	2.3	1.6	1.1	4.0	1.3
Chest	129.9	24.5	4.1	17.8	21.7	25.0	18.7	15.0	10.2	2.5	29.4	23.9	15.2	13.9	26.2
Back	88.9	5.2	6.9	42.4	6.1	4.4	10.7	15.7	2.3	2.2	26.2	18.1	4.6	10.2	18.3
Upper arm(L)	13.5	3.3	30.1	1.8	8.0	1.4	10.7	4.7	2.2	2.1	6.0	4.2	3.9	11.0	14.2
Upper arm(R)	17.2	26.2	20.8	2.7	20.1	24.3	7.5	5.3	24.0	19.9	29.2	8.1	3.0	5.4	30.2
Forearm(L)	23.0	6.0	3.4	2.2	11.8	11.2	5.3	3.6	2.2	2.2	5.4	2.9	1.4	24.3	14.7
Forearm(R)	29.3	24.2	19.2	1.4	19.3	9.6	12.0	0.9	5.4	2.5	16.9	2.5	2.0	5.3	15.8
Thigh(L)	70.8	31.0	25.5	11.3	35.5	17.7	20.8	22.8	19.5	14.3	25.0	20.3	19.8	34.2	57.4
Thigh(R)	44.0	63.0	60.4	11.6	32.4	26.8	17.4	43.8	49.4	21.9	37.5	16.6	15.7	67.4	50.6
Shin(L)	96.6	49.3	44.3	14.9	16.6	0.5	3.7	11.5	20.6	13.2	15.7	7.7	10.9	16.1	13.3

Shin(R)	76.2	66.5	55.5	15.7	12.4	2.9	45.1	16.2	11.8	14.2	19.3	12.8	2.5	25.7	20.6
Face	6.6	4.5	2.9	2.9	3.1	2.2	2.8	0.9	1.5	0.6	2.5	0.7	1.0	1.5	1.4
Hands	7.5	6.7	1.0	14.8	21.3	28.9	19.8	6.2	5.2	15.5	4.9	1.4	26.0	27.0	0.9
Feet	3.9	3.2	2.1	2.4	2.4	3.4	2.4	2.4	2.4	0.2	2.1	2.2	2.5	2.9	4.1
Sum	679.1	331.3	315.7	160.8	236.9	179.7	215.3	165.9	190.1	130.7	246.6	126.4	112.9	291.2	295.2

Risk assessment

Because it takes average 48 min for preparation and application of 1,000L (500L x 2) spray suspension, workers could conduct about ten working activities with speed sprayer for 8 h per day. The reliable exposure estimates were determined from dermal and inhalation exposure amount on several assumptions, including day-to-day activity, penetration rate through clothes, skin absorption and body weight for adult male Korean (Table 14). The AQEs were 2.1×10-4 mg/day (95%CI 5.0×10-6–7.2×10-4) and 2.3 mg/day (95%CI 1.2–3.8) for mixer/loader and applicator, respectively. The 95th percentile AQEs for mixer/loader and applicator were 7.2×10-4 and 3.4 mg/day, which were higher 1.5–3.4 times than average AQEs, respectively. The ratio of PIE to AQE during application was 0.003% (95%CI 0.0003–0.004%), so inhalation exposure was negligible as the other study (PIE 0.02–0.06 μg/day) with acetamiprid (Kim et al., 2013). The ADE during application was similar to the study with methomyl (1.1–2.0 mg/day) and higher 3–12 times than the study with acetamiprid (0.8 mg/day) and fenvalerate (0.2–0.4 mg/day) (Kim et al., 2012b; Kim et al., 2013; Moon et al., 2013). Meanwhile, the AQE during mixing/ loading was 0.009% of that during application. The predicted distribution of MOS for kresoxim-methyl was 30.4 (95%CI 15.4–55.2) (Table 5, Figure 4). The proportion of the general Korean workers under the threshold for concern (MOS <1) was almost zero, indicating workers using SS on apple orchard are considered to be safe from kresoxim-methyl exposure. This was due to low toxicity (AOEL, 0.9 mg/kg) and low skin absorption rate (13%) of kresoxim-methyl, despite high exposure occurrence and high a.i. content of pesticide products (50%).

Figure 3. Correlation coefficients of body parts to dermal exposure during application

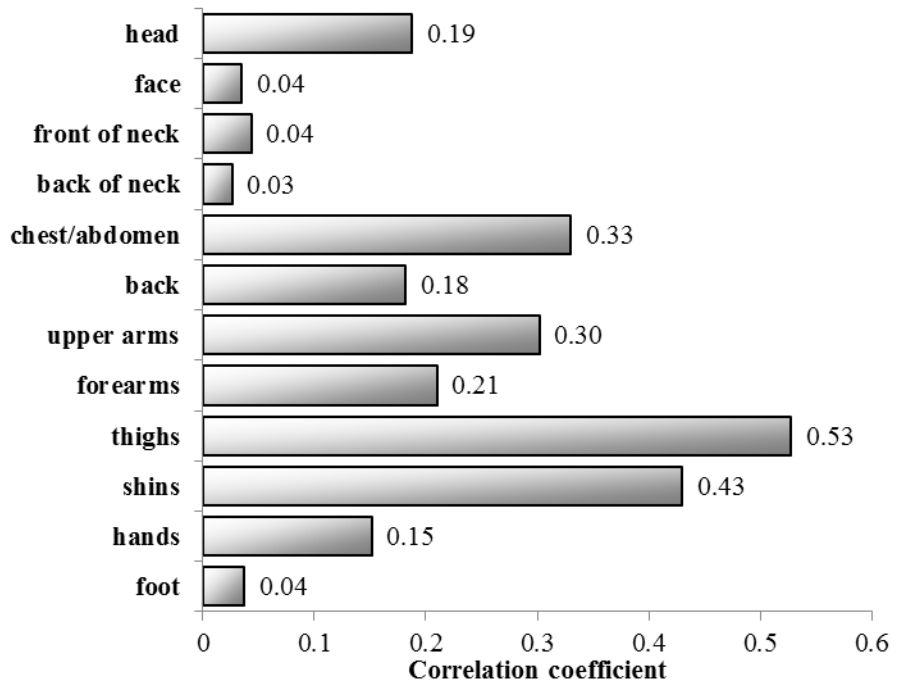

head
face
front of neck
back of neck
chest/abdomen
back
upper arms
forearms
thighs
shins
hands
foot
0.19
0.04
0.04
0.03
0.33
0.18
0.30
0.21
0.53
0.43
0.15
0.04
0
0.1
0.2
0.3
0.4
0.5
0.6
Correlation coefficient

Table 6. Dermal exposure on body parts during mixing/loading and application

body parts		Dermal exposure (mg)		Best fitted distribution
		Average	95%CI[a]	
mixing	Hands	0.7	0.02–2.4	Expon
application	Head	1.4	0.2–4.0	Gamma
	Face	0.2	0.04–0.5	Invgauss
	front of neck	0.2	0.02–0.8	Invgauss
	back of neck	0.2	0.06–0.6	Invgauss
	chest/abdomen	1.8	0.04–6.5	Expon
	Back	1.0	0.1–4.5	Lognorm
	upper arms	2.2	0.2–5.2	Triang
	Forearms	1.2	0.03–4.5	Expon
	Thighs	4.9	0.9–10.2	Triang
	Shins	3.3	0.5–11.0	Loglogistic
	Hands	0.8	0.1–3.8	Invgauss
	Foot	0.3	0.05–0.9	Loglogistic
	Total	17.5	9.3–28.9	

[a] 95% confidence interval

Table 7. Probabilistic risk estimation during mixing/loading and application of kresoxim-methyl on apple orchard[a]

working type	mix/loading	application
PDE[b] (mg/day)	6.5 (0.2–24.1)[d]	174.7 (93.2–289.2)
ADE[c] (mg/day)	2.0×10^{-4} (5.0×10^{-6}–7.2×10^{-4})	2.3 (1.2–3.8)
PIE[d] (μg/day)	-	6.8×10^{-2} (3.7×10^{-3}–0.17)
AQE (mg/day)	2.1×10^{-4} (5.0×10^{-6}–7.2×10^{-4})	2.3 (1.2–3.8)
AQE per unit body weight (mg/kg bw/day)	7.7×10^{-7} (7.1×10^{-8}–1.1×10^{-5})	0.03 (0.02–0.06)
MOS	1.2×10^{6} (8.4×10^{4}–1.3×10^{7})	30.4 (15.4–55.2)

[a]PDE: potential dermal exposure, ADE: actual dermal exposure, PIE: potential inhalation exposure, AQE: absorbable quantity of exposure, MOS: margin of safety.

[b,d]PDE and PIE were calculated on assumption of working number: mixing/loading and application are conducted 10 times a day with SS.

[c]ADE = [PDE × 1% (mix/loading) or 10% (application) of penetration rate through clothes] × 0.3% (mix/loading) or 13% (application) of skin absorption.

[d]95% confidence interval

Part 2

Exposure and Risk Assessment of Operators to Insecticide Acetamiprid during Treatment on Apple Orchard

Introduction

Apple is one of the most produced and consumed fruits in Korea. The cultivated area and amount of production is 31,167 ha and 379,541 ton in 2011, respectively (KOSIS, 2011). Pesticides are indispensable chemicals that are widely used to control insects, diseases and weeds in agricultural field, and 165 pesticides were registered for apple (KFDA, 2012). Thus operator, who handle and apply pesticides in fields, can be exposed to pesticides during mixing/loading and spraying in apple orchard. Safety and health of operator during mixing/loading and application is a major concern in agricultural activities (Ramos et al., 2010) because direct contact with pesticides by operators who handle and apply these agents can lead to harmful effects depending on the toxicity of pesticides. In orchard, 64.6% of famers prefer to use speed sprayer than power sprayer for the application of pesticides (Hong et al., 2007). They wear long-sleeve shirts and long trouser instead of protective garments while spraying. Therefore there could be significant dermal exposure to operators to results in the unintended field-poisoning. During spraying, the representative routes of human exposure are dermal deposition and inhalation (Kim et al., 2012). Potential dermal exposure (PDE), measured by analyzing the quantity of a pesticide that deposited on body regions, provides the information on the amount of exposure. But PDE data by itself cannot be used as a risk indicator because it must be related to acceptable exposure limit. For this purpose, the margin of safety (MOS) has been proposed as a useful risk indication (Machado-Neto, 2001) that relates the acceptable exposure to a pesticide with the mass absorbed by the body, which can be estimated from PDE. MOS value higher than 1 are considered as an indication of safe working condition. Acetamiprid is a systemic neonicotinoid insecticide which is intended to control sucking insects on crops such as leafy vegetables, citrus fruits, pome fruits, grapes, cotton, and ornamental plants. It has a low acute and

chronic toxicity in mammals with no evidence of carcinogenicity, neurotoxicity endocrine disruption or mutagenicity (Marín et al., 2004). However, there is no previous exposure and risk assessment of operator to insecticide acetamiprid in apple orchard, to our knowledge. Thus, in this study, dermal and inhalation exposure amounts were measured during mixing/loading and application of acetamiprid after analytical methods were fully validated. Body parts of major exposure were identified and MOS for operator was calculated based on PDE to find out whether work condition is safe or not.

Materials and Methods

Reagents and materials

Acetamiprid (99.9%) of analytical standard grade was purchased from Sigma-Aldrich (MO, USA). All solvents were HPLC-grade, and were purchased from Fisher Scientific Korea Ltd (Ansung, Korea). Stock solution (100 μg/mL) was prepared HPLC-grade acetonitrile. The working standard solutions were prepared by serial dilution of stock solution in acetonitrile. Acetamiprid wettable powder (WP) (8%) was purchased from pesticide vender for field study.

Exposure matrices

Dermal patches for dermal exposure measurement were made by putting cellulose paper (Whatman 17CHR, 46 × 57 cm, 1 mm thickness; Kent, UK) in the patch pocket (10 cm × 10 cm), which has circular exposure part (50 cm2). Safety pins were used to attach patches on protective garment (SP protective, KleenGuard, Yuhan-kimberly Korea Ltd, Seoul, Korea) of operator. Cotton mask (face exposure; 200 cm2), cotton socks (feet exposure), cotton gloves (hands exposure) were purchased from local markets. A personal air monitor (PAM) consists of air pump (Gillian Model 224-PCXR7, MSA, Dong Ha Trading Co. Ltd, Seoul, Korea), glass fiber filter (37 mm, SKC, Eighty Four, PA) in open-faced cassette (SKC) and solid sorbent (ORBOTM 609 Amberlite XAD-2 400/200 mg, Supelco, MO, USA). U-shape glass tube for trapping efficiency test was manufactured by Dae-Jung Chemical (Daejon, Korea).

Experimental sites

All the field studies were carried out in apple research institute orchards (Table 8). The unit size of site for repetition was 0.2 ha. The apple crop height was 2.5 and 5 m, and rows were separated by 4-6 m. During the experiment, ambient temperature was 9-24 ºC and relative humidity was 16-61%. Wind velocity did not exceed 4 m/s (Kester 3000, Nielsen-Kellerma, USA).

Chromatographic condition

All chromatographic analysis were performed on a High Performance Liquid Chromatograph (Agilent 1100 HPLC; CA, USA) with automatic injector, DAD (diode array detector), and a Shiseido C18 column (250 mm × 4.6 mm, 5 µm particle; Kyoto, Japan) at 40 ºC. The mobile phases A and B were water and acetonitrile, respectively. A gradient system was employed for 15 min at the flow rate of 1.0 mL/min with A:B as follows: initial 0 min, 50:50; 1 min, 50:50; 10 min 30:70; 10.5 min 0:100; 12 min, 0:100; final 15 min 50:50. Injection volume was 10 µL, and elution of acetamiprid was monitored at 250 nm.

Table 8. Field conditions, application data, and meteorological conditions of operators of acetamiprid

Applicator	Op. 1	Op. 2	Op. 3	Op. 4
Applicator				
Years of experience	20	20	25	30
Repetition,	8	9	4	7
Sex	male	male	male	male
Height (cm)	172	173	165	175
Weight (kg)	65	60	65	68
Field				
Area (ha)	0.2	0.2	0.2	0.2
Crop age (years)	4-30	3-35	3-26	5-25
Crop growth stage	Before blossom stage			
Crop height (m)	3-4	3.5	2.5-4	3-4
Inner row crop distance (m)	0.5-1	1-2	1-3	1-4
Row distance (m)	4-5	4	4-6	4-6
Pesticide				
AI content used (g)	40			
Spray volume (L)	1,000			
Application				
Type of spray application	Speed sprayer			
Boom length (m)	10	8	8	7
Application time (min)	35-45	40-45	35-40	35-45
Nozzle size	Small	Small	Small	Small
Climate				
Temperature (℃)	10-24	9-21	7-20	9-24
Relative humidity (%)	22-54	21-61	16-62	19-50
Wind speed (m/sec)	0-2	0-4	0-1	0-1
Precipitation	None			

Limit of detection (LOD), limit of quantitation (LOQ), reproducibility and linearity of calibration curve

Aliquots (10 μL) of acetamiprid standard solution (0.01~1.0 μg/mL) were analyzed for LOD determination before LOQ calculation. For reproducibility test, three levels (LOQ, 10 LOQ, and 50 LOQ) of acetamiprid standard solution were analyzed six times by HPLC to calculate coefficient of variation (C.V). Various acetamiprid standard solutions (0.1-10 μg/mL) were analyzed for establishment of calibration curve. After 1 and 3 days of storage, the linearity (coefficient determination, R^2) of the curve was investigated again

Trapping efficiency and breakthrough tests

Trapping efficiency test was repeated three times by spiking of a standard solution (10 LOQ) on the bottom of U-shape glass tube connected with solid sorbent, and air was passed through the system at 60 L/h for 4 h for trapping of vaporized acetamiprid. To help the volatilization of compounds, U-shape glass tube was heated to 70℃. The residue in U-shape glass tube and the amount trapped in solid sorbent were analyzed for mass balance.

For breakthrough test, acetamiprid at 10 LOQ level was spiked in the first part before passing air through the solid sorbent at 60 L/h for 4 h. Subsequently, first part and second part were analyzed separately. This test was repeated three times.

Recovery (Matrix extraction efficiency) test

Three levels (LOQ, 10 LOQ and 100 LOQ) of standard solution were spiked in dermal patches, gloves, socks, mask, glass fiber filter, and solid sorbent. The

extraction and analysis of acetamiprid were repeated three times according to the method described in the above analytical procedure.

Extraction of acetamiprid from exposure matrices

The dermal patches, gloves, mask, and socks were placed in glass bottle (100 or 500 mL) and extracted using 50 or 300 mL of methanol, depending on matrix size. Solid sorbent and glass fiber filter were placed into a 20 mL vial, and 10 mL of methanol was added. Those containers were shaken for 1 h in a shaker (Wooju Scientific, Kimpo, Korea) at room temperature. A sample of 1~2 mL of each extract was filtered through a 0.2 μm pore syringe filter (4 mm, Milipore, Bill, USA) before aliquots were analyzed using HPLC.

Field trials and sampling procedure

For exposure measurement during mixing/loading, only gloves were used and operators prepared spray mixture by mixing acetamiprid WP with water after weighing a specific amount of powder. The mixture was stirred mechanically or by stick with hands on speed sprayer (Model TLD ASS-555, Asia Motors, Daegu, Korea). After mixing/loading, gloves were removed for analysis. In the case of spraying, dermal patches (Kim et al., 2012) were attached on 13 parts of body: head (1), front of neck (1), back of neck (1), chest/stomach (1), back (1), upper arm (2), forearm (2), thigh (2), and lower leg (2). And the operator wore cotton gloves, cotton socks, and mask (face exposure; 200 cm^2). Application of spray mixture to apple trees was carried out using speed sprayer in field for 35~45 min. The operator started spraying following their usual technique, with no other instructions. The application was carried out by

driving along the row. After the application was finished, the all materials were carefully removed avoiding accidental contamination of the different part and all samples were placed in individual plastic bags.

PAM was used to monitor inhalation exposure. A glass fiber filter cassette and a solid sorbent were attached to the breathing zone with clip, and a personal air pump was fastened on waist by belt. After mixing/loading or spraying, solid sorbent and filter were removed for analysis. The flow rate of personal air pump was 60 L/h.

Calculation of potential dermal and inhalation exposure

PDE per body part were calculated multiplying the unit amount of acetamiprid (μg/cm^2) on dermal patch or mask with each surface area (cm^2) of body region for Korean, which recently suggested by Kim et al. 2011. The inhalation exposure rate (μg/h) was obtained by dividing the inhalation exposure amount (μg) by work time (h), and it was extrapolated to medium exercise (1,270 L/h) breathing rate for Korean male (Kim et al., 2011) to calculate potential inhalation exposure (PIE).

Risk assessment

The risk assessment was carried out by modifying MOS formula in report of Huges et al. 2008. The MOS in obtained as follows: MOS = AE / [(PDE x AF) + PIE] x SF. Where AE (acceptable exposure) = AOEL (0.124 mg/kg/d) x average body weight (60 kg); PDE = potential dermal exposure; AF = absorption factor and SF = safety factor. The PDE and PIE values were obtained by extrapolating of application time to 4 h for effective exposure per day. AF

of 0.01 was used by the assumption of 10% of dermal absorption and 10% of cloth penetration. Thus, the actual formula used was: MOS = 0.124 x 60 / [(PDE x 0.01) + PIE] x 1.

Results and discussion

Method Validation

The analytical and sampling methods were fully validated through the various experiments as follows. LOQ was calculated as 4 times greater than LOD and the values were low enough for the detection of acetamiprid (Table 9). Good reproducibility (C.V. < 4%) showed that instrument was stable for analysis. Acetamiprid concentration and the response of the detector was linearly correlated ($R^2 > 0.999$) over the range of 0.1~10 mg/L for 3 days. Recovery tests of acetamiprid from various sample matrices were conducted and ranged from 85.3 to 118.2 % with the small relative standard deviation values (1.1~9.1), indicating the analytical procedures are reliable (Table 10).

To validate the sampling method in inhalation exposure monitoring, trapping efficiency test and breakthrough test were performed. The trapping efficiency test was to measure the efficiency of solid sorbent for the trapping of pesticide in air. This experiment allowed a mass balance of over 96.4% by adding up the acetamiprid trapped in the solid sorbent and the residue at the bottom of the U-shaped glass tube. Most of acetamiprid remained at the bottom of the U-shaped glass tube because it was not volatile. The breakthrough test evaluates the capacity of the solid sorbent to retain pesticide. The result of over 93.8% recovery from first part of resin without escaping to second part suggested that first part of resin has enough capacity to retain the corresponding amount of acetamiprid (Table 11). The validated approaches have been applied to assess potential exposure of the operator spraying acetamiprid in apple orchards.

Table 9. LOD, LOQ, reproducibility of analysis and linearity of calibration curve of acetamiprid

LOD	LOQ	Reproducibility (Area)			Linearity (R^2) [b]	
		LOQ level	Ave-rage	CV[a] (%)		
		1	4.4	3.6	Day of preparation	1.0000
0.25 ng	1 ng	10	46.8	1.0	After 1 day	0.9999
		50	238.6	0.9	After 3 day	0.9999

[a]Coefficient of variation.

[b]Coefficient of determination.

Table 10. Recovery of acetamiprid from dermal patches, gloves, masks, socks, glass fiber filter, and solid sorbent

Treated level	Recovery (%)					
	Dermal patch	Gloves	Mask	Socks	Glass fiber filter	Solid sorbent
1LOQ[a]	108.5 ±2.2[b]	101.1 ±1.1	98.9 ±6.5	118.2 ±2.1	95.9 ±1.7	96.3 ±4.1
10LOQ	95.9 ±8.3	88.8 ±6.9	89.8 ±8.0	94.8 ±9.1	91.2 ±2.0	85.3 ±5.1
100LOQ	103.9 ±6.7	94.3 ±7.3	94.3 ±8.0	99.0 ±8.4	90.9 ±6.2	85.8 ±5.1

[a]LOQ: limit of quantitation.

[b]Mean ± SD (n = 3).

Table 11. Trapping efficiency and breakthrough test of solid sorbent for acetamiprid

Test[a]	Recovery (%)	
Trapping efficiency	Residue[b]	96.4
	Trapped[c]	0
Breakthrough	First part[d]	93.8
	Second part[e]	0

[a]The tests were carried out at 10 limit of quantitation level.

[b]Amount left on the bottom of U-shaped tube.

[c]Amount trapped in solid sorbent.

[d]First part of solid sorbent.

[e]Second part of solid sorbent.

PDE and PIE

In this study, cotton gloves were used for measurement of potential dermal exposure to the operator's hands (Capri et al., 1999; Vercruysse et al., 1999) because they can be changed very quickly and that the provided some degree of safety for the operators, especially when using solid formulations (Tuomainen et al., 2002) even though exposure maybe overestimated if cotton is more absorbent than skin (Fenske et al., 1989). Only hands exposure were measured using gloves since the hands were reported to be significantly more exposed than the other body parts of the mixer and loaders (Kuye et al., 2008).

Data for hands exposures amount during mixing/loading were expressed total amount (μg) and not time rate, as this step was not time dependent(Hughes et al., 2006). Exposure amount of hands during mixing/loading acetamiprid showed variable results (33~1,132 μg) (Table 12).

Exposure amount of operator 3 among 4 workers was noticeably high because he opened the WP package, rinsed the package in the water, and stirred it by stick while other workers opened WP package and mixed it with water by machine. The results also show that application habit can give rise to the high variability of the operator's exposure (Table 12). As shown in Table 13, the average PDE of acetamiprid during application ranged from 535 to 1,235 mL/h which is corresponding to 0.03~0.08% of the applied amount. The level of exposure is known to be variable, depending on many different factors such as the type of crop, crop size, application technique, weather conditions, personal protective equipment, etc. (Machera et al., 2002b; Tuomainen et al., 2002; Hughes et al., 2006). In the cases of operator 1 and operator 2, higher exposure rate was found than those of operator 3 and operator 4. This result could be due to the narrower space between the trees and more windy condition when

compared to other operators. Thigh was the most contaminated part with a rate of 224.9 mL/h (26.6%) then the next was chest (16.8%). In the same sense, when exposure was studied in apple orchard during application of methomyl, thighs and chest were identified as the most exposed body parts (Kim et al., 2012b). Other majorly exposed parts were upper arms, forearms and lower legs. Head and face exposures were also relatively high (Fig. 4). Inhalation exposure was monitored during the application and found to be around 10-8% of applied amount (Table 14). The highest inhalation exposure was observed with operator 2, probably due to relatively more windy weather during application. However, Cattani et al. (2001) reported that inhalation of chlorpyrifos was influenced by the ambient air temperature.

Table 12. Hands exposure during mixing/loading of acetamiprid

Operator	Hands exposure (mL/h)			
	Minimum	Maximum	Average	Ratio to applied a.i[a] (%)
1	65.8	542.7	84.3	4.6×10^{-4}
2	45.3	400.3	169.2	4.2×10^{-4}
3	168.7	1132.1	649.3	1.6×10^{-3}
4	32.9	327.8	147.8	3.7×10^{-4}

[a]Active ingredient.

Table 13. Dermal exposure of each body part during application of acetamiprid

Body part	Dermal exposure (mL/h)				
	Operator 1	Operator 2	Operator 3	Operator 4	Average
Head	58.6	62.8	44.2	35.8	50.3
Face	5.8	22.1	7.7	2	9.4
Front of neck	11.4	15	11	6.2	10.9
Back of neck	9.9	7.1	5.3	3.4	6.4
Chest/stomach	258.3	142.5	75.3	75	137.8
Back	104.3	52.4	73.7	49.9	70.1
Left upper arm	59.9	54.6	36	16.8	41.8
Right upper arm	67.4	67.6	70.9	56.3	65.5
Left forearm	53.6	43.8	41.1	15.4	38.5
Right forearm	47.7	33.1	33	28.4	35.6
Hands	36.1	38.5	16.8	28.3	29.9
Left thigh	161.8	123.3	75.3	61.3	105.4
Right thigh	177.3	131.6	101.8	67.4	119.5
Left lower leg	102.9	65	42.7	52.6	65.8
Right lower leg	75.9	66.2	54.1	35.5	57.9
Feet	4.1	1	1.9	1.2	2.1
Total	1235.1	926.6	690.5	535.3	846.9
Ratio to applied a.i[a] (%)	$8.0x10^{-2}$	$5.0x10^{-2}$	$3.0x10^{-2}$	$3.0x10^{-2}$	$5.0x10^{-2}$

[a]Active ingredient.

Figure 4. Acetamiprid dermal exposure (%) distribution in head, face, torso, arms, hands, legs, and feet

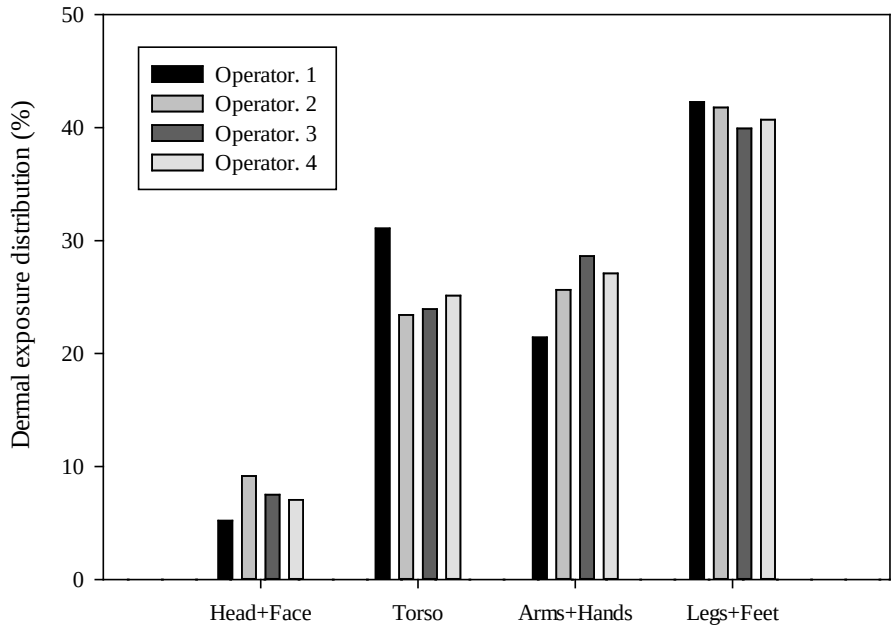

Dermal exposure distribution (%)
50
40
30
20
10
0
Operator. 1
Operator. 2
Operator. 3
Operator. 4
Head+Face
Torso
Arms+Hands
Legs+Feet

Table 14. Inhalation exposure amount during application of acetamiprid

Operator	Inhalation exposure (μg)		
	Average	Ratio to applied a.i[a] (%)	Ratio to dermal exposure (%)
1	0.009	2.3×10^{-8}	2.8×10^{-5}
2	0.014	3.6×10^{-8}	6.9×10^{-5}
3	0.006	1.5×10^{-8}	5.2×10^{-5}
4	0.005	1.2×10^{-8}	4.0×10^{-5}

[a]a.i: Active ingredient.

MOS and Risk Assessment

In risk assessment PDE values are considered together with the toxicological data for insecticide, then the resulting MOS values indicates that the spray procedure evaluated can be considered safe or not (Hughes et al., 2006). A value of MOS ≥ 1 would indicate safe working conditions, whereas a MOS < 1 would mean unsafe conditions. In MOS calculation (Table 15), AE values are calculated on the basis of appropriate toxicological end point such as AOEL (Acceptable Operator Exposure Level) or NOAEL (No Observed Adverse Effect Level). If AOEL is available, NOAEL is not applicable and SF is 1. AOEL was found to be 0.124 mg/kg/d (EC, 2004). Average body weight for man is 60 kg (Choi et al., 2006; Kim et al., 2012). For dermal absorption, 10% of dermal absorption (Choi et al., 2006; Hughes et al., 2008; Ramos et al., 2010; Kim et al., 2012) and 10% of cloth penetration (Choi et al., 2006; Kim et al., 2012) were assumed, while 100% penetration was applied for inhalation (Oliveira and Machado-Neto 2003; Kim et al., 2012). When no inhalation data was available, Huges et al. (2008) assumed a dermal exposure of 1%. However, many study reported around from 10^{-4} to 10^{-2}% levels of dermal exposure including this study (Capri et al., 1999; Machera et al., 2003; Oliveira and Machado-Neto 2003; Kim et al., 2012). Calculated MOS values (5.6~15.2) was much higher than 1 in all cases, indicating that the working condition was of least risk.

Database for model

The data for the model were calculated as mL/h and is shown in Table 16.

Table 15. Calculation of MOS for application of acetamiprid

	Operator				
	Operator 1	Operator 2	Operator 3	Operator 4	Average
Potential dermal exposure (mg/day)[a]	132.10	83.92	49.06	50.11	78.80
Potential inhalation exposure (µg/day)	0.04	0.06	0.02	0.02	0.03
MOS per operator[b]	5.6	8.9	15.2	14.8	11.1

[a]Including mixing/loading

[b]Calculated as MOS (margin of safety) = 0.124 mg/kg/d x 60 kg / [(PDE x 0.01) + PIE] x 1

Table 16. Dermal exposure per hour of acetamiprid (mL/h)

Trials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Head	95.6	79.4	61.1	47.9	72.6	47.1	40.2	27.1	22.4	53.2	55.7	65.8	98.6	43.3	80.7
Neck of front	9.2	19.1	13.2	4.6	16.0	7.0	20.6	3.8	8.0	14.2	18.7	16.1	34.4	10.8	15.4
Neck of back	14.9	16.7	8.1	5.3	15.7	5.7	10.1	3.1	1.9	8.9	6.9	8.1	8.3	5.6	7.3
Chest	302.3	576.3	204.0	84.3	374.0	114.3	363.1	19.3	38.9	129.7	31.1	97.9	287.5	122.9	171.5
Back	248.2	198.0	27.6	30.4	73.5	67.2	138.2	23.0	7.2	33.1	60.6	70.1	45.6	95.1	52.4
Upper arm(L)	3.0	7.7	2.1	1.3	2.6	7.5	20.5	1.6	5.5	7.0	7.1	13.5	104.9	10.9	16.2
Upper arm(R)	58.6	140.8	78.9	15.0	53.6	57.0	66.3	36.8	13.9	55.6	39.2	60.0	89.3	13.4	60.9
Forearm(L)	43.8	129.2	79.5	46.6	121.9	48.7	23.1	8.3	23.1	33.0	42.7	91.3	120.7	32.3	63.8
Forearm(R)	62.9	82.0	57.7	26.6	81.1	42.3	68.5	32.3	19.2	40.3	20.6	70.5	85.4	20.2	46.8
Thigh(L)	34.7	96.5	46.1	21.2	77.1	31.3	58.2	12.7	1.9	44.6	45.7	52.2	15.3	39.5	22.2
Thigh(R)	316.9	242.9	160.8	27.1	167.0	82.1	281.4	21.6	40.6	82.8	102.7	139.2	206.3	72.1	149.9
Shin(L)	256.7	255.1	167.6	92.9	287.7	120.5	195.7	17.2	46.1	63.9	135.9	87.6	148.2	146.8	205.2

Shin(R)	165.2	182.7	85.4	31.9	119.0	76.9	90.4	29.7	28.9	97.1	72.4	75.4	107.6	31.2	64.5
Face	67.9	139.6	55.2	20.9	108.3	80.4	98.8	14.3	32.6	100.6	76.3	48.5	90.1	48.1	67.9
Hands	6.0	14.8	2.4	1.6	2.7	1.0	2.3	0.2	1.9	0.4	0.7	0.5	1.0	0.7	1.8
Feet	32.6	52.5	27.1	19.4	58.9	28.2	53.1	11.0	8.0	26.7	24.0	34.8	54.1	41.8	56.5
Sum	1,718.5	2,233.3	1,076.8	477	1,631.7	817.2	1,530.5	262	300.1	791.1	740.3	931.5	1,497.3	734.7	1,083

Table 16. Continued

Trials	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Head	82.0	58.5	9.6	23.3	74.7	69.0	60.8	25.2	21.5	21.3	35.7	23.9	67.1	29.9	51.4
Neck of front	9.0	12.4	0.9	7.0	20.8	15.1	15.3	1.6	2.1	3.9	3.7	2.9	14.2	3.8	13.1
Neck of back	10.5	5.3	1.4	5.4	12.5	2.0	11.7	2.8	3.1	2.0	2.0	2.3	7.6	3.0	3.6
Chest	289.6	133.5	13.8	50.8	151.7	84.7	112.8	47.7	45.4	53.6	100.0	29.2	165.3	34.8	96.8
Back	32.8	58.6	5.4	41.4	105.3	142.8	28.0	51.5	19.2	26.4	50.8	40.6	107.8	30.9	73.5
Upper arm(L)	23.3	14.7	1.5	1.1	18.4	9.7	16.7	1.6	0.3	0.9	0.8	1.9	3.7	1.5	4.8
Upper arm(R)	111.0	25.2	4.5	48.2	38.9	52.3	86.5	8.8	4.0	8.2	24.0	10.1	24.5	10.3	36.3
Forearm(L)	135.3	80.5	11.9	45.7	120.7	105.1	86.5	45.9	23.1	30.8	26.2	64.4	116.1	49.7	83.6
Forearm(R)	49.1	29.4	2.3	45.6	52.6	63.9	58.6	7.7	6.0	6.3	22.5	6.1	24.2	9.2	33.1
Thigh(L)	23.6	42.1	14.6	33.0	61.2	23.2	49.2	16.8	8.8	5.1	24.7	27.9	73.5	8.9	49.8
Thigh(R)	232.6	102.8	7.3	72.0	116.0	105.8	160.4	16.4	58.7	31.4	58.4	61.8	88.9	43.9	85.8
Shin(L)	223.7	155.7	12.6	69.5	224.7	100.5	224.2	42.4	41.9	44.1	24.4	55.4	145.4	60.8	99.8

Shin(R)	90.3	16.4	1.0	28.8	40.4	100.4	164.6	71.8	27.1	13.9	40.8	38.5	147.2	28.4	72.0
Face	79.8	70.7	13.9	26.8	100.5	75.0	119.2	36.0	23.9	12.6	38.9	12.4	120.4	17.7	22.3
Hands	1.8	2.1	0.4	0.5	3.2	3.4	1.0	2.4	0.3	0.6	0.0	0.9	2.1	1.0	3.6
Feet	52.7	45.0	5.2	12.8	5.9	43.3	75.2	17.4	12.0	18.1	22.1	22.9	42.7	24.4	55.5
Sum	1,447.1	852.9	106.3	511.9	1,147.5	996.2	1,270.7	396	297.4	279.2	475	401.2	1,150.7	358.2	785

Part 3

Comparative Exposure of Operators to Fenthion during Treatment in Paddy Field

Introduction

Cultivation of rice is important in Korea, because it is the staple food of the country. Furthermore, 214 insecticides are registered for rice cultivation; therefore, many kinds of pesticides are applied from seed stage for successful harvest. Thus, the appropriate exposure assessment is required to assure the operator's health (or healthy agricultural practices).

Fenthion, one of the insecticides for rice, has been used for many years in Korea to control fruit flies, leafhoppers, leaf miners, leaf-eating, larvae, stem borers, cereal bugs, and other insect pests (Tomlin, 2009). Its mode of action is through the inhibition of acetylcholine esterase. It is known to affect the central nervous, cardiovascular, and respiratory systems of mammals including humans. Being similar with other organophosphates, fenthion is readily absorbed through the skin (Kerem et al., 2007).

In the present study, the exposure of fenthion to operators was monitored during mixing/loading and during the application of pesticide. Operators wore typical paddy field garment consisting of long-sleeved polyester shirts and impermeable trousers under rain boots. To monitor exposure in application, patches (Tuomainen et al., 2002; Kuye et al., 2007) were placed on the outer clothing over the following body parts: head, front of neck, back of neck, upper arms, forearms, thighs, lower legs, back, and chest. Hand exposure was monitored by means of cotton gloves (González et al., 1999; Ramwell et al., 2005), whereas face exposure was measured using cotton mask (200 cm^2) (Machado-Neto 2001; Choi et al., 2006). For inhalation exposure, personal air monitor was employed (Cattani et al., 2001; Machera et al., 2003). During

mixing/loading of pesticide, only gloves were used for exposure measurement, because hand is expected to be exposed the most (Kim et al., 2012).

Materials and Methods

Reagents and materials

Fenthion (WG, 50%, Sungbo Chemical, Seoul, Korea) was obtained through the local vendor. Analytical standard of fenthion (purity, 98.3%) was purchased from the Sigma-Aldrich. (West chester, PA). All solvents were HPLC grade, and purchased from Burdick & Jackson (SK Chemical, Ulsan, Korea).

Sampling methodology

The potential dermal exposure and inhalation exposure was measured using the patch technique as part 2.

Calculation of dermal and inhalation exposure

The potential dermal exposure and inhalation exposure was measured using the patch technique as part1 reported.

Analytical condition

For the analysis of fenthion in various exposure matrices, an Agilent 6890 gas chromatograph (GC; SantaClara, CA, USA) with a nitrogen phosphorus detector was used with a DB-5 column (30 m × 0.25 mm, 0.25 μm; J&W Scientific, Folsom, CA, USA). Sample injections (1 μL) were performed in split mode (20:1) at 260 °C. The column temperature was maintained at 150 °C for 1 min, elevated at a rate of 10 °C/min to a temperature of 210°C for 2 min, elevated at a rate of 20 °C/min to a final temperature of 300°C. Nitrogen gas was pumped as carrier gas at 1 mL/min

Method validation

Aliquots of standard solutions from 0.01 to 5 mg/L were analyzed for determination of instrumental limits of detection (ILODs) and instrumental limits of quantitation (ILOQs). The method limits of quantitation (MLOQs) were calculated from ILOQs, injection volume and extract solvent volume in analytical method. After injecting three levels (MLOQ, 10MLOQ and 100 MLOQ) of standard solution 7 times, C.V. values of the integrated peak area was determined to validate instrumental repeatability. Various standard solutions (LOQ level) were analyzed and the linearity of the curve was investigated after one day and three days of preparation. For recovery test, control exposure samples were fortified in three levels (MLOQ, 10 MLOQ and 100 MLOQ) of standard solution. The trapping efficiency was tested by spiking two levels of standard solution (10 MLOQ and 100 MLOQ) on the bottom of U-shape glass tube (Daejung Chemical, Daejeon, Korea) connected with solid sorbent, and passing air through the system at 1 L/min for 4 h. U-shape glass tube was heated to 70^{o}C to help volatilization of compounds. The residue in U-shape glass tube and the amount trapped in XAD-2 resin were analyzed for mass balance. Breakthrough test was conducted by spiking two levels of standard solution (10 MLOQ and 100 MLOQ) in the 1^{o}-resin part of the solid sorbent tube and passing air through the tube at 1 L/min for 4 h. Subsequently, 1^{o}- and 2^{o}-part of resin were analyzed separately. All analysis and test were repeated 3 times.

Sampling and field experiment

Field study was conducted in paddy field, and four operators, who have similar experiences in agricultural practices (over 40 years) participated in exposure

study. Each of them carried out mixing/loading and the application of pesticide by repeating two times.

Operators prepared spray mixture by mixing 500 mL of fenthion EC (emulsifiable concentrate, 50%) in 500 L water to spray it on rice plants during growing stage. Temperature and relative humidity during this field study ranged from 29 to 31℃ and 47 to 60%, respectively. All exposure samples were kept frozen when not analyzed immediately. Dermal exposure estimates were calculated by extrapolating patch deposition values to the total surface area (cm^2) of the appropriate body region for Koreans (Kim et al., 2011). Potential inhalation exposure was calculated as a rate of 1,270 L/h (Kim et al., 2011).

Results and Discussion

Method validation

Method validation for exposure monitoring of fenthion was carried out. LOD and LOQ were 0.01 and 0.05 ng, respectively. Calibration curve linearity (R2>0.999) and reproducibility (CV<3%) were also excellent. Recovery at LOQ, 10LOQ and 100LOQ levels from gloves, socks, mask, patch, solid sorbent, glass fiber filter was 76~113% (C.V<3%). Trapping efficiency was 95~105% while no breakthrough was observed. Method validation for the exposure monitoring was established successfully through several experiments. Such method validation can be usually performed in laboratory and not much different for each pesticide so that, this techniques will be applied widely in research for pesticide exposure monitoring by combination with body surface area and respiration rates.

Potential dermal exposure and inhalation assessment

Hand exposure during mixing/loading was 0.04~33.06 mg (Table 17), which suggests personal habit or mistake can result in great difference of exposure, even though they have similar work experiences. In the case of operator 2, difference of approximately 600-fold was observed in repetition. Such difference is very important result, because that situation suggests that careful handling is essential in mixing/loading procedure, and many repetitions of experiment are critical to obtaining statistically significant exposure results. During spray of fenthion, exposure differences were also observed between repetition and operators, but it was not as serious as in the case of mixing/loading. However, operator 2 still shows the greatest difference

between repetitions, suggesting he is generally not careful in treatment of pesticide. When the exposure pattern on body was examined based on the average data of 7 repetitions (data of first repetition of operator 3 was discarded due to the missing of dermal patches of legs and arms), most of the exposure occurred in applicators' legs and hands (Table 18). Leg exposure accounted for 54% of total average exposure, whereas hands accounted for 28%. Torso, arms, and head/face parts accounted for 6, 10, and 2%, respectively (Fig. 5). Leg exposure was considerably higher than that in the upper body, probably due to direct contact with the contaminated rice plants during spraying. Under comparable field conditions, Farahat et al. (2010) reported similar exposure patterns of higher exposure rate in thighs than in forearms. These results again emphasize the importance of wearing impermeable protective trousers and gloves for operators' safety. In most of cases, inhalation exposure is estimated as 1% of dermal exposure when inhalation exposure data are not available (Machdo-Neto et al., 1998; Hughes et al., 2008). However, in the present study, inhalation exposure was observed at 0.001~0.2% of dermal exposure. Such findings are in agreement with other studies, which reported normally respiratory exposure occupies less than 1% in the field environment (Capri et al., 1999; Machera et al., 2003; Oliveira and Machado-Neto 2003; Kim et al., 2012). In conclusion, high variability of dermal exposure level and its distribution on the body parts were observed between repetitions and operators, indicating exposure results vary widely depending on applicator's habit, condition of field, weather, and other different factors as mentioned by Hughes et al. (2006). It is strongly recommended for operators to wear personal protective equipment to avoid direct and extreme exposure to pesticides during

treatment of pesticide. Due to the high variability of exposure data, a great numbers of repetitions are necessary to establish comprehensive exposure database and predicting models for reliable and quantitative risk assessment.

Table 17. Exposure amount and ratios during mixing/loading and application of fenthion in paddy field

	Operator 1		Operator 2		Operator 3		Operator 4	
	1	2	1	2	1	2	1	2
Mixing/applied amount (g)	250							
Dermal exposure during mixing/loading								
Total exposure amount (mg)	0.17	0.10	0.05	33.06	0.03	0.04	0.04	12.02
Ratio to prepared amount (%)	7×10^{-5}	4×10^{-5}	2×10^{-5}	1×10^{-2}	1×10^{-5}	2×10^{-5}	2×10^{-5}	5×10^{-3}
Dermal exposure during application								
Total exposure amount (mg)	27.2	44.9	36.3	288.3	1.3	44.7	20.3	26.5
Ratio to applied amount (%)	0.01	0.02	0.01	0.12	0.001	0.02	0.01	0.01
Inhalation exposure during application								
Total exposure amount (μg)	1.7	8.5	7.1	8.4	2.6	9.2	0.3	2.3
Ratio to applied amount (%)	7×10^{-7}	3×10^{-6}	3×10^{-6}	3×10^{-6}	1×10^{-6}	4×10^{-6}	1×10^{-7}	9×10^{-7}
Ratio to dermal exposure (%)	0.01	0.02	0.02	0.003	0.20	0.02	0.001	0.01

Figure 5. Dermal exposure distribution of fenthion in paddy field

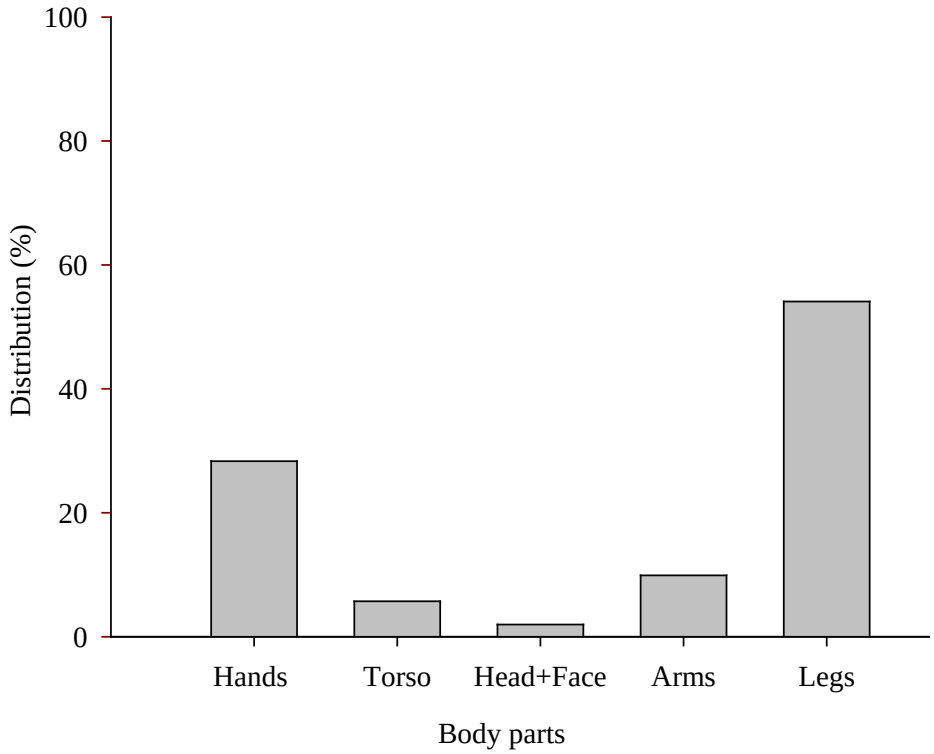

100
80
60
40
20
0
Distribution (%)
Hands
Torso
Head+Face
Arms
Legs
Body parts

Table 18. Dermal exposure (mg) during application of fenthion in paddy field

	Operator 1			Operator 2			Operator 3			Operator 4		
	1	2	Av	1	2	Av	1	2	Av	1	2	Av
Head	0.88	0.21	0.54	0.20	0.55	0.37	0.07	0.00	0.03	0.05	0.83	0.44
Front of neck	0.20	0.03	0.12	0.10	0.29	0.20	0.00	0.01	0.01	0.03	0.13	0.08
Back of neck	0.58	0.08	0.33	0.01	0.04	0.03	0.02	0.00	0.01	0.04	0.72	0.38
Chest/stomach	3.37	0.97	2.17	0.65	1.43	1.04	0.13	4.12	2.12	0.30	3.32	1.81
Back	3.39	0.71	2.05	0.71	2.02	1.37	0.27	0.12	0.20	0.47	3.75	2.11
Face	1.28	0.26	0.77	0.21	0.17	0.19	0.00	0.60	0.30	2.27	2.03	2.15
Upper arm (left)	1.17	0.18	0.67	0.14	0.47	0.30	0.08	0.15	0.11	0.10	0.69	0.39
Upper arm (right)	1.20	0.16	0.68	0.31	0.79	0.55	0.22	0.00	0.11	0.00	0.43	0.22
Forearm(left)	1.17	0.30	0.73	0.08	0.13	0.11	— [a]	31.18	15.59	0.79	1.00	0.89
Forearm(right)	2.61	0.29	1.45	0.32	1.05	0.68	0.00	2.14	1.07	0.72	0.61	0.67
Thigh(left)	2.92	4.01	3.47	4.33	92.84	48.58	0.01	2.10	1.05	4.33	6.09	5.21
Thigh(right)	6.08	5.41	5.75	5.35	96.85	51.10	0.50	2.22	1.36	6.26	5.37	5.81
Lower leg (left)	0.83	0.68	0.75	2.05	2.93	2.49	— [a]	0.92	0.46	1.81	0.61	1.21
Lower leg (right)	0.69	1.02	0.85	2.21	2.56	2.38	— [a]	0.40	0.20	1.72	0.40	1.06
Feet	0.02	0.04	0.03	0.02	0.12	0.07	0.00	0.27	0.13	0.75	0.12	0.43
Hand	0.86	30.48	15.67	19.59	86.11	52.85	0.00	0.51	0.25	0.73	0.39	0.56
Total	27.24	44.85	36.05	36.29	288.35	162.32	1.30	44.73	23.02	20.35	26.49	23.42

[a]Patches lost during application

Risk assessment

Health risk to operators was evaluated by margin of safety (MOS) calculation (Choi et al., 2006; Ramos et al., 2010) using 5 mg/kg of 2 year rat short term dietary NOEL (Tomlin, 2009) for fenthion and 4 h of the regular spray time per day (Table 19). In calculation of MOS, repetition 2 of operator 2 was not considered due to its extremely high value, which probably resulted from careless handling of pesticide. Repetition 1 of operator 3 also was not considered due to loss of dermal patches during application. Margin of safety shows similar tendency over 1, indicating that application works were safe enough to be carried out.

Database for model

The data for the model were calculated as mL/h and is shown in Table 20.

Table 19. Calculation of MOS for application of fenthion in paddy field

	PDE[a]	EDE[b]	IDE[c]	PIE[d]	AQE[e]	MOS
Operator 1	217.07	21.71	2.17	20.33	2.19	1.37
Operator 2	218.00	21.80	2.18	28.34	2.21	1.36
Operator 3	268.64	26.86	2.69	36.67	2.72	1.10
Operator 4	176.67	17.67	1.77	5.15	1.77	1.69

*Data of repetitions 2

[a]Potential dermal exposure, exposure amount x 4hr (mg/day), including mixing/loading

[b]External dermal exposure, PDE x 10% (mg/day)

[c]Internal dermal exposure, EDE x 10% (mg/day)

[d]Potential inhalation exposure, exposure amount x 4hr (μg/day)

[e]Absorbable quantity of exposure, IDE + PIE

Table 20. Dermal exposure per hour of fenthion (mL/h)

Trials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Head	0.51	0.00	0.18	2.63	0.24	0.23	0.39	0.59	1.65	0.64	0.22	5.26	0.09	0.00	0.00
Neck of front	0.05	0.00	0.12	0.61	0.43	0.05	0.07	0.30	0.88	0.09	0.05	0.53	0.00	0.00	0.09
Neck of back	0.10	0.02	0.05	1.75	0.08	0.05	0.21	0.03	0.13	0.25	0.04	0.12	0.04	0.00	0.00
Chest	1.30	9.07	3.32	10.10	3.89	1.14	0.00	1.96	4.30	2.91	1.08	20.80	11.07	2.05	7.01
Back	0.08	0.02	0.00	10.17	0.07	0.06	0.00	2.14	6.06	2.14	0.06	4.54	0.96	0.00	0.16
Upper arm(L)	0.18	0.60	3.44	3.83	1.22	1.37	0.30	0.64	0.50	0.79	0.87	4.25	0.09	0.87	1.40
Upper arm(R)	0.24	0.00	0.52	3.50	0.41	0.13	137.73	0.41	1.40	0.55	0.00	0.19	0.00	0.76	0.00
Forearm(L)	0.20	0.17	0.26	3.60	0.22	0.32	2.55	0.92	2.36	0.49	0.18	0.26	0.03	0.01	0.01
Forearm(R)	1.24	3.29	2.18	3.50	0.28	3.19	7.16	0.25	0.39	0.91	0.31	3.16	0.90	1.42	1.56
Thigh(L)	0.77	0.53	1.13	7.83	0.27	0.74	171.58	0.96	3.15	0.87	0.24	5.80	2.04	3.85	1.54
Thigh(R)	241.23	18.89	241.44	8.77	13.61	14.08	0.00	12.98	278.52	12.03	9.56	5.47	274.80	9.80	4.25
Shin(L)	228.54	7.19	256.66	18.24	12.42	20.16	0.00	16.06	290.56	16.24	9.88	3.88	509.92	8.89	2.31

Shin(R)	4.27	3.50	5.77	2.48	6.19	3.69	2.74	6.15	8.78	2.05	3.71	2.28	14.27	3.95	0.29
Face	1.86	1.52	4.83	2.06	6.52	4.85	4.11	6.63	7.67	3.05	2.13	2.09	14.11	3.47	0.42
Hands	2.18	0.06	0.05	0.05	0.16	60.40	71.39	0.06	0.35	0.12	0.07	0.13	0.00	53.15	3.42
Feet	95.21	1.82	119.47	2.59	67.55	152.05	13.45	58.78	258.33	91.44	217.42	125.91	1.65	1.15	1.84
Sum	577.95	46.69	639.44	81.72	113.54	262.49	411.68	108.86	865.04	134.55	245.82	184.67	829.98	89.37	24.32

Table 20. Continued

Trials	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Head	0.38	0.00	0.41	0.12	0.18	0.09	1.64	0.16	0.57	0.20	0.00	0.01	0.00	0.60	0.16
Neck of front	0.06	0.03	0.00	0.19	0.10	0.09	0.40	0.00	0.12	0.00	0.00	0.04	0.00	0.14	0.08
Neck of back	0.08	0.07	0.10	0.02	0.01	0.00	1.29	0.00	0.08	0.05	0.01	0.01	0.05	0.42	0.11
Chest	1.30	5.92	0.33	6.75	0.48	2.11	9.88	0.17	10.10	0.38	3.40	12.35	1.87	14.32	0.90
Back	2.30	0.47	1.15	0.50	0.22	0.51	5.17	0.18	1.02	0.82	0.29	0.37	0.00	2.28	1.41
Upper arm(L)	0.62	0.17	0.97	0.46	0.47	0.30	0.75	0.70	0.13	0.00	0.27	1.81	0.50	0.72	6.80
Upper arm(R)	0.44	0.00	0.04	0.12	0.24	0.01	1.54	0.00	0.00	0.24	0.12	0.44	0.02	0.42	0.30
Forearm(L)	0.28	0.01	0.08	0.02	0.27	0.01	1.52	0.00	0.28	0.66	0.01	0.01	0.00	0.29	0.01
Forearm(R)	0.09	0.25	0.62	0.28	0.11	0.54	97.74	0.00	2.20	0.00	5.52	93.55	0.00	0.99	2.36
Thigh(L)	0.23	2.45	0.71	0.96	0.00	0.29	3.74	0.08	3.12	0.00	104.86	6.41	5.54	5.02	2.17
Thigh(R)	11.14	769.76	4.83	6.39	17.91	8.52	13.72	18.01	15.24	0.02	5.34	6.29	17.35	585.16	12.98
Shin(L)	5.98	467.39	5.79	9.20	17.33	6.32	13.64	210.19	16.07	1.51	4.57	6.65	217.10	530.70	18.77

Shin(R)	8.54	8.65	1.35	2.10	166.51	3.38	4.38	8.54	4.34	0.00	1.02	2.75	8.22	8.23	5.43
Face	4.00	8.72	0.95	2.36	14.67	1.62	2.75	4.18	2.42	0.00	0.98	1.21	10.93	12.72	5.15
Hands	0.00	0.00	0.00	0.06	0.05	0.08	0.55	0.06	0.22	0.00	0.30	0.80	0.11	0.39	2.24
Feet	129.51	1.02	0.32	1.81	0.19	0.64	1.33	0.82	2.48	0.00	1.32	1.52	0.92	1.90	2.19
Sum	164.97	1264.92	17.63	31.34	218.76	24.52	160.04	243.12	58.39	3.89	128.02	134.20	262.61	1164.31	61.04

Part 4

Operator Exposure to Indoxacarb Wettable Powder and Water Dispersible Granule during Mixing/loading and Risk Assessment

Materials and Methods

Experimental materials

Indoxacarb (98.3%) of analytical standard grade was purchased from Sigma-Aldrich (St. Louis, MO, USA). Wettable powder (WP) (10%) and water dispersible granule (WG) (30%) were purchased from a pesticide vender for field study.

Exposure study samples and analytical conditions

Exposure sample analysis were performed on a High Performance Liquid Chromatograph (Agilent 1100 HPLC; CA, USA) with automatic injector, DAD (diode array detector), and a YMC C18 column (250 mm × 4.6 mm, 5 μm particle; Kyoto, Japan) at 40℃. The mobile phases A and B were water and acetonitrile, respectively. An isocratic system was A 70: B30. Injection volume was 10 μL, and elution of indoxacarb was monitored at 310 nm.

Extraction of exposure matrices

The gloves was placed in glass bottle (500 mL) and extracted using 300 mL of methanol. Solid sorbent and glass fiber filter were placed into a 20 mL vial, and 10 mL of methanol was added. Those containers were shaken for 1 h in a shaker (Wooju Scientific, Kimpo, Korea) at room temperature. A sample of 1-2 mL of each extract was filtered through a 0.2 μm pore syringe filter (4 mm, Milipore, Bill, USA) before aliquots were analyzed using HPLC. In case of solid sorbent and glass fiber filter, after removal of 1.5 mL, it was dissolved in 100 μL of methanol.

LOD, LOQ, and reproducibility

Aliquots (10 μL) of indoxacarb standard solution (0.01~0.5 μg/mL) were analyzed for LOD determination before LOQ calculation. For reproducibility test, two levels (LOQ and 10 LOQ) of indoxacarb standard solution were analyzed six times by HPLC to calculate coefficient of variation (C.V). Various standard solutions (0.05~10 μg/mL) were analyzed for establishment of calibration curve. After 1 and 3 days of storage, the linearity (coefficient determination, R^2) of the curve was investigated again.

Recovery (Matrix extraction efficiency) test

Three levels (LOQ, 10 LOQ and 20 LOQ) of standard solution were spiked in gloves, glass fiber filter, and solid sorbent. The extraction and analysis of indoxacarb were repeated three times according to the method described in the above analytical procedure.

Trapping efficiency and breakthrough tests

The trapping efficiency was tested by spiking standard solution (10 LOQ) on the bottom of U-shape glass tube (Daejung Chemical, Daejeon, Korea) connected with solid sorbent, and passing air through the system at 1 L/min for 4 h. U-shape glass tube was heated to 70°C to help volatilization of compounds. The residue in U-shape glass tube and the amount trapped in XAD-2 resin were analyzed for mass balance. Breakthrough test was conducted by spiking standard solution (50 LOQ) in the 1°-resin part of the solid sorbent tube and passing air through the tube at 1 L/min for 4

h. Subsequently, 1º- and 2 º -part of resin were analyzed separately. All analysis and test were repeated 3 times.

Field study, calculation of exposure, and risk assessment

Hand exposure was monitored using cotton gloves and for the analysis in exposure matrices. A personal air monitor (PAM) consists of air pump (Gillian Model 224-PCXR7, MSA, Dong Ha Trading Co. Ltd, Seoul, Korea), glass fiber filter (37 mm, SKC, Eighty Four, PA) in open-faced cassette (SKC) and solid sorbent (ORBOTM 609 Amberlite XAD-2 400/200 mg, Supelco, MO, USA) (Kim et al., 2012b). A glass fiber filter cassette and a XAD-2 resin tube were attached with clips on breathing zone of worker, and air pump was fastened on the belt. The air flow rate was 1 L/min. After mixing/loading or spraying, exposure samples were collected by preventing contamination. Workers prepared spray mixture by mixing indoxacarb WP and WG with water after weighing a specific amount of powder (62 g of WP in 250 L water and 41.6 g of WG in 250 L). The mixture was stirred using a long stick. After experiment, gloves were sampled in a zip lock bag and transferred to laboratory immediately analysis. Hand exposure amount was determined by multiplying extraction solvent amount. Inhalation exposure amount (ng) was obtained by extrapolating pesticide residue (ng) in an inhalation exposure matrix to the ratio of the air volume collected to the respiration rate of working situation (1,270 L/h) for adult male Korean (Kim et al., 2011). The risk assessment was carried out by (Kim et al., 2012b). AOEL is 0.004 mg/kg b.w./day. If MOS $\geq$ 1, the working condition is considered to be safe.

Results and Discussion

Method Validation

Method validation for the exposure monitoring was established successfully through several experiments. LOQ was calculated as 4 times greater than LOD and the values were low enough for the detection of indoxacarb. LOD and LOQ were 0.25 and 1 ng, respectively. R^2 of calibration curve linearity was more than 0.9999. Good reproducibility (C.V < 6%) showed that instrument was stable for analysis. Recovery of indoxacarb from gloves, solid sorbent and glass fiber filter at three different levels was 81.5~108.8% (Fig. 6). To validate the sampling method in inhalation exposure monitoring, trapping efficiency test and breakthrough test were performed. The trapping efficiency test was to measure the efficiency of solid sorbent for the trapping of pesticide in air. This experiment allowed a mass balance of over 112.0% by adding up the indoxacarb trapped in the solid sorbent and the residue at the bottom of the U-shaped glass tube. Because it was not volatile, most of indoxacarb remained at the bottom of U-shaped glass tube. The breakthrough test evaluates the capacity of the solid sorbent to retain pesticide. The recovery of the first adsorbent was 89.5% and the recovery of the second adsorbent was 0.9%. The validated approaches have been applied to assess potential exposure of pesticide operator mixing/loading indoxacarb for power sprayer.

Hand exposure, inahalation exposure and MOS

Exposure to the dermal during the preparation of the pesticide spray solution was done hands because it was the most important route of exposure pathway (Vercruysse et al., 1999). According to a study by Machado-Neto et al. (1998), 86% exposure of body parts was hands. In addition, in the case of methomyl wetting powder, 19.0~99.9% of hand exposure was observed (Kim et al., 2012b).

It has also been reported that the exposure of the hand during mixing/loading was more than the hand exposure amount when spraying (Vercruysse et al., 1999; Ramwell et al., 2005). Therefore, evaluation of hand exposure of pesticides is very important, Hand exposure measurement methods have cotton gloves(Calumpang and Medina, 1996; Egea González et al., 1999; Cattani et al., 2001; Kim et al., 2012a), hand rinse/washes, and hand wipe methods. During mixing/loading procedure, hand exposure amount (75 percentile of 30 repetitions) for indoxacarb WP was 6 folds (459.8 mg/kg a.i) than that of WG (81.4 mg/kg a.i) (Table 21). This result indicates that WG has less drift than WP thanks to its granular type of formulation. Inhalation amount was 10^{-8}~10^{-7}% of spray mixture prepared and 10^{-4}~10^{-3}% of hand exposure (Table 22). In inhalation case, no significant differences were observed between two formulations. Margin of safety was calculated for risk assessment using male Korean average body weight and acceptable operator exposure level as the important exposure factors. Mixing/loading procedures for both of the formulations were considered to be of least risk because calculated MOS values were more than 1.

Figure 6. Extraction efficiency of indoxacarb from cotton gloves, XAD-2 resin and glass fiber filter.

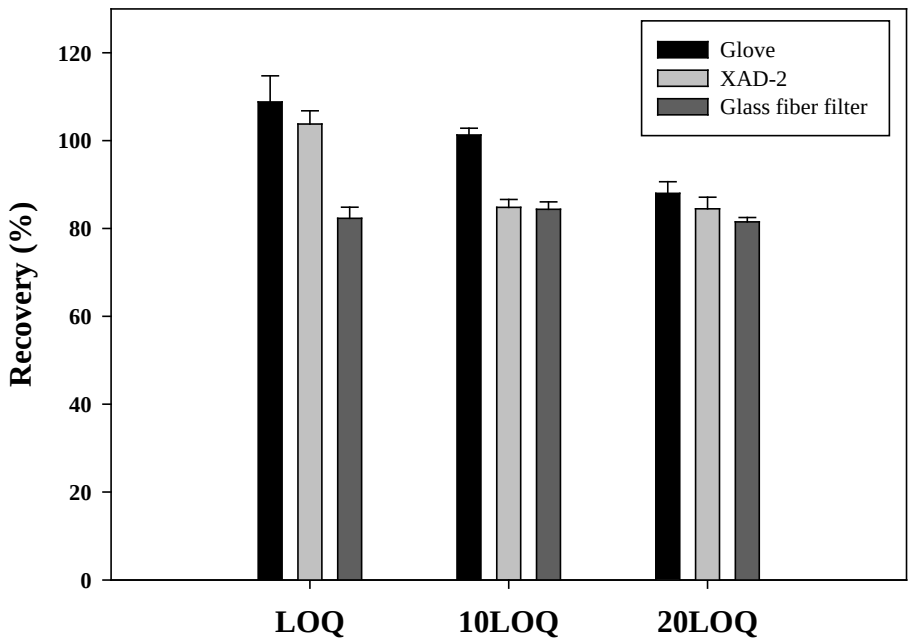

Glove
XAD-2
Glass fiber filter
Recovery (%)
120
100
80
60
40
20
0
LOQ
10LOQ
20LOQ

Table 21. Hand exposure during mixing/loading of indoxacarb

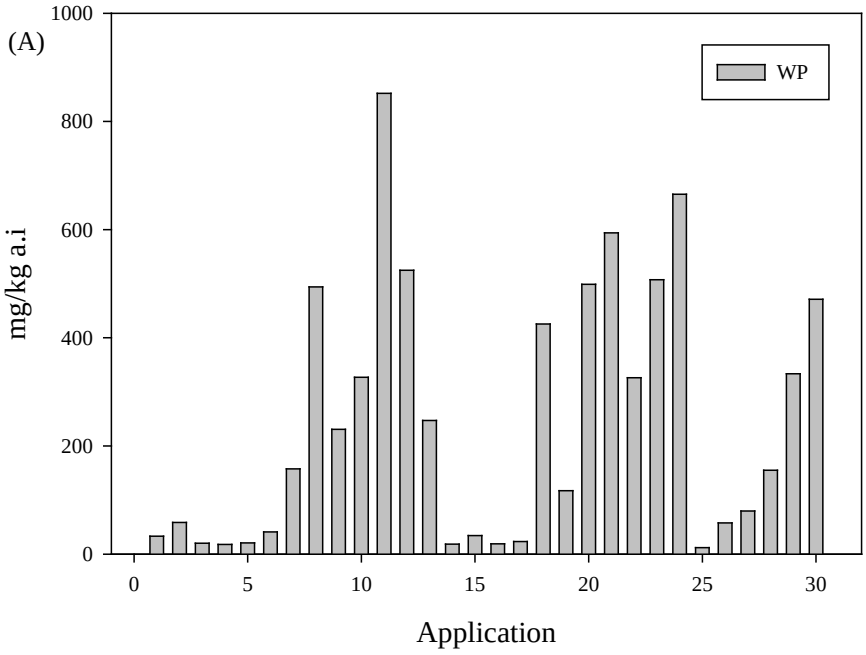
Hand exposure	WP (mg/kg)	WG (mg/kg)
75 percentile	459.8	81.4
min value	12.1	2.5
max value	852.0	194.1

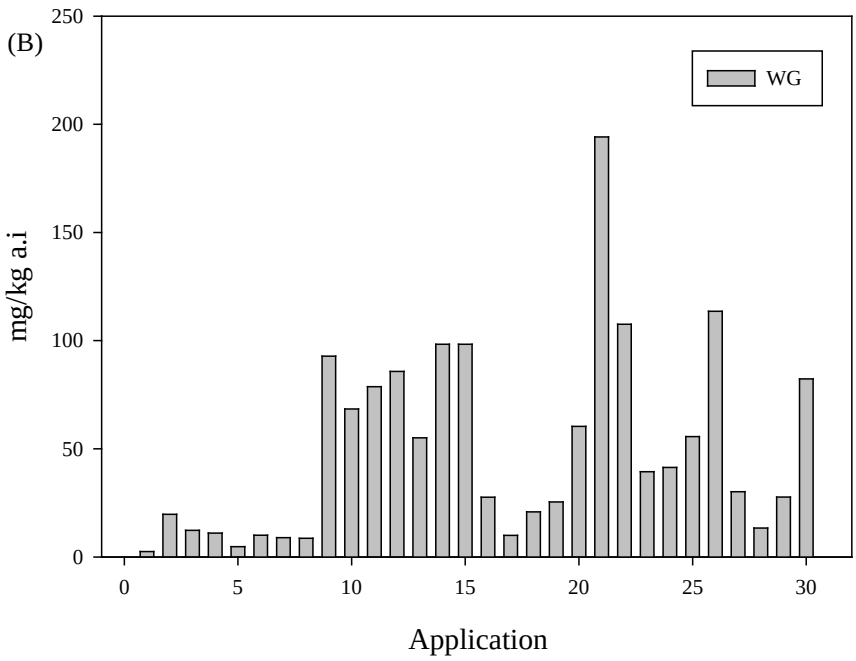
Table 22. Inhalation exposure during mixing/loading of indoxacarb

	WP	WG
Exposure amount (μg, 75percentile)	0.014	0.012
Applied a.i[a] (g)	6.2	12.5
Ratio to applied a.i.[a] (%)	2.3×10^{-7}	9.6×10^{-8}
Hand exposure (μg)	2,851	1,018
Ratio to hand exposure (%)	4.9×10^{-4}	1.2×10^{-3}

[a]a.i.; Active ingredient

Figure 7. Hand exposure of indoxacarb wettable powder (A) and water dispersible granule (B) during mixing/loading.


(A)
WP
mg/kg a.i
1000
800
600
400
200
0
0
5
10
15
20
25
30
Application


(B)
WG
mg/kg a.i
250
200
150
100
50
0
0
5
10
15
20
25
30
Application

Part 5

Hand Exposure of Operator to Chlorpyrifos during Mixing/loading and Risk Assessment

Materials and Methods

Reagents and materials

Chlorpyrifos (99.9%) of analytical standard grade was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chlorpyrifos emulsion concentrate (EC) (20%) was purchased from a pesticide vender for field study. Hand exposure was monitored using cotton gloves and for the analysis in matrices, an Agilent 6890 gas chromatograph (GC; SantaClara, CA, USA) with an electron capture detector.

Analytical method validation

This method validation was carried out in accordance with the method referred by Kim et al. (2011). Aliquots (1 μL) of standard solutions from 0.01 to 1 ppm were analyzed to determine the limit of detection (LOD) and limit of quantitation (LOQ). To validate instrumental reproducibility, 3 levels of standard solution (LOQ, 10LOQ, and 100LOQ) were analyzed 7 times, and the coefficient of variation (C.V.) was calculated. Various standard solutions were analyzed to construct a calibration curve, and the linearity of the curve was investigated again after 1 and 3 days of storage. For the recovery test, 3 levels of standard solution (LOQ, 10LOQ, and 100LOQ) were spiked in gloves by shaking, prior to the extraction with the 300 mL of acetone by 1 h shaking.

Measurement of hand exposure and risk assessment

Before the exposure assessment, all operators washed hands. Workers prepared the spray mixture by mixing EC (500 mL for SS) with 500 L of water in a mixing tank.

3 Workers made spray suspension for about 3 min. Each replicate consisted of two 500L mixing/ loadings or applications and 30 replicates were made (60 spray of 500L tank). After experiment, gloves were sampled in a zip lock bag and transferred to laboratory immediately analysis. Hand exposure amount was determined by multiplying extraction solvent amount.

The risk assessment was carried out by comparing the pesticide exposure forecasted to the relevant risk value, AOEL. For this, the margin of safety (MOS) (Hughes et al., 2008a) for workers was calculated using following modified formula: MOS = AOEL / AQE per unit Korean male body weight (69.2 kg). AOEL of chlorpyrifos is 0.01 mg/kg b.w./day. (PPDB, 2005). If MOS $\geq$ 1, the working condition is considered to be safe. The hand exposure was calculated using the 75th percentile of 30 repetitions. The potential dermal exposure (PDE) values was obtained by extrapolating corresponding exposure 8 times. The IDE (internal dermal exposure) value was based on assumptions of 10% skin absorption for PDE after 10% of penetration through clothes was considered.

Results and Discussion

Method validation

Exposure and risk assessment during mixing/loading of chlorpyrifos emulsifiable concentrate (EC, 20%) were carried out. Limit of detection and limit of quantitation were 0.02 and 0.1 ng, respectively. Good instrumental repeatability (C.V. < 6%) and consistent linearity of calibration curves for 3 days (R^2 >0.999) showed the analytical instrument was precise and stable. Recovery of chlorpyrifos from gloves was 72.3-103.4% (Fig. 8).

Hand exposure and risk assessment

During mixing/loading procedure, average hand exposure amount of chlorpyrifos was 3.9 mg which is corresponding to 0.004% of total active ingredient in the prepared spray mixture. In calculation of MOS (Margin of Safety) for risk assessment, male Korean average body weight and AOEL (Acceptable Operator Exposure Level) were used. Nine events of mixing/loading procedure were assumed per day. And 75 percentile of 30 repetition (4.6 mg) was used as for the worst case (Table 23). MOS was more than 1 for total repetition, indicating mixing/loading work was of least risk (Table 24). However, MOS of individual repetition was examined, two cases were less than 1 suggesting careful work habit is essential in mixing/loading procedure.

Figure 8. Extraction efficiency of chlorpyrifos from cotton gloves

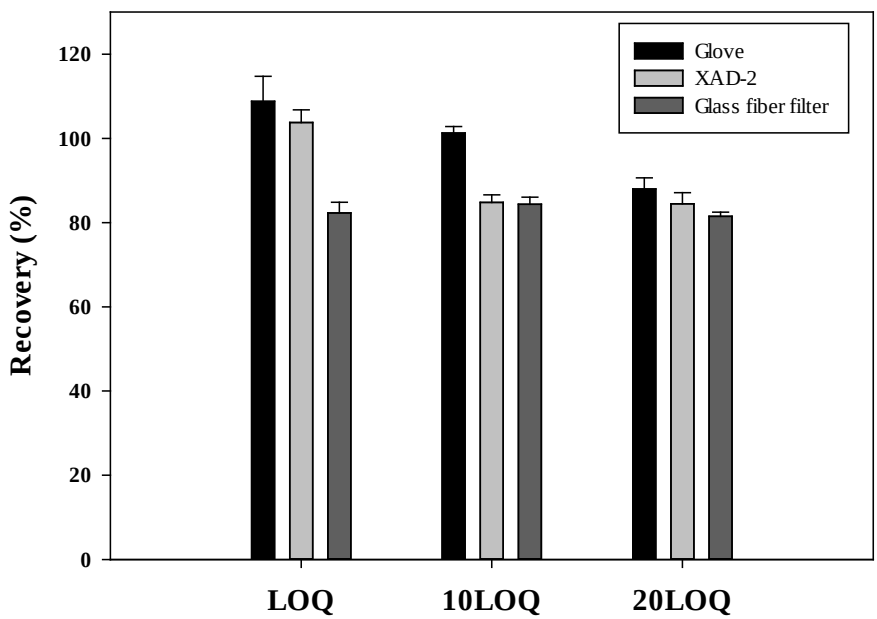

Glove
XAD-2
Glass fiber filter
Recovery (%)
120
100
80
60
40
20
0
LOQ
10LOQ
20LOQ

Table 23. Amount of hand exposure during mixing/loading.

Trial	1	2	3	4	5	6	7	8	9	10
Exposure (mg)	0.9	2.6	0.7	0.4	2.8	3.2	1.8	0.5	1.7	1.6
Trial	11	12	13	14	15	16	17	18	19	20
Exposure (mg)	3.3	7.0	1.4	1.9	4.9	2.2	24.2	2.6	1.5	1.8
Trial	21	22	23	24	25	26	27	28	29	30
Exposure (mg)	6.6	1.6	0.9	3.6	6.5	8.0	10.3	5.1	2.9	2.6
Minimum	0.4 mg									
Maximum	24.2 mg									
Average	3.9 mg									
75 percentile	4.6 mg									

Table 24. Calculation of MOS for mixing/loading of chlorpyrifos.

PDE[a] (mg/day)	IDE[b] (mg/day)	MOS[c]
39.2	0.4	5.4

[a]Potential dermal exposure

[b]Internal dermal exposure

[c]Margin of safety

Chapter II

In vitro metabolism of kresoxim-methyl by human liver microsome

Introduction

In vitro human metabolism studies of pesticides (biotransformation)

In vitro human metabolism studies of pesticides are very important and useful for prediction and management of the hazard and risk arising from introduction of pesticides in that understanding metabolism of pesticides. It should be emphasized that, although pesticides and their use have many positive attributes, in terms of their interactions with living organisms, pesticides are xenobiotics and are processed (metabolized) in the same way as other xenobiotics such as clinical drugs and industrial chemicals (Hodgson, 2010). The metabolism of pesticides involves three phase process; in first phase process (phase I) the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent, in second phase process (phase II) involves conjugation of a pesticide to a sugar or amino acid, which increases the water solubility and reduces toxicity compared with the parent pesticide, and in third phase process helps in conversion of second phase metabolites into secondary conjugates, which are also non-toxic (Van Eerd et al., 2003; Verma et al., 2014). Phase I reactions involve hydrolysis, reduction, and oxidation. These reactions expose or introduce a functional group (–OH, $–NH_2$, –SH or –COOH), and usually result in only a small increase in hydrophilicity. The cofactors for these reactions react with functional groups that are either presented on the xenobiotic or are introduced/exposed during phase I biotransformation (Parkinson, 2001). During the recent years, a large number of papers have been published on the metabolism of pesticides by human CYPs and HLMs involved in (Table 25).

Table 25. Metabolism studies of pesticides by human CYPs and HLMs

Pesticide	Chemical class	Usage	Reference
2,4-D	Phenoxy	Herbicide	(Ohkawa et al., 1998)
Acetochlor	Chloroacetanilide	Herbicide	(Kale et al., 2008) (Coleman et al., 2000)
Alachlor	Chloroacetanilide	Herbicide	(Coleman et al., 1999) (Kale et al., 2008)
Ametryne	Triazine	Herbicide	(Cresteil et al., 1979)
Atrazine	Triazine	Herbicide	(Cresteil et al., 1979) (Joo et al., 2010)
Azinphos-methyl	Organophosphate	Insecticide	(Buratti et al., 2003)
Benfuracarb	Carbamate	Insecticide	(Abass et al., 2014a) (Abass et al., 2014b)
Bifenthrin	Pyrethroid	Insecticide	(Scollon et al., 2009)
Bioresmethrin	Pyrethroid	Insecticide	(Scollon et al., 2009)
Butachlor	Chloroacetanilide	Herbicide	(Coleman et al., 2000)
Carbaryl	Carbamate	Insecticide	(Tang et al., 2002)
Carbosulfan	Carbamate	Insecticide	(Abass et al., 2009)
Chlorfenvinphos	Organophosphate	Insecticide	(Hutson and Logan, 1986)
Chlorpyrifos	Organophosphate	Insecticide	(Tang et al., 2001) (Buratti et al., 2003) (Sams et al., 2004) (Mutch and Williams, 2006)
Chlorpyrifos	Organophosphate	Insecticide	(Choi et al., 2006) (Foxenberg et al., 2007) (Croom et al., 2010) (Smith et al., 2011)
β-Cyfluthrin	Pyrethroid	Insecticide	(Scollon et al., 2009)
λ-Cyhalothrin	Pyrethroid	Insecticide	(Scollon et al., 2009)
Cypermethrin	Pyrethroid	Insecticide	(Scollon et al., 2009)
Deltamethrin	Pyrethroid	Insecticide	(Godin et al., 2006) (Godin et al., 2007)
Diazinon	Organophosphate	Insecticide	(Kappers et al., 2001) (Buratti et al., 2003) (Sams et al., 2004) (Mutch and Williams, 2006) (Ellison et al., 2012)
Dimethoate	Organophosphate	Insecticide	(Buratti and Testai, 2007)

Pesticide	Chemical class	Usage	Reference
Disulfoton	Organophosphate	Insecticide	(Usmani et al., 2004)
Diuron	Phenylurea	Herbicide	(Abass et al., 2007)
Endosulfan	Cyclodiene	Insecticide	(Lee et al., 2006)
Esfenvalerate	Pyrethroid	Insecticide	(Godin et al., 2006) (Godin et al., 2007)
Fenthion	Organophosphate	Insecticide	(Furnes and Schlenk, 2005) (Leoni et al., 2008)
Fipronil	Phenylpyrazole	Insecticide	(Tang et al., 2004) (Joo et al., 2007)
Flucetosulfuron	Sulfonylurea	Herbicide	(Lee et al., 2014)
Furametpyr	Anilide	Fungicide	(Nagahori et al., 2000)
Imidacloprid	Neonitotinoid	Insecticide	(Schulz-Jander et al., 2002)
Isocarbofos	Organophosphate	Insecticide	(Zhuang et al., 2014)
Malathion	Organophosphate	Insecticide	(Buratti et al., 2005)
Methiocarb	Carbamate	Insecticide	(Usmani et al., 2004)
Methoxychlor	Organochlorine	Insecticide	(Stresser and Kupfer, 1998) (Hu and Kupfer, 2002)
Metolachlor	Chloroacetanilide	Herbicide	(Coleman et al., 2000)
Molinate	Thiocarbamate	Herbicide	(Jewell and Miller, 1999)
Myclobutanil	Triazole	Fungicide	(Barton et al., 2006)
Parathion	Organophosphate	Insecticide	(Butler and Murray, 1997) (Buratti et al., 2003) (Sams et al., 2004) (Mutch and Williams, 2006) (Foxenberg et al., 2007)
Parathion-methyl	Organophosphate	Insecticide	(Ellison et al., 2012)
Permethrin	Pyrethroid	Insecticide	(Scollon et al., 2009) (Lavado et al., 2014)
Phorate	Organophosphate	Insecticide	(Hodgson, 2003) (Usmani et al., 2004)
Sulprofos	Organophosphate	Insecticide	(Usmani et al., 2004)
Terbuthylazine	Triazine	Herbicide	(Cresteil et al., 1979)
Terbutryne	Triazine	Herbicide	(Cresteil et al., 1979)
Thiamethoxam	Neonicotinoid	Insecticide	(Swenson and Casida, 2013)

Human liver microsomal CYP450

The CYP superfamily consisted of more than 7,000 named sequences in animals, plants, bacteria and fungi. The human genome has 57 CYP genes, and the function for most of the corresponding enzymes is known at least to some degree. Fifteen individual CYP enzymes in families 1, 2 and 3 metabolize xenobiotics, including the majority of small molecule drugs currently in use. A typical feature of these CYPs is broad and overlapping substrate specificity (Guengerich, 2003). Cytochrome P450 enzymes are found in practically all tissues, with highest abundance and largest number of individual CYP forms present in the liver. CYPs reside also in the intestine, lung, kidney, brain, adrenal gland, gonads, heart, nasal and tracheal mucosa, and skin. In human liver CYP enzymes comprise approximately 2% of total microsomal protein (0.3–0.6 nmol of total CYP per mg of microsomal protein). The content of drug-metabolizing CYPs is much lower in other tissues. While extrahepatic metabolism may have clinically significant local effects, systemic metabolic clearance of drugs occurs in the liver with a significant contribution by the gut wall in special cases (Pelkonen et al., 2008). Ten individual CYP forms in the adult human liver carry out virtually the whole CYP-mediated metabolism. CYP3A4 is the highest abundance form and it metabolizes the greatest number of drugs and a very large number of other xenobiotics. Together CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are responsible for more than 90% of known oxidative drug metabolism reactions (Pelkonen et al., 2008). CYP1 Family of microsomal cytochrome P450s consists of three members, CYP1A1, CYP1A2 and CYP1B1. The two members of the CYP1A family (CYP1A1 and CYP1A2) show greater than 70% amino acid sequence identity but display very

different patterns of tissue expression. CYP1A1 is expressed primarily in extrahepatic tissues such as the lungs, lymphocytes and placenta while only low-level expression has been reported in liver tissue. In contrast, CYP1A2 is expressed primarily in the liver with little if any detectable expression in the extrahepatic tissues (Danielson, 2002). The CYP1A enzymes bioactivate several procarcinogens. CYP 1A1 activate benzo pyrene and other polyaromatic hydro carbons. CYP 1A2 activate aromatic amines, such as 2-acetylaminofluorene, heterocyclic amines, and aflatoxin B1 (Omiecinski et al., 1999). CYP 1B1 is a more recently characterized member of the CYP1 family. CYP1B1 is constitutively expressed in most tissues but also inducible through the Ah receptor pathway. CYP1B1 is involved in the metabolism of endogenous estrogens, as well as active in the biotransformation of heterocyclic amines found in charcoal broiled meats (Crofts et al., 1997). The third member of the CYP1 family, CYP1B1 is constitutively expressed at low levels in a broad range of tissues including brain, colon, heart, kidney, leukocytes, liver, lung, ovary, placenta, prostate, skeletal muscle, small intestine, spleen, and thymus CYP 1A2 is universally expressed in human liver (Danielson, 2002). CYP2 family is the largest family of cytochrome P450s in humans comprising approximately one third of human cytochrome P450s sequences. This family of microsomal cytochrome P450s is classified into 13 subfamilies that consist of 16 functional genes (CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1) and 13 confirmed pseudogenes (CYP2A7PT (telomeric), CYP2A7PC (centromeric), CYP2A18P, CYP2B7P1, CYP2B7P2, CYP2B7P3, CYP2D7AP, CYP2D8P, CYP2F1P, CYP2G1P, CYP2G2P,

CYP2T2P, CYP2T3P). Members of the CYP2 family of cytochrome P450s play a significant role in drug metabolism although only CYP2A6 appears to be expressed to any significant degree in hepatic tissue. Other members of the CYP2 family are expressed in a sex-specific manner and thus it is not unexpected that these enzymes support the hydroxylation of steroids including sex-specific steroids. The CYP2A proteins, in particular are expressed at much higher levels in the olfactory mucosa of fetal tissues at gestational days 91- 125 than in corresponding hepatic tissues. It has been suggested that such prenatal expression of xenobiotic metabolizing cytochrome P450s indicates that the human fetal olfactory mucosa may be an important target site for chemical toxicity during early development (Gu et al., 2000). CYP 2A6 may participate in procarcinogen activation but has a relatively minor role in drug biotransformation and account for only approximately 4% of total immune quantified human hepatic CYP. CYP 2A6 participates in nicotine metabolism. CYP 2B6 was previously described as a low abundance isoform in human liver, large inter-individual variations are observed in expression, with hepatic microsomal content varying from as low as 0.3 pmol/mg protein to as high as 82 pmol/mg protein (Code et al., 1997)). CYP 2C subfamily consists of 2C8, 2C9, 2C18 and 2C19. CYP 2C8 is only known to play a major role in the metabolism of the anticancer agent taxol and it contributes to carbamazepine ephydroxylation. CYP 2C9 is the primary enzyme responsible for the metabolism of tolbutamide, S-warfarin, phenytoin, losartan, ibuprofen, and celecoxib. CYP 3A subfamily consist of 3A4, 3A5, 3A7 and 3A43. CYP 3A isoforms are the most abundant in both human liver and small intestine, accounting for an average of 28.8% of total human hepatic CYP. The majority

of oxidatively bio-transformed therapeutic agents are metabolized at least in part by this subfamily of enzymes. CYP 3A4 is the major adult isoform and is universally expressed both in human liver and in the small intestine. CYP 3A5 is polymorphically expressed in approximately 20% of the population, while CYP 3A7 is expressed only in fetal liver. CYP 3A43 has only been recently identified, and its importance in human drug metabolism remains to be established. Expression and activity of CYP 3A isoforms in humans show wide individual variability (Venkatakrishnan et al., 2001).

Enzyme kinetics in metabolism

Enzyme kinetics is study of the rate (velocity) of the enzyme-catalyzed reaction. It is the central approach to studying the mechanism of an enzyme-catalyzed reaction. A key factor affecting the reaction rate by a specific enzyme is the concentration of substrate, [S]. However [S] is in dynamic changes and converted to [P], which is complicated to studying the effects of substrate concentration by the fact that [S] changes. One simplifying approach in kinetics experiment is to measure the initial velocity, V_0, at high substrate concentration. A simple enzymatic reaction might be written

$$E + S \leftrightarrow ES \leftrightarrow EP \leftrightarrow E + P$$

E, S, and P represent the enzyme, substrate, and product, respectively. ES and EP are transient complexes of the enzyme with the substrate and with the product. Enzyme exists in two forms, E and ES. At low substrate concentrations, $V_0 = k[S]$. Where V_0 is the initial velocity, at high substrate concentrations, $V_0 = V_{max}$ (maximum velocity).

Enzyme reaction is divided in three states. In the short time prior to the steady state, there is a burst of ES complex formation as substrate is quickly bound by empty enzyme. The submaximal rate of substrate utilization and product formation reflects the fact that it takes some time for the ES complexes to form; Pre-steady state. While the initial [S] is much greater than [E]t, a steady state is maintained where ES is formed at the same rate as it is decomposed: $d[ES]/dt \approx 0$. Here, the rate of product formation is at $V_{max.}$ [S] decreases at it are converted to P. When [S] approaches [E]t, [ES] begins to drop as there is not enough S to keep E saturated, and the steady state assumption is no longer valid. Note that this region is not describing equilibrium, just a lack of readily available substrate for the enzyme, for I are still ignoring the reverse reaction for product formation. The measured V_0 is generally reflects the steady state, even though V_0 is limited to the early part of the reaction, and analysis of these initial rate is referred to as steady-state kinetics. The curve expressing the relationship between [S] and V_0 has the same general shape for most enzymes, which can be expressed algebraically by the Michaelis-Menten equation. The equation is

$$V_0 = (V_{max} \cdot [S]) / (K_m + [S])$$

The important terms are [S], initial velocity V_0, maximum velocity V_{max}, and Michaelis constant K_m. The Michaelis constant is equal to the substrate concentration that sufficient to give half the maximum velocity for the enzyme. One should get an intuitive feeling for the magnitude of K_m. A small concentrations of substrates are sufficient to saturate the enzyme and to reach the maximum catalytic efficiency of the enzyme. These K_m and V_{max} values were

then used to calculate the intrinsic clearance value ($Cl_{int} = V_{max} / K_m$), the functional ability of the enzyme. Most metabolite kinetics studies involve the use of hepatic microsomes, which contain a mixture of several CYP isoforms with overlapping specificity, and the observed rates of metabolism reflect the net effect of several protein-drug interactions. In some cases there may be a smoothing over of any irregularities and the kinetics may look hyperbolic due to the 'candling out' of different kinetic features. In other cases, complications can arise due to the differing impact of several isoforms at different substrate concentrations. Such complications are absent when purified and recombinant enzymes are used, and for many drugs metabolic kinetics can be analyzed appropriately by the Michaelis-Menten equation (Tassaneeyakul et al., 1993; Veronese et al., 1993). However, the full capacity of the organ will only be estimated when appropriate allowance is made for the consequences of both parallel and sequential pathways of metabolism. The integration of the total hepatocellular activity with the other physiological determinants of liver clearance, namely, blood flow and drug binding on the blood matrix, requires the use of a pharmacokinetic model. Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion of chemicals to describe the time course of the chemical in the body. The aim is to provide data to enable an understanding to be made of the safety of the compound to the manufacturer, user and consumer of treated produce. Basic pharmacokinetic parameters will provide information on the potential for accumulation of the test substance in tissues and/or organs and the potential for induction of biotransformation as a result of exposure to the test substance.

Materials and Methods

Chemicals and reagents

Pooled HLMs (Table 26), and 10 different cDNA-expressed human recombinant P450s, CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5 (Supersomes) were purchased from BD Gentest (Woburn, MA, USA). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate ($NADP^+$), nicotinamide adenine dinucleotide phosphate reduced (NADPH), potassium phosphate monobasic/dibasic, and magnesium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was HPLC grade (Fisher Scientific CO., Pittsburgh, PA, USA) and the other chemicals were of the highest quality available.

Analytical instruments and conditions

LC–MS/MS analysis was performed on LCMS-8050 (Shimadzu, Japan) coupled to Nexera UHPLC (Shimadzu, Japan) with electrospray (ESI, positive mode). The analytical column was a Kinetex C18 (100 × 2.1 mm i.d., 2.6 μm, Phenomenex®, USA) and the column oven temperature was 40ºC. The injection volume was 2 μL and the mobile phases were eluted at a 0.2 mL/min. Mobile phases were 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). For gradient elution, the initial combination was 30 : 70 (A : B, v/v) and the B solution was increased to 100 % in duration of 2 min, holding for 1 min. The full scan condition on LCMS-8050, scan range was 80 to 500 m/z.

Table 26. Characteristics of BD Ultra Pool HLM 150[*]

Enzyme Measured	Assay	Enzyme Activity [in pmol/(mg×min)]
Total P450	Omura and sato	350 pmol/mg
OR[**]	Cytochrome c Reductase	270
Cyt. b_5	Spectrophotometric	520 pmol/mg
CYP1A2	Phenacetin O-deethylase	690
CYP2A6	Coumarin 7-hydroxylase	830
CYP2B5	(S)-Mephenytoin N-demethylase	40
CYP2C8	Paclitaxel 6α-hydroxylase	170
CYP2C9	Diclofenac-4'-hydroxylase	3100
CYP2C19	(S)-Mephenytoin N-hydroxylase	57
CYP2D6	Bufuralol 1'-hydroxylase (The amount of activity inhibited by 1 μM quinidine)	71
CYP2E1	Chlorozoxazone 6-hydroxylase	2400
CYP3A4	Testosterone 6β-hydroxylase	4600
CYP4A11	Lauric acid 12-hydroxylase	1700
FMO	Methyl p-Tolyl Sulfide Oxidase	1100
UGT1A1	Estradiol 3-Glucuronidation	940
UGT1A4	Trifluoperazine Glucuronidation	580
UGT1A6	Serotonin Glucuronidation	10000
UGT1A9	Propofol Glucuronidation	3700
UGT2B7	AZT Glucuronidation	760

* : Provided from BD Biosciences

** : OR, Oxido-reductase

Metabolism of kresoxim-methyl in HLMs (Phase I reaction)

To determine the metabolite formation from kresoxim-methyl, the incubation mixtures containing 50 mM potassium phosphate buffer (pH 7.4), 10 mM magnesium chloride, pooled HLMs (0.5 mg/mL), NADPH-generating system (1 mM $NADP^+$, 5 mM glucose-6-phosphate, 0.25 U glucose-6-phosphate dehydrogenase, and 1 mM NADPH), and 10 μM kresoxim-methyl were prepared in a total incubation volume of 200 μL. The reaction mixtures were incubated at 37°C for 0, 30, 60, and 120 min in a shaking water bath before terminating the reaction by the addition of 200 μL of acetonitrile on ice. The reaction mixture was centrifuged at 13,000 rpm for 7 min at 4°C, and the 10 μL of supernatant was subsequently analyzed with HPLC. Control incubations were conducted in the absence of an NADPH-generating system or with the denatured HLMs at 80°C. HLMs were heated for 30 min at 45°C before the incubation to confirm whether FMOs are involved in the metabolite formation or not.

Metabolite identification

The acetonitrile supernatant (200 μL) of the pooled HLMs reaction mixture was dried with the gentle nitrogen stream, and the residue was dissolved with a 50 μL of acetonitrile to be analyzed with LC-MS/MS scan mode.

Optimization of metabolic conditions and kinetic studies

The metabolic reactions were performed with various concentrations of HLMs (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL) for 0, 5, 10, 15, 20, and 30 min in the same manner as the above method to determine the optimal reaction conditions.

To analyze enzyme kinetics such as V_{max}, K_m, and CL_{int}, a range of kresoxim-methyl concentrations (5, 10, 20, 50, 100, and 200 μM) were used in metabolic reactions under the optimized conditions (HLMs concentration of 0.2 mg/mL and an incubation time of 10 min).

Metabolism of kresoxim-methyl by cDNA-expressed CYP450 isoforms

The metabolic reactions were performed with ten types of cDNA-expressed CYP450 isoforms (10 pmol) and 10 μM of kresoxim-methyl for 10 min to identify CYP isoforms responsible for metabolite formation.

Determination of crystal structure

Small clear crystals were obtained by slow evaporation in a mixture of acetone and hexane. The structure of kresoxim-methyl was determined by single crystal X-ray diffraction methods by Professor Hoseop Yun group of Ajou University. Data collection was performed with Mo Kα1 on an RIGAKU R-ASXIS RAPID diffractometer.

Results and Discussion

Formation of the kresoxim-methyl metabolite by HLMs

HLMs incubation of kresoxim-methyl in the presence of NADPH resulted in the formation of two metabolite (Fig. 9). No metabolites were observed in control reactions with denatured HLMs or with the absence of the NADPH generating system, suggesting that metabolites were formed from kresoxim-methyl by the HLMs metabolic reaction. The metabolites, M1 and M2 from HLMs incubation gave $[M+H]^+$ at *m/z* 330 and $[M+H]^+$ at *m/z* 312. Judging from molecular weight and mass fragment pattern, M1 must be hydroxyl-KM. consequentially, the formation of M1 from kresoxim-methyl is resulted in the metabolic reaction by CYPs, resulting in oxidation.

Optimization of metabolic conditions and kinetic studies

A range of HLMs protein concentrations and incubation time were used in metabolic reactions to obtain 0.2 mg/mL of protein and 10 min of incubation time as the optimal metabolic conditions of kresoxim-methyl in HLMs (Fig. 11). Under these optimized metabolic conditions, the formation pattern of M1 from kresoxim-methyl by HLMs was best fitted to a Michaelis-Menten equation [V = Vmax × [S] / (Km + [S])] to yield 16.8 counts/min/mg proteins of Vmax and 10.2 μM of Km values. M2 In endosulfan sulfate formation from α-endosulfan and β-endosulfan by HLMs, Vmax were 1.48 ± 0.07, 4.40 ± 0.18 pmol/min/ pmol P450, respectively while Km were 7.34 ± 1.29 μM, 6.37 ± 0.88 μM, respectively (Lee et al. 2006). The Clint (mL/min/ mg proteins) is a pure measure of enzyme activity towards a compound and acts as a proportionality constant to describe the relationship between rate of metabolism of a drug and its concentration at the enzyme site. It is not influenced by other physiological

determinants of liver clearance such as hepatic blood flow or drug binding within the blood matrix (Rane et al., 1977; Wilkinson, 1987; Houston, 1994).In this study, the CLint value of M1 was 1.64 μL/min/mg proteins for cyazofamid, while those of the other studies was 48.02~51.20 μL/min/mg proteins for erythro, threo-flucetosulfuron (Lee et al. 2014).

Metabolism of kresoxim-methyl in cDNA-expressed CYP450 isoforms

When metabolites formation from kresoxim-methyl was studied with 10 different human cDNA-expressed CYP isoforms (CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4 and 3A5). The cDNA-expressed CYP isoforms were pre-incubated for 5 minutes at 37℃ in the presence of the NADPH-generating system. The formation of M1 was observed in CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 (Fig. 12). The formation of M2 was observed only in CYP1A2, 2C8, 2C9, 2C19, 3A4, and 3A5 (Fig. 12). In 63 pesticide metabolism studies using human recombinant cytochrome P450 isoforms, CYP 2C19 was involved in metabolism by 15%, CYP2B6 by 12%, CYP2C9 by 10%, and 24% by CYP3A4, which is the most abundant isoform (Abass et al., 2014b).

Figure 9. Formation of metabolite M1 and M2 from kresoxim-methyl when it was incubated with human liver microsomes and NADPH-generating system for 10 min at 37°C. A : Control incubation, B : 10 min of incubation

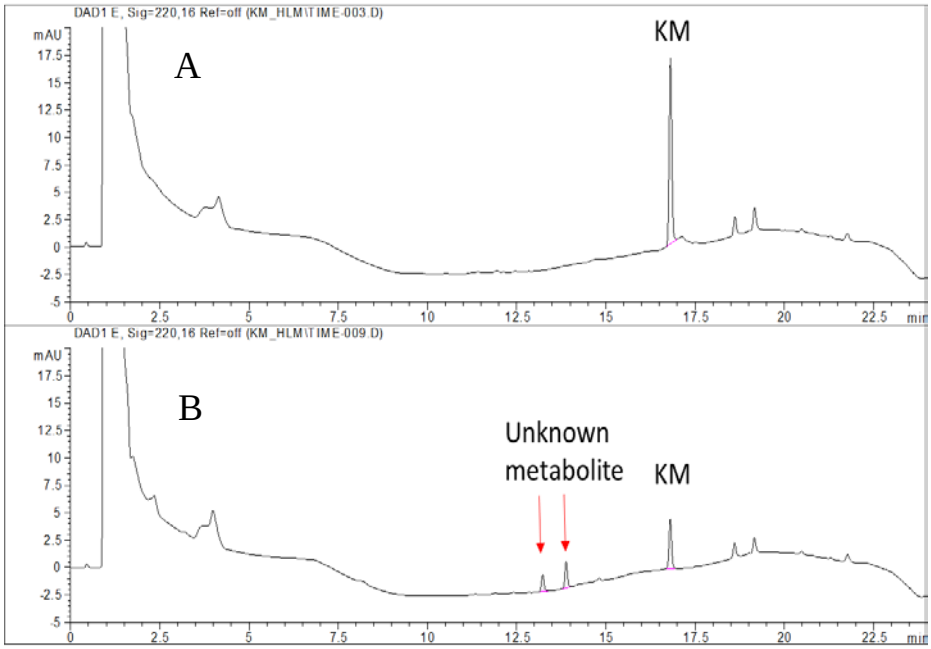

DAD1 E, Sig=220,16 Ref=off (KM_HLM\TIME-003.D)
mAU
A
KM
DAD1 E, Sig=220,16 Ref=off (KM_HLM\TIME-009.D)
B
Unknown
metabolite
KM

Figure 10. LC-MS/MS product ion mass spectrum of M1 from kresoxim-methyl by metabolism of HLM with NADPH-generation system.

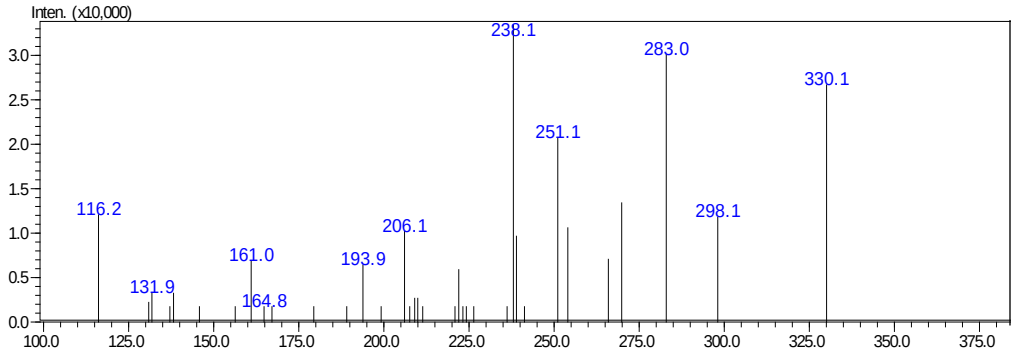
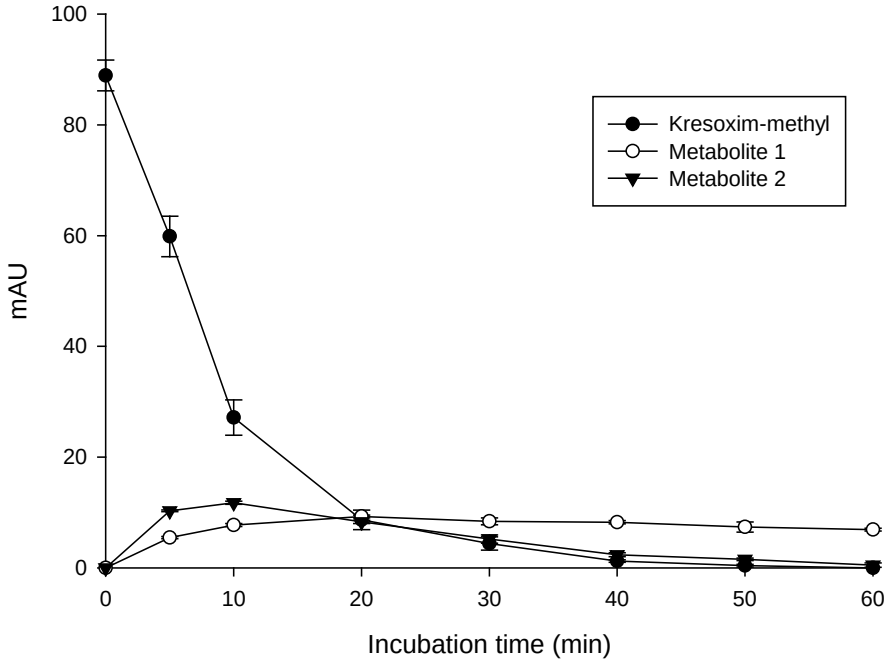

Inten. (x10,000)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
100.0
125.0
150.0
175.0
200.0
225.0
250.0
275.0
300.0
325.0
350.0
375.0
238.1
283.0
330.1
251.1
116.2
298.1
206.1
161.0
193.9
131.9
164.8

Figure 11. Formation of metabolites from kresoxim-methyl depending on the incubation time (A) and protein concentration (B) with human liver microsomes

A


100
80
60
40
20
0
mAU
0
10
20
30
40
50
60
Incubation time (min)
Kresoxim-methyl
Metabolite 1
Metabolite 2

B

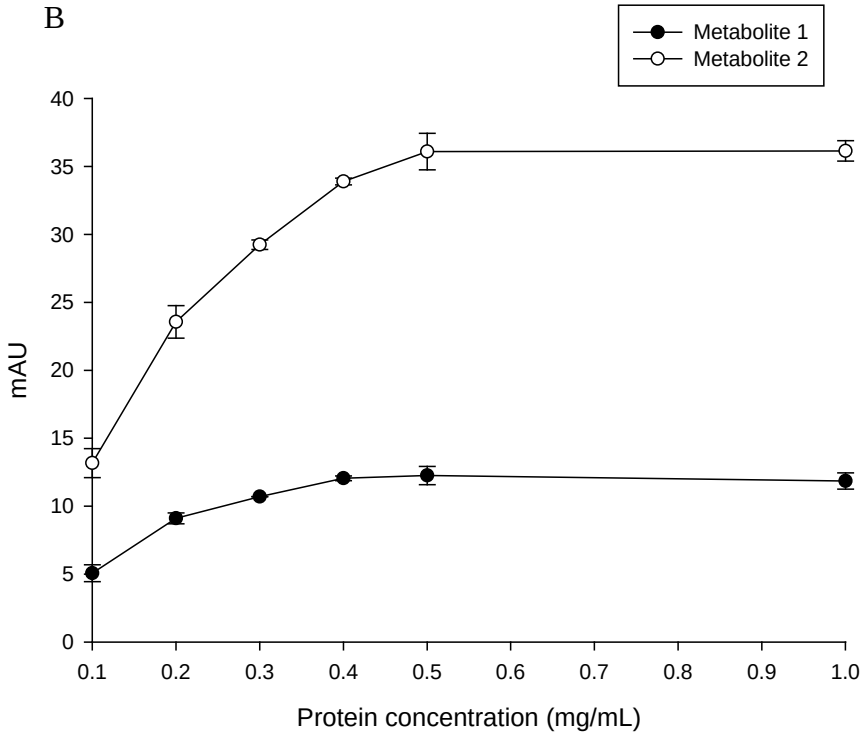
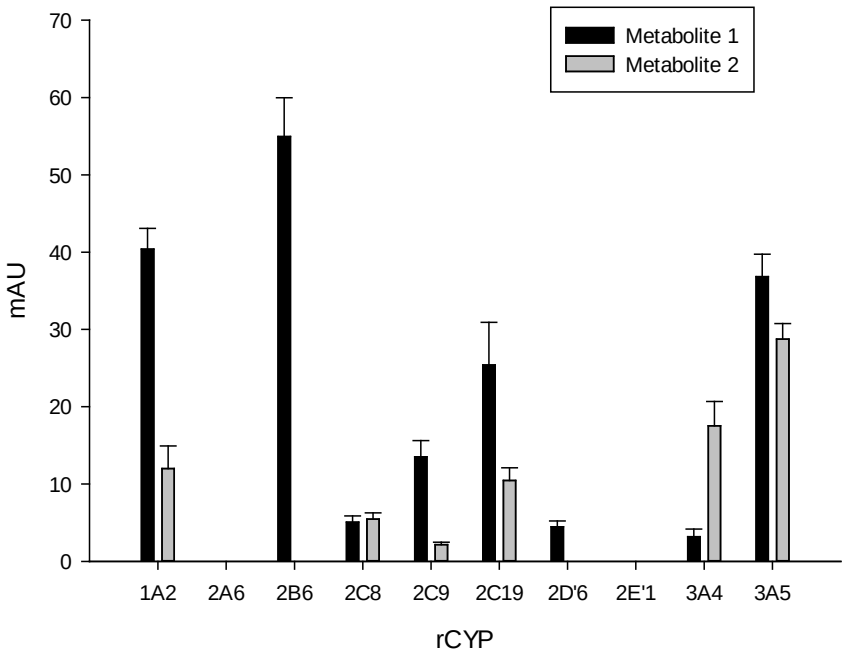

40
35
30
25
20
15
10
5
0
mAU
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Protein concentration (mg/mL)
Metabolite 1
Metabolite 2

Figure 12. The formation of metabolites from kresoxim-methyl by cDNA-expressed P450 isoforms when those were incubated with 10 μM of kresoxim-methyl at 37°C for 10 min. Data shown are averages of triplicate experiments.


Metabolite 1
Metabolite 2
mAU
70
60
50
40
30
20
10
0
1A2
2A6
2B6
2C8
2C9
2C19
2D'6
2E'1
3A4
3A5
rCYP

Determination of crystal structure for kresoxim-methyl

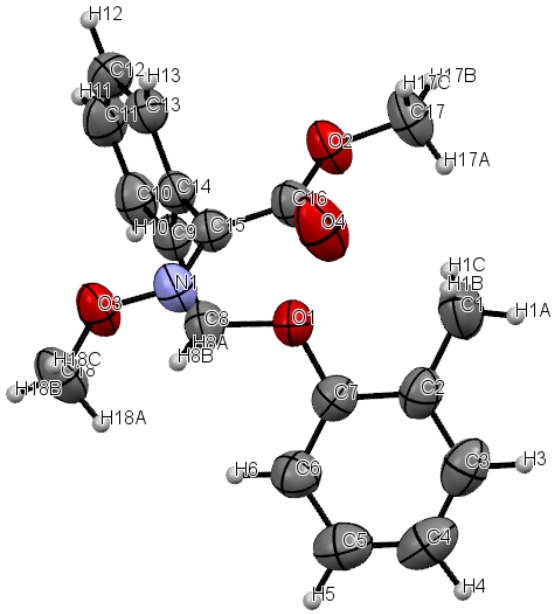
An ORTEP diagram and the packing mode for kresoxim-methyl shown in Fig. 13. The structure of the title compound were determined by single crystal X-ray diffraction methods. A summary of the crystallographic data, data collection and structure refinement for the kresoxim-methyl is given in Table 27. Preliminary examination and data collection were performed with Mo $K\alpha_1$ radiation (λ=0.71073 Å) on a RIGAKU R-ASXIS RAPID diffractometer. The cell constants and an orientation matrix were determined from least-squares, using the setting angles in the range $3.0^{\circ} < \theta < 25.0^{\circ}$. Intensity data were collected with the ω scan technique. The intensity statistics and systematic absences are consistent with the monoclinic space group, *C2/c*. The initial positions for all atoms were obtained by using direct methods of the SHELXS-86 program. The structure was refined by full-matrix least-squares techniques with the use of the SHELXL-97 program. The data were corrected for absorption using the multi-scan method. The final cycle of refinement performed on F_o^2 with 2914 unique reflections afforded residuals wR2=0.1698 and the conventional R index based on the reflections having $F_o^2 > 2\sigma (F_o^2)$ is 0.0484. A difference Fourier synthesis calculated with phases based on the final parameters shows no peak heights greater than 0.327 e/$Å^3$. No unusual trends were found in the goodness of fit as a function of F_o, $\sin\theta/\lambda$ and Miller indices. Anisotropic displacement parameters and complete tabulations on the X-ray studies can be found in CIF format in the Supporting Information Section.

Table 27. Crystal data and structure refinement for kresoxim-methyl

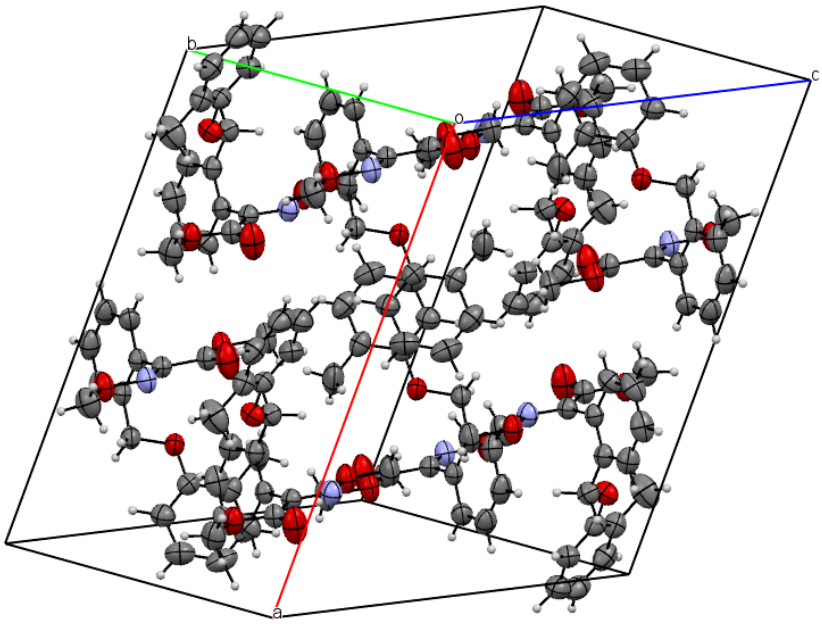
Empirical formula	$C_{18}H_{19}NO_4$
Formula weight	313.34
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group	C2/c
Unit cell dimensions (a, b, c (Å), α, β, γ (°))	a = 16.9569(10), b = 15.5663(8), c = 13.7592(8) α = 90, β = 114.461(2), γ = 90
Volume ($Å^3$)	3305.8(3)
Z	8
Calculated density (Mg/m^3)	1.259
Absorption coefficient (mm^{-1})	0.089
F(000)	1328
θ range for data collection (°)	3.08 to 25.00
Limiting indices	$-20 \leq h \leq 20$, $-18 \leq k \leq 18$, $-16 \leq l \leq 14$
Reflections collected / unique	12796 / 2914 [R(int) = 0.0319]
Completeness to θ = 25.00 (%)	99.8
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	2914 / 0 / 285
Goodness-of-fit on F^2	1.102
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0484, wR_2 = 0.1296
R indices (all data)	R_1 = 0.0887, wR_2 = 0.1698
Extinction coefficient	0.0019(6)
Largest diff. peak and hole (e $Å^{-3}$)	0.327 and -0.314

Figure 13. ORTEP diagram and numbering scheme (A) and packing diagram (B) for kresoxim-methyl

A


H12
C12
H13
H11
C11
C13
C10
C14
H10
C9
C15
C16
O4
O2
C17
H17C
H17B
H17A
N1
O3
C8
O1
H8A
H8B
H18C
C18
H18B
H18A
H1C
H1B
C1
H1A
O7
C2
C3
H3
H6
C6
C5
C4
H5
H4

B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Supplementary Materials

Table S1. During mixing/loading hand exposure amount (mg)

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
1	0.205	0.031	29.201	0.910	0.210	0.036
2	0.363	0.246	0.361	2.620	0.219	0.052
3	0.125	0.154	0.295	0.735	0.099	0.067
4	0.111	0.138	0.166	0.387	0.067	0.065
5	0.129	0.060	0.251	2.800	0.105	0.113
6	0.254	0.125	0.016	3.243	0.543	0.109
7	0.978	0.112	0.033	1.823	0.165	0.995
8	3.063	0.108	0.046	0.525	0.248	0.778
9	1.430	1.160	33.060	1.738	0.265	2.200

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
10	2.028	0.855	0.100	1.643	0.127	0.581
11	5.282	0.984	11.400	3.328	0.237	0.321
12	3.253	1.072	84.792	6.953	0.045	0.642
13	1.533	0.689	0.025	1.407	0.212	0.434
14	0.115	1.229	104.287	1.865	0.069	2.948
15	0.213	1.229	40.832	4.930	0.400	1.181
16	0.117	0.345	13.848	2.239	0.097	0.038
17	0.144	0.124	54.934	24.183	0.088	0.084
18	2.639	0.261	0.019	2.582	0.410	0.445
19	0.726	0.318	0.035	1.451	0.887	1.257
20	3.093	0.754	0.128	1.824	0.169	0.083

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
21	3.682	2.427	224.538	6.584	1.132	1.383
22	2.022	1.345	20.837	1.607	0.340	1.085
23	3.145	0.492	51.831	0.901	0.066	0.152
24	4.126	0.517	0.261	3.629	0.033	0.211
25	0.075	0.696	0.034	6.527	0.223	0.633
26	0.358	1.420	0.404	8.001	0.038	0.167
27	0.494	0.377	0.039	10.304	0.328	0.249
28	0.962	0.167	0.024	5.134	0.203	1.196
29	2.068	0.346	34.968	2.933	0.109	0.240
30	2.921	1.029	0.040	2.618	0.101	1.869

Table S2. During mixing/loading, ratio of exposure (%)

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
1	0.00164	0.00025	0.00091	0.01168	0.00053	0.00002
2	0.00290	0.00197	0.00262	0.00014	0.00055	0.00003
3	0.00100	0.00123	0.00073	0.00012	0.00025	0.00004
4	0.00089	0.00110	0.00039	0.00007	0.00017	0.00004
5	0.00103	0.00048	0.00280	0.00010	0.00026	0.00007
6	0.00203	0.00100	0.00324	0.00001	0.00136	0.00007
7	0.00782	0.00089	0.00182	0.00001	0.00041	0.00063
8	0.02450	0.00087	0.00053	0.00002	0.00062	0.00049
9	0.01144	0.00928	0.00174	0.01322	0.00066	0.00140

10	0.01622	0.00684	0.00164	0.00004	0.00032	0.00037
11	0.04226	0.00787	0.00333	0.00456	0.00059	0.00020
12	0.02603	0.00857	0.00695	0.03392	0.00011	0.00041
13	0.01226	0.00551	0.00141	0.00001	0.00053	0.00028
14	0.00092	0.00983	0.00186	0.04171	0.00017	0.00187
15	0.00170	0.00983	0.00493	0.01633	0.00100	0.00075
16	0.00094	0.00276	0.00224	0.00554	0.00024	0.00002
17	0.00115	0.00100	0.02418	0.02197	0.00022	0.00005
18	0.02111	0.00209	0.00258	0.00001	0.00102	0.00028
19	0.00581	0.00254	0.00145	0.00001	0.00222	0.00080
20	0.02474	0.00603	0.00182	0.00005	0.00042	0.00005
21	0.02946	0.01941	0.00658	0.08982	0.00283	0.00088
22	0.01618	0.01076	0.00161	0.00833	0.00085	0.00069
23	0.02516	0.00394	0.00090	0.02073	0.00016	0.00010

24	0.03301	0.00414	0.00363	0.00010	0.00008	0.00013
25	0.00060	0.00557	0.00653	0.00001	0.00056	0.00040
26	0.00286	0.01136	0.00800	0.00016	0.00010	0.00011
27	0.00395	0.00302	0.01030	0.00002	0.00082	0.00016
28	0.00769	0.00134	0.00513	0.00001	0.00051	0.00076
29	0.01654	0.00277	0.00293	0.01399	0.00027	0.00015
30	0.02337	0.00823	0.00262	0.00002	0.00025	0.00119

Table S3. During mixing/loading, hand exposure volume (mL)

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
1	4.1	0.6	4.5	58.4	5.3	0.2
2	7.3	4.9	13.1	0.7	5.5	0.3
3	2.5	3.1	3.7	0.6	2.5	0.4
4	2.2	2.8	1.9	0.3	1.7	0.4
5	2.6	1.2	14.0	0.5	2.6	0.7
6	5.1	2.5	16.2	0.0	13.6	0.7
7	19.6	2.2	9.1	0.1	4.1	6.3
8	61.3	2.2	2.6	0.1	6.2	4.9
9	28.6	23.2	8.7	66.1	6.6	14.0

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
10	40.6	17.1	8.2	0.2	3.2	3.7
11	105.6	19.7	16.6	22.8	5.9	2.0
12	65.1	21.4	34.8	169.6	1.1	4.1
13	30.7	13.8	7.0	0.0	5.3	2.8
14	2.3	24.6	9.3	208.6	1.7	18.7
15	4.3	24.6	24.6	81.7	10.0	7.5
16	2.3	6.9	11.2	27.7	2.4	0.2
17	2.9	2.5	120.9	109.9	2.2	0.5
18	52.8	5.2	12.9	0.0	10.2	2.8
19	14.5	6.4	7.3	0.1	22.2	8.0
20	61.9	15.1	9.1	0.3	4.2	0.5

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
21	73.6	48.5	32.9	449.1	28.3	8.8
22	40.4	26.9	8.0	41.7	8.5	6.9
23	62.9	9.8	4.5	103.7	1.6	1.0
24	82.5	10.3	18.1	0.5	0.8	1.3
25	1.5	13.9	32.6	0.1	5.6	4.0
26	7.2	28.4	40.0	0.8	1.0	1.1
27	9.9	7.5	51.5	0.1	8.2	1.6
28	19.2	3.3	25.7	0.0	5.1	7.6
29	41.4	6.9	14.7	69.9	2.7	1.5
30	58.4	20.6	13.1	0.1	2.5	11.9

Table S4. During mixing/loading, hand exposure amount per active ingredient (mg/kg a.i)

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
1	16.44	2.51	116.80	9.10	5.26	0.14
2	58.48	19.69	1.44	26.20	0.88	0.21
3	20.11	12.31	1.18	7.35	0.40	0.27
4	17.90	11.02	0.67	3.87	0.27	0.26
5	20.82	4.78	1.00	28.00	0.42	0.45
6	40.99	10.04	0.06	32.43	2.17	0.44
7	157.69	8.92	0.13	18.23	0.66	3.98
8	494.04	8.65	0.18	5.25	0.99	3.11
9	230.62	92.83	132.24	17.38	1.06	8.80

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
10	327.11	68.43	0.40	16.43	0.51	2.32
11	851.96	78.72	45.60	33.28	0.95	1.29
12	524.75	85.73	339.17	69.53	0.18	2.57
13	247.21	55.08	0.10	14.07	0.85	1.74
14	18.51	98.32	417.15	18.65	0.27	11.79
15	34.30	98.32	163.33	49.30	1.60	4.72
16	18.94	27.63	55.39	22.39	0.39	0.15
17	23.24	9.95	219.74	241.83	0.35	0.34
18	425.60	20.85	0.08	25.82	1.64	1.78
19	117.14	25.44	0.14	14.51	3.55	5.03

Trials	Power sprayer			Speed sprayer		
	WP indoxacarb	WG indoxacarb	EC fenthion	EC chlorpyrifos	WP acetamiprid	WG kresoxim-methyl
20	498.86	60.33	0.51	18.24	0.67	0.33
21	593.95	194.14	898.15	65.84	4.53	5.53
22	326.18	107.60	83.35	16.07	1.36	4.34
23	507.22	39.40	207.32	9.01	0.26	0.61
24	665.45	41.38	1.05	36.29	0.13	0.84
25	12.12	55.65	0.13	65.27	0.89	2.53
26	57.76	113.60	1.62	80.01	0.15	0.67
27	79.70	30.19	0.16	103.04	1.31	1.00
28	155.12	13.39	0.10	51.34	0.81	4.78
29	333.50	27.70	139.87	29.33	0.44	0.96
30	471.15	82.30	0.16	26.18	0.40	7.48

Sum	7,346.86	1,504.9	2,827.22	1,154.24	33.35	78.46
Average	244.9	50.2	94.24	38.48	1.11	2.62
75percentile	459.8	81.4	128.38	46.04	1.25	4.25
Minimum	12.1	2.5	0.06	3.87	0.13	0.14
Maximum	852.0	194.1	898.15	241.83	5.26	11.79

Table S5. During mixing/loading, inhalation exposure amount (mg)

Trials	Power sprayer		Speed sprayer	
	WP indoxacarb	WG indoxacarb	WP acetamiprid	WG kresoxim-methyl
1	<LOQ	<LOQ	<LOQ	<LOQ
2	<LOQ	0.004	<LOQ	<LOQ
3	<LOQ	<LOQ	2.807	<LOQ
4	<LOQ	0.004	<LOQ	<LOQ
5	<LOQ	<LOQ	<LOQ	<LOQ
6	0.026	<LOQ	<LOQ	<LOQ
7	0.001	<LOQ	<LOQ	<LOQ
8	<LOQ	0.006	<LOQ	<LOQ
9	0.003	0.001	<LOQ	<LOQ
10	<LOQ	0.008	<LOQ	<LOQ

11	0.005	0.007	<LOQ	<LOQ
12	0.016	0.010	<LOQ	<LOQ
13	0.007	<LOQ	<LOQ	<LOQ
14	<LOQ	0.009	<LOQ	<LOQ
15	0.018	<LOQ	<LOQ	<LOQ
16	<LOQ	0.004	<LOQ	<LOQ
17	<LOQ	0.092	<LOQ	<LOQ
18	0.005	0.000	0.003	0.003
19	0.003	0.039	<LOQ	<LOQ
20	0.008	0.024	<LOQ	<LOQ
21	0.004	0.020	<LOQ	<LOQ
22	0.011	0.007	0.002	0.002
23	0.016	0.006	<LOQ	<LOQ
24	0.018	0.003	0.003	0.003

25	0.006	0.010	<LOQ	<LOQ
26	0.006	0.008	<LOQ	<LOQ
27	0.030	0.016	<LOQ	<LOQ
28	0.012	0.019	<LOQ	<LOQ
29	<LOQ	<LOQ	<LOQ	<LOQ
30	0.004	<LOQ	<LOQ	<LOQ
Average	0.01	0.01	0.70	0.00
Sum	0.199	0.297	2.815	0.008

Table S6. During mixing/loading, ratio to inhalation exposure (%)

Trials	Power sprayer		Speed sprayer	
	WP indoxacarb	WG indoxacarb	WP acetamiprid	WG kresoxim-methyl
1	N.D	N.D	N.D	N.D
2	N.D	N.D	N.D	N.D
3	N.D	N.D	7.02E-06	N.D
4	5.04E-10	5.04E-10	N.D	N.D
5	2.52E-10	2.52E-10	N.D	N.D
6	2.09E-07	2.09E-07	N.D	N.D
7	1.13E-08	1.13E-08	N.D	N.D
8	N.D	N.D	N.D	N.D
9	2.44E-08	2.44E-08	N.D	N.D
10	3.02E-09	3.02E-09	N.D	N.D

11	3.7E-08	3.7E-08	N.D	N.D
12	1.25E-07	1.25E-07	N.D	N.D
13	5.59E-08	5.59E-08	N.D	N.D
14	2.52E-10	2.52E-10	N.D	N.D
15	1.45E-07	1.45E-07	N.D	N.D
16	N.D	N.D	N.D	N.D
17	N.D	N.D	N.D	N.D
18	3.78E-08	3.78E-08	N.D	1.76E-09
19	2.49E-08	2.49E-08	N.D	N.D
20	6.07E-08	6.07E-08	N.D	N.D
21	3.02E-08	3.02E-08	N.D	N.D
22	8.74E-08	8.74E-08	N.D	1.03E-09

23	1.25E-07	1.25E-07	N.D	N.D
24	1.4E-07	1.4E-07	N.D	2.12E-09
25	4.43E-08	4.43E-08	N.D	N.D
26	4.96E-08	4.96E-08	N.D	N.D
27	2.36E-07	2.36E-07	N.D	N.D
28	9.25E-08	9.25E-08	N.D	N.D
29	N.D	N.D	N.D	N.D
30	3.2E-08	3.2E-08	N.D	N.D
Average	5.24E-08	5.24E-08	2.34E-07	1.64E-10

Table S7. During mixing/loading, hand exposure amount per active ingredient (mg/kg a.i)

Trials	Power sprayer		Speed sprayer	
	WP indoxacarb	WG indoxacarb	WP acetamiprid	WG kresoxim-methyl
1	N.D	N.D	N.D	N.D
2	N.D	0.000017	N.D	N.D
3	N.D	0.000001	0.011226	N.D
4	N.D	0.000015	N.D	N.D
5	N.D	0.000002	N.D	N.D
6	0.000104	N.D	N.D	N.D
7	0.000006	N.D	N.D	N.D
8	N.D	0.000024	N.D	N.D
9	0.000012	0.000003	N.D	N.D
10	0.000002	0.000030	N.D	N.D

11	0.000019	0.000030	N.D	N.D
12	0.000063	0.000041	N.D	N.D
13	0.000028	N.D	N.D	N.D
14	N.D	0.000035	N.D	N.D
15	0.000073	N.D	N.D	N.D
16	N.D	0.000015	N.D	N.D
17	N.D	0.000369	N.D	N.D
18	0.000019	N.D	N.D	0.000011
19	0.000012	0.000158	N.D	N.D
20	0.000030	0.000098	N.D	N.D
21	0.000015	0.000080	N.D	N.D
22	0.000044	0.000026	N.D	0.000006

23	0.000062	0.000023	N.D	N.D
24	0.000070	0.000011	N.D	0.000013
25	0.000022	0.000041	N.D	N.D
26	0.000025	0.000032	N.D	N.D
27	0.000118	0.000066	N.D	N.D
28	0.000046	0.000076	N.D	N.D
29	N.D	N.D	N.D	N.D
30	0.000016	N.D	N.D	N.D
Sum	0.000786	0.001193	0.011226	0.00003
Average	0.000026	0.000040	0.000374	0.000001
75percentile	0.000040	0.000039	N.D	N.D
Minimum	12.1	2.5	0.13	0.14
Maximum	852.0	194.1	5.26	11.79

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Abstract in Korean

다양한 노출 경로 및 노출 상황에 대한 농작업자의 노출의 정량적 평가는 농약의 합리적 농약의 안전성과 위해성 평가를 가능하게 한다. 그러나 다종의 농약과 다양한 농작업에 해당하는 노출량을 모두 측정하기는 현실적으로 어렵다. 이에 따라 유럽 및 미국 등 다른 나라의 경우 자국에 맞는 대표경우를 선발, 다년간 반복 포장 노출 실험결과를 토대로 노출량 예측 모델을 개발하고 이를 이용하여 농약 노출량을 예측, 평가하고 있다. 현재 우리나라는 UK-POEM 을 이용하여 농약의 노출량을 예측 평가하고 있다. 하지만 이는 유럽의 농약의 사용 양상, 농업 및 농작업 형태를 반영하여 개발된 것이라서 모델들 간에도 노출요소의 여러 면에서 서로 차이가 있는 문제점을 갖고 있다. 본 연구자는 우리나라 농약의 종류 및 사용 양상과 농작업 및 농업 형태에 적합한 노출요소를 포함한 '한국형 농약노출량 예측모델'을 개발 연구를 수행하였다. 대표 작물로 벼, 사과를 선정하였으며 각 농업형태에 맞는 살포기기인 SS 기와 PS 기를 사용하였고 농약 제형은 유제, 수화제, 입상수화제를 사용하였다. 살포액 조제 및 살포 시 노출 시나리오로 패치법을 이용한 피부 노출 및 XAD-2 레진을 이용한 호흡 노출량을 측정하였다. 또한 검출한계, 정량한계, 회수율, 재현성, 검량선의 직선성, 포집효율, 파과시험의 분석법 검증을 통해 노출 시험 수행에 적합한 합리적인 결과를 얻었다. 이에 따라 시나리오에 따른 농약 살포액 조제 및 살포 시 75 백분위수 노출량을 산출하여 한국형 모델의 노출요소로 도출하였다. 도출된 노출량을 토대로 위해성 평가를 실시하여 농작업자의 위해성 여부를 확인한 결과 위해성을 나타내는 MOS 값이 1 보다 큰 값으로 위해 가능성이 적은 것으로 나타났다. kresoxim-methyl 의 크리스탈 구조를 확인하고 인간 간 마이크로좀에 의한 대사연구를

통하여 대사 양상과 대사물을 확인하였고 rCYPs 2A6 와 rCYPs 2E1 을 제외한 8 종의 rCYPs 가 대사물을 생성함을 확인하였다.

주요어: 농작업자 노출, 대사, 농작업자 모델, 위해성평가, 피부노출, 호흡노출

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