applied sciences

MDPI

Article

Sequential Indoor Use of Pesticides: Operator Exposure via Deposit Transfer from Sprayed Crops and Contaminated Application Equipment

Angelos N. Tsakirakis [1], Konstantinos M. Kasiotis [1,*], C. Richard Glass [2,†], Agathi N. Charistou [1], Pelagia Anastasiadou [1], Rianda Gerritsen-Ebben [3] and Kyriaki Machera [1,*]

1 Laboratory of Pesticides' Toxicology, Benaki Phytopathological Institute, 8 St. Delta Str., Kifissia, 14561 Athens, Greece; angtsak@hotmail.com (A.N.T.); a.charistou@bpi.gr (A.N.C.); b.anastasiadou@bpi.gr (P.A.)
2 Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK; glass.richard@gmail.com
3 TNO, Princetonlaan 6, 3584 CB Utrecht, The Netherlands; rianda.gerritsen@tno.nl
* Correspondence: k.kasiotis@bpi.gr (K.M.K.); k.machera@bpi.gr (K.M.); Tel.: +30-2108180357 (K.M.K.); +30-2108180201 (K.M.)
† Current address: Crop Health and Protection, York Biotech Campus, Sand Hutton, York YO41 1LZ, UK.

Abstract: Dermal transfer of pesticide residues to human skin due to contact with treated crops, treated surfaces, or contaminated surfaces is an important route of exposure for operators, workers and possibly for bystanders and residents. However, information on dermal transfer data is limited and mainly available for workers. The aim of the present study has been to generate both dermal exposure and transfer data related for operators involved in sequential tasks of mixing/loading and application of pesticides in a southern EU zone greenhouse. Exposure measurements were based on the principles of the whole-body dosimetry (WBD) method involving the use of cotton coveralls and gloves as dosimeters. Six field trials were conducted in three tomato greenhouses, on the island of Crete, Greece. The study results showed that the contribution of existing pesticide deposits on the treated crops, i.e., from an application conducted earlier the same day, was in the range of 8–16% for the application task and 0.9–18% for the mixing/loading task in relation to the measured total exposure to this pesticide during a short-term sequential application. The results of this study have been incorporated in the GAOEM (Greenhouse Agricultural Operator Exposure Model) included in the updated EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products. The low values of the pesticide amount penetrating the coverall (actual dermal exposure) in all cases highlight and confirm the need for the use of appropriate personal protective equipment (PPE) for operator safety.

Keywords: greenhouse; pesticide transfer; operator exposure; sprayed crop

Citation: Tsakirakis, A.N.; Kasiotis, K.M.; Glass, C.R.; Charistou, A.N.; Anastasiadou, P.; Gerritsen-Ebben, R.; Machera, K. Sequential Indoor Use of Pesticides: Operator Exposure via Deposit Transfer from Sprayed Crops and Contaminated Application Equipment. *Appl. Sci.* **2022**, *12*, 3909. https://doi.org/10.3390/app12083909

Academic Editors: Giuseppe Manetto, Emanuele Cerruto, Domenico Longo and Rita Papa

Received: 11 February 2022
Accepted: 11 April 2022
Published: 13 April 2022

1. Introduction

Operator exposure levels during mixing/loading and application of plant protection products (PPPs) in many cases are of critical importance for the human non-dietary risk assessment during the authorization process of a PPP. Although there has been a noteworthy improvement towards harmonization in the area of non-dietary exposure assessment for field spray applications of pesticides [1], this has not yet been achieved for greenhouse applications. Since 2014, additional data have been produced on the direct dermal exposure of operators during greenhouse applications; however, the information on the indirect exposure of operators owed to dermal transfer of residues from the sprayed crop during re-entry of the operator, for a sequential application, is still limited. In addition, the issue of cumulative and aggregated exposure to pesticides has stressed the need for further investigation regarding the different sources of occupational exposure to one pesticide and/or to multiple pesticides.

Re-entry exposure to pesticides commonly refers to dermal exposure of workers that may occur while entering the treated field to carry out different tasks. The re-entry exposure depends on the crop and the growth stage during application. These tasks vary from inspection or maintenance tasks and irrigation to harvesting and related activities (e.g., leaf pulling and tying, reach/pick in case of vegetables, etc.). Considering the agricultural practices in the different areas of EU Member States and the fact that most PPPs do not have a label recommendation to be used as tank mixtures, operators may need to apply more than one PPP on the same day or on consecutive days. Thus, re-entry dermal exposure may also refer to operators that are exposed not only to the pesticide currently applied, but also to the residues of the pesticide previously sprayed [2,3]. In general, operator exposure studies are conducted, aiming to investigate the exposure to a pesticide while being mixed/loaded or during its application [4–6]. The standard practice followed for the measurements in case of operator exposure studies for pesticide applications in both the field and greenhouse is well documented in the literature and predominantly involves measurements of exposure to one pesticide [7–11]. The exposure to just one pesticide (the one last applied) has been the objective for many worker exposure/dislodgeable foliar residue (DFR) studies (indicatively see [12]).

Although dermal transfer of pesticide residues due to contact with either treated crops, treated surfaces, or contaminated surfaces is an important route of exposure for operators, workers, as well as bystanders and residents, any available information on dermal transfer data refers mainly to worker tasks, e.g., re-entry for crop inspection or maintenance tasks, irrigation, harvesting. In particular, there are limited data available regarding the operator (pesticide handler) exposure to pesticides previously mixed/loaded and/or applied. The limitations and uncertainties in the assessment of the re-entry exposure have been extensively discussed by Dong and Beauvais (2013) [13], while it is indicated that re-entry exposure may contribute significantly to the exposure of persons involved in agricultural tasks, either as workers or as operators. Although there are studies/projects showing that based on the agricultural practices followed around EU and worldwide, operators and workers are exposed to multiple pesticides within a short period [14–16], the available operator and worker exposure assessment tools/models for both field and greenhouse applications calculate exposure under the aforementioned "conventional" tasks [17].

Considering the above, the present study aimed to generate both dermal exposure and transfer data related to mixing/loading and application tasks in a southern EU zone greenhouse. The field experiments conducted involved the measurement of the operator dermal exposure to a pesticide not only during the actual mixing/loading (M/L) and hand-held spray application tasks, but also due to the transfer of existing deposits of a pesticide from the crop and application equipment (exposure via bulk/splashes, transfer, and deposition jointly). This could provide new information regarding the combined exposure of operators resulting from a specific kind of re-entry task, which is a common agricultural practice for indoor crops.

2. Materials and Methods

2.1. Study Design Consideration

The field experiments were designed to meet the objective of the study described above, as well as to comply with the respective Organization for Economic Co-operation and Development (OECD) guidelines for studies of occupational exposure to pesticides [18].

Six field trials (identified as trials I–VI) were conducted in three greenhouses (GHs) to determine operator exposure. In each trial, one M/L and one application task was considered, both carried out by the same operator. It is noted that the term "operator" is used in the present work to define the volunteers participating in the study, regardless of their M/L or application tasks. Thus, to specifically address operators during their M/L or application tasks, the terms "mixer/loader" and "applicator" are used, respectively, throughout the text hereafter.

In total, three operators took part in the study, each of whom carried out a set of two trials, i.e., one in the morning and one in the afternoon. To isolate the trials, each time, one operator treated one GH. More specifically, the first three trials (identified as trials I, II and III) involved monitoring of M/L and application of one pesticide (pesticide A) in the morning. Respectively, trials IV, V, VI involved monitoring of M/L and application of a different pesticide (pesticide B) in the afternoon; therefore, corresponding to re-entry treatment. In all six trials conducted, the exposure was determined separately for mixer/loaders and applicators.

The study design and experimental scheme followed are presented in Table 1.

Table 1. Study design and sampling strategy scheme.

Trial ID	Date	Greenhouse (GH)	Task Monitored	Pesticide	Volunteer ID (Task-Related)	Remarks
Trial I	2 October 2012 morning	GH-1	Mixing/Loading (M/L) Application (AP)	A	ML-1 AP-1	ML-1, AP-1, ML-4, AP-4 were the same the person (operator 1)
Trial II	2 October 2012 morning	GH-2	Mixing/Loading Application	A	ML-2 AP-2	ML-2, AP-2, ML-5, AP-5 were the same the person (operator 2)
Trial III	2 October 2012 morning	GH-3	Mixing/Loading Application	A	ML-3 AP-3	ML-3, AP-3, ML-6, AP-6 were the same the person (operator 3)
Trial IV	2 October 2012 afternoon	GH-1	Mixing/Loading Application	B	ML-4 AP-4	ML-1, AP-1, ML-4, AP-4 were the same the person (operator 1)
Trial V	2 October 2012 afternoon	GH-2	Mixing/Loading Application	B	ML-5 AP-5	ML-2, AP-2, ML-5, AP-5 were the same the person (operator 2)
Trial VI	2 October 2012 afternoon	GH-3	Mixing/Loading Application	B	ML-6 AP-6	ML-3, AP-3, ML-6, AP-6 were the same the person (operator 3)

2.2. Field Phase

All field trials took place on the island of Crete, Greece (Tympaki region). Greenhouse tomato crops were selected for the applications, grown with standard protected cropping systems for the region, e.g., narrow inter-row width. The treated area in each one of the three GHs was 0.13 ha, while the duration of application ranged between 36 to 47 min (see data in Table 2). The spray liquids (s.l.) were applied using a spray gun connected via a hose to the tank. The distance of the operators from the tank was sufficient (>2 m) to avoid any cross-contamination due to the proximity to the tank. Field trial parameters and data are presented in Table 2. In addition, since applications took place indoors, the impact of environmental conditions was negligible.

Table 2. Field trial parameters and data.

Parameters	ML-1	AP-1	ML-2	AP-2	ML-3	AP-3	ML-4	AP-4	ML-5	AP-5	ML-6	AP-6
Location	Tympaki GH-1		Tympaki GH-2		Tympaki GH-3		Tympaki GH-1		Tympaki GH-2		Tympaki GH-3	
GH area (ha)	0.13		0.13		0.13		0.13		0.13		0.13	
Crop	tomato		tomato		tomato		tomato		tomato		tomato	
Duration of task (min)	n/a	36	n/a	43	n/a	47	n/a	36	n/a	47	n/a	39
Row distance (m)	1.90		1.90		1.60		1.90		1.90		1.60	
Plant distance (m)	0.60		0.60		0.60		0.60		0.60		0.60	
Crop height (m)	1.95		1.95		1.95		1.95		1.95		1.95	
Dilution of formulation (mL/L s.l.)	1.625		1.625		1.625		0.525		0.525		0.525	
Amount of s.l. mixed (L)	200	n/a	200	n/a	200	n/a	200	n/a	200	n/a	200	n/a
Amount of spray liquid left in tank (L)	n/a	32	n/a	15	n/a	0	n/a	30	n/a	2	n/a	32
Amount of spray liquid applied (L)	n/a	168	n/a	185	n/a	200	n/a	170	n/a	198	n/a	168
Application rate (L/min)	n/a	4.02	n/a	4.14	n/a	4.02	n/a	4.14	n/a	4.14	n/a	4.14

n/a: not applicable. s.l.: spray liquid.

More specifically, the procedure implemented was based on the principles of the whole-body dosimetry (WBD) method [8,18–20], as adapted and designated in detail in a previous study [11]. The coverall type used was a cotton coverall (100% cotton, see Figure 1), serving both as operator's standard working clothing and concurrently as an outer exposure dosimeter. Inner dosimeters (100% cotton shirts and long pants) were used for the monitoring of actual exposure. Head and hand exposure were measured in accordance with the sampling method already used in a previous study [10] using cotton caps and gloves (inner cotton, outer nitrile) as dosimeters, respectively. Quality control samples of all dosimeters used were fortified in the field at two fortification rates (plus blanks) as a measure of the active substance (a.s.) stability and recovery according to the previously described procedure [10,21]. The nitrile gloves extraction, both for field- and spiked samples, was conducted in the field immediately after sampling, as this matrix is known to retain analytes, impacting recovery efficiency if stored for any length of time [22]. All samples were stored in freezers (−18 °C) shortly after the trial. Exposure term definitions (potential, actual, etc.) and clarifications regarding the various dosimeters considered for the respective exposure calculations are provided in Section 2.4.

Figure 1. Application of the spray liquid in tomatoes.

Two different pesticides (authorized and normally used either as a tank mix or in sequential application on the same day) were applied. Mixing and loading (M/L) and application procedures were in accordance with local practices and the principles of Good Agricultural Practice (GAP). The experimental work followed the design and sampling scheme described in the respective section above and depicted in Table 1. The pesticide A was an emulsifiable concentrate (EC) fungicide formulation containing the a.s. bupirimate at 25% w/v. Since pesticide A was the first one applied, it also served as the pesticide tested for the study of the potential transfer of residues from the equipment and the treated crop to the operator. Pesticide B was a suspension concentrate (SC) fungicide formulation containing the a.s. tebufenozide at 24% w/v. Both pesticides were purchased from a local distributor and the respective containers were checked for expiry dates and intact packaging by the field scientists, and found to comply with the quality requirements and foreseen label.

During the application of the first pesticide (pesticide A), in the morning, the potential dermal exposure (PDE) and the actual dermal exposure (ADE) for the M/L and for the applicators were measured using standard whole-body techniques (see Section 2.4 below) following published procedures [11]. The volunteers wore outer coveralls (jacket and trousers 100% cotton) and inner coveralls (long-sleeved T-shirt and long johns, 100% cotton).

In the course of application of the second pesticide (pesticide B), the afternoon of the same day, a new clean set of dosimeters was provided to the operators, and the dermal exposure to pesticide B, but also to pesticide A, applied in the morning, due to potential transfer of residues from the equipment and the already treated crop, was measured. The re-entry of the operators to apply pesticide B took place six hours after the application of pesticide A, which was adequate time for drying of the spray solution (also confirmed by visual inspection of the crop).

2.3. Laboratory Phase

The laboratory phase of the study was carried out at the Laboratory of Pesticides' Toxicology of Benaki Phytopathological Institute, Greece. Stock and working solutions (used for the validation of analytical method) of the analytical standards tebufenozide (obtained from ChemService 99.5% purity) and bupirimate a.s. (acquired from ChemService, 99.5% purity) were prepared as described in previous related publication [12]. All the aforementioned solutions were stored at −18 °C.

For both pesticides used, the a.s. residues from the dosimeters were extracted with methanol according to the procedure described earlier [10]. Especially for the outer gloves (nitrile), the extraction had already been performed in the field after the end of each application based on the procedure defined in the aforesaid study, since it was known that recovery of the a.s. reduces over time with this matrix [23]. The chemical analysis (liquid chromatography electrospray mass spectrometry, LC-ESI/MS) was conducted using previously described conditions and the same instrumentation [12].

Considering that no matrix effect from fabric and glove dosimeters was observed during the validation process (for this purpose, a series of calibration standard solutions was fortified with 40% concentrated extract of cotton matrix and analyzed, showing no evidence of influence on the detector response), all the standard solutions prepared and used in the analyses were prepared in methanol. The calibration standards were mixtures of equivalent concentration of both a.s. in this solvent. As the analytical routine procedure for completion of all the sample extraction and measurements took several weeks, calibration curves were prepared every two weeks, during this period, to ensure that possible baseline drifting and instrumental variations were detected. Acceptable linearity ($r^2 > 0.997$) was observed in the range of 0.001 μg/mL to 0.050 μg/mL for bupirimate and up to 1 μg/mL for tebufenozide. The rest of validation criteria (accuracy, precision, specificity) were fulfilled as well.

The limit of quantification (LOQ) values for fabric (inner, outer) and glove dosimeters were equal to the lowest amount of a.s. that could be extracted and analyzed from a given dosimeter surface area with satisfactory recovery (>70%), providing a final extract solution concentration not less than the lowest point of the calibration curve (i.e., 0.001 μg/mL). Thus, LOQs corresponded for both a.s. to 0.007 μg/cm^2 for coverall fabrics and 0.6 μg/dosimeter for cap and for nitrile glove.

2.4. Exposure Considerations

The exposure was determined as the amount of a.s. residues (expressed per kg a.s. applied, i.e., mg/kg a.s.) detected on the field samples, as documented in the respective OECD guidelines [18]. Thus, the PDE corresponds to the totality of a.s. residues on the outer and inner dosimeters, while the ADE to the respective a.s. amount on the inner dosimeters only. Residues found on the gloves (outer or inner) are similarly considered to represent the hand exposure (potential and actual). For the head exposure, potential and actual levels coincide and are calculated from the a.s. amount detected on the cap multiplied by a factor of 2 to extrapolate for the whole head area [11]. The aforementioned exposure terms, along with the calculations considered for the expression of results, have been described in detail in a previously published study [11].

2.5. Quantitative Risk Assessment

To proceed to a quantitative risk assessment, the systemic exposure (*SE*) in mg kg^{-1} bw day^{-1} was calculated using Equation (1), i.e., based on the measured ADE and considering a dermal penetration factor (*Pf*) for the a.s.

$$SE = ADE \times AR \times TA \times Pf / BW \quad (1)$$

ADE = mass (mg) of the a.s. per kg of a.s. applied;
AR = application rate, kg a.s./ha;
TA = treated area, i.e., 1 ha; in line with EFSA Guidance of 2022 (see below);
Pf = penetration factor (%);
BW = body weight (kg), 60 kg.

Then, the hazard quotient (*HQ*) was calculated (Equation (2)) by dividing *SE* with a health-based guidance value (*HBGV*, in this case the acceptable operator exposure level (AOEL)).

$$HQ = SE/HBGV \quad (2)$$

When *HQ* is greater than 1, then an unacceptable risk is concluded.

In case of combined exposure to more than one pesticide, the hazard index (HI) was calculated by adding the individual HQs. Again, when HI is greater than 1, then an unacceptable risk is concluded for the combined exposure.

2.6. Ethics

The greenhouse workers were informed about the scope of the study by the scientific personnel before the study was initiated. After studying all aspects of the study, they signed the respective consent form. The complete study was approved by Benaki Phytopathological Institute's ethical committee.

3. Results and Discussion

3.1. Field Spiked Samples

Field spiking results are presented in Table 3 showing recoveries well above the cut off value of 70% [18]. Relative standard deviation (%RSD) values for concentrations levels tested were <4% (figures not presented) with tolerable threshold being <20% [18]. The above results verify the credibility of the field phase method and sampling procedures in the field, as well as the stability of the a.s. studied.

Table 3. Field spiking recovery results for a.s. bupirimate and tebufenozide.

Pesticide	Mean Recovery of Pesticides a.s. (n = 3)							
	LOQ * Level				10 × LOQ Level			
	Outer Coverall [a]	Inner Coverall [a,b]	Cap	Nitrile Glove	Outer Coverall [a]	Inner Coverall [a,b]	Cap	Nitrile Glove
A	84	88	85	79	83	90	89	78
B	83	90	88	78	86	94	90	80

* Limit of Quantification, [a] 30 × 30 cm^2 pieces of fabric. [b] The field spiking for 'Inner coverall cotton' is applicable for 'Inner cotton gloves' as well, since the fabric is the same.

3.2. Exposure Levels for the Monitored Tasks

The PDE, as well as the ADE, for the different body parts, were determined from the residues found on the dosimeters. The total PDE is the summation of the potential exposure of the body adding the potential exposure of the hands plus the head exposure [11]. Respectively, the total ADE is the sum of the actual exposure of the body plus the actual exposure of the hands plus the head exposure [11].

The results for each body part, as well as for total dermal operator exposure, are presented in Tables 4 and 5 for pesticides A and B, respectively. Summary data expressing exposure results as PDE and ADE for the body, hands, head, and total are depicted in Tables 6 and 7 for mixing/loading and application tasks, respectively. In the case of operators handling pesticide A, the levels of total PDE from M/L ranged from 4 to 27 mg a.s./kg a.s. applied (average 12 mg a.s./kg a.s., geometric mean 9 mg a.s./kg a.s., n = 3), while the respective exposure during application ranged from 106 to 130 mg a.s./kg a.s. applied (average 121 mg a.s./kg a.s., geometric mean 120 mg a.s./kg a.s., n = 3). Regarding pesticide B (afternoon application/re-entry), the PDE from M/L ranged from 65 to 77 mg a.s./kg a.s. applied (average 70 mg a.s. /kg a.s., geometric mean 70, n = 3), and for application from 55 to 114 mg a.s./kg a.s. applied (average 75 mg a.s./kg a.s., geometric mean 71, $n = 3$) for application.

Table 4. Exposure results for pesticide A in mg a.s./kg a.s. handled for mixer/loaders and applicators *.

Pesticide A	Amount per Quantity Handled (mg a.s./kg a.s.)											
	ML-1	**ML-2**	**ML-3**	**ML-4**	**ML-5**	**ML-6**	**AP-1**	**AP-2**	**AP-3**	**AP-4**	**AP-5**	**AP-6**
outer jacket	0.602	18.482	0.817	0.136	0.227	0.086	39.4	34.3	60.9	10.9	4.95	9.81
outer trousers	0.344	0.461	0.126	0.81	<LOQ	0.050	80.0	65.7	64.3	5.47	4.96	9.28
inner shirt	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.075	0.268	0.125	0.131	<LOQ	0.362
inner pants	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.384	0.434	0.177	0.071	0.097	0.152
cap	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.026	0.204	0.104	0.009	0.020	0.021
inner gloves	<LOQ	3.653	0.373	0.077	0.007	0.061	0.007	0.019	0.022	0.115	<LOQ	0.044
protective gloves	4.899	4.415	2.680	0.058	<LOQ	0.016	6.800	5.497	4.188	1.331	0.490	0.710

* ML1, ML2, ML3 and AP1, AP2, AP3 handled pesticide A (morning) while ML4, ML5, ML6 and AP4, AP5, AP6 handled pesticide B (afternoon) on the same crop, and thus have been exposed to pesticide A existing deposits.

Table 5. Exposure results for pesticide B in mg a.s./kg a.s. handled for mixer/loaders and applicators *.

Pesticide B	Amount per Quantity Handled (mg/kg a.s.)					
	ML4	**ML5**	**ML6**	**AP4**	**AP5**	**AP6**
outer jacket	4.478	3.997	1.092	39.9	25.4	53.6
outer trousers	<LOQ	3.404	0.210	10.5	25.3	52.6
inner shirt	<LOQ	<LOQ	0.210	0.126	0.146	0.258
inner pants	0.122	0.124	0.409	0.196	0.240	0.346
cap	<LOQ	<LOQ	<LOQ	0.074	0.621	0.258
inner gloves	0.359	9.47	4.00	0.063	0.023	0.447
protective gloves	59.9	51.6	70.7	4.327	4.898	6.665

* ML4, ML5, ML6 and AP4, AP5, AP6 handled pesticide B (afternoon).

Table 6. PDE * and ADE * values for mixer/loaders (mg a.s./kg a.s. handled).

Pesticide A	Bupirimate mg a.s./kg a.s. Handled					
	ML1	**ML2**	**ML3**	**ML4**	**ML5**	**ML6**
PDE body	0.95	18.94	0.94	0.95	0.23	0.14
PDE hands	4.90	8.07	3.05	0.14	0.007	0.077
PDE head	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Total PDE	5.85	27.0	4.00	1.08	0.23	0.21
ADE body	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
ADE hands	<LOQ	3.65	0.37	0.077	0.007	0.061
ADE Head	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Total ADE	<LOQ	3.65	0.37	0.077	0.007	0.061
Pesticide B	**Tebufenozide mg a.s./kg a.s. Handled**					
	**n/a ** **	**n/a ** **	**n/a ** **	**ML4**	**ML5**	**ML6**
PDE body	-	-	-	4.60	7.53	1.92
PDE hands	-	-	-	60.3	61.1	74.7
PDE head	-	-	-	<LOQ	<LOQ	<LOQ
Total PDE	-	-	-	64.9	68.6	76.6
ADE body	-	-	-	0.12	0.12	0.62
ADE hands	-	-	-	0.36	9.47	4.00
ADE head	-	-	-	<LOQ	<LOQ	<LOQ
Total ADE	-	-	-	0.48	9.59	4.62

* Potential Dermal Exposure (PDE), Actual Dermal Exposure (ADE), ** ML1, ML2, ML3 handled pesticide A (morning), while ML4, ML5, ML6 handled pesticide B (afternoon), and thus have been exposed to pesticide A existing deposits.

Table 7. PDE and ADE values for applicators (mg a.s./kg a.s. handled).

Pesticide A	Bupirimate mg a.s./kg a.s. Handled					
	AP1	**AP2**	**AP3**	**AP4**	**AP5**	**AP6**
PDE body	120	101	126	16.6	10.0	19.6
PDE hands	6.81	5.52	4.21	1.45	0.49	0.75
PDE head	0.05	0.41	0.21	0.02	004	0.04
Total PDE	127	107	130	18.0	10.5	20.4
ADE body	046	0.71	0.30	0.20	0.097	0.51
ADE hands	0.007	0.019	0.022	0.115	<LOQ	0.044
ADE head	0.05	0.41	0.21	0.018	0.04	0.04
Total ADE	0.52	1.13	0.53	0.34	0.14	0.60
Pesticide B	**Tebufenozide mg a.s./kg a.s. handled**					
	n/a *	**n/a ***	**n/a ***	**AP4**	**AP5**	**AP6**
PDE body	-	-	-	50.7	51.1	107
PDE hands	-	-	-	4.39	4.92	7.11
PDE head	-	-	-	0.15	1.24	0.52
Total PDE	-	-	-	55.3	57.2	114
ADE body	-	-	-	0.32	0.39	0.60
ADE hands	-	-	-	0.063	0.023	0.45
ADE head	-	-	-	0.15	1.24	0.52
Total ADE	-	-	-	0.53	1.65	1.57

* AP1, AP2, AP3 handled pesticide A (morning), while AP4, AP5, AP6 handled pesticide B (afternoon) on the same crop, and thus have been exposed to pesticide A existing deposits.

It is evident that the above values for the two pesticides are in the same order of magnitude for the application part. However, when comparing the two pesticides for the M/L part, the exposure for the pesticide B is higher by a factor of 6 or 8 considering the arithmetic and geometric means, respectively. Although this factor is not significant when contemplating the variation of exposure observed in such trials [21,24], a possible cause for this difference might be the anticipated tiredness of the volunteers in the afternoon shift. In the frames of risk analysis, tiredness and associated deterioration of performance and alertness could be regarded as a factor that impacts protective behavior, rendering persons more susceptible to being exposed due to individual cross-contamination incidents (such incidents are more often and intense during handling of undiluted products). Fatigue, due to several shifts, overtime, and prolonged work, as a factor increasing exposure to chemicals, was also reviewed and verified in an effort to examine the associations of long working hours with injuries, performance and health effects ([25] and references therein). It is noted, however, that the results obtained in the present work are comparable to the ones of a previous greenhouse study addressing similar application scenario and coveralls with the value of exposure levels (geometric mean, n = 10) being 141 mg a.s./kg a.s. [24].

Despite the variability observed during M/L pesticide A and during application of pesticide B, overall, the maximum total PDE levels measured in the study for both pesticides A and B are lower than the exposure of 200 mg/kg a.s. considered in the Dutch Greenhouse Model widely used in the exposure assessment to pesticides for regulatory purposes in the EU. The same conclusion has been derived when comparing the study results to the PDE levels considered for the development of the first version of the Greenhouse Agricultural Operator Exposure Model [17].

It is noted that the results of the present study have been considered for the revision of the Greenhouse Agricultural Operator Exposure Model [26] included in the updated EFSA Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products [27].

3.3. PDE Added via Pesticide Transfer from Sprayed Crop

Regarding the PDE contribution from residues of pesticide A that can be transferred to the operator due to contact with the treated crop, the results showed that the respective exposure values were 10–20 mg a.s./kg a.s. (average 16.3 mg a.s./kg a.s., $n = 3$). This fact indicates that in the conditions of the present study, there was a contribution to the operator's PDE at a rate of 8–15%. Consequently, in cases where operators experienced contact with treated crops and other sources of pesticide cross-contamination, as for example, during re-entry applications, the potential of being additionally exposed due to transfer of pesticide residues deposited on the sprayed leaves should be considered as well. On the contrary, there was minimal pesticide transfer during M/L (average 0.5 mg a.s./kg a.s., $n = 3$). This was reasonably foreseen due to the lack of contact with the crop during the specific task, and also due to the fact that the equipment, which could be a potential source of contamination, was properly cleaned as soon as the previous application was completed. Both remarks highlight the importance of reduced chemical exposure in conjunction with hygienic behavior, implementing proper handling and cleaning of the application equipment. In the same context, the need for following the principles of GAP is indispensable. Last but not least, the aforementioned observation is also supported by the fact that the determined ADE levels were very low in all cases; a fact that is mostly attributed to the satisfactory protection provided to the operators by the PPE and standard working clothing used under the conditions, and the moderately short duration of the specific applications.

3.4. Quantitative Risk Assessment

Based on the available experimental data, the systemic exposure (SE) for a whole working day (assuming application to 1 ha) has been estimated to be lower than the respective health-based guidance value for both bupirimate (pesticides A) and tebufenozide (pesticide B) (Table 8). Regarding the combined exposure of mixer/loaders and applicators to both pesticides, the hazard index calculated was lower than 1, indicating that there is no risk anticipated under the specific conditions examined.

Table 8. Systemic exposure to bupirimate (pesticide A) and tebufenozide (pesticide B): operator risk assessment.

	Pesticide A (Bupirimate)					
	ML1/AP1	**ML2/AP2**	**ML3/AP3**	**ML4/AP4**	**ML5/AP5**	**ML6/AP6**
SE* (mg a.s./kg bw/day)—AP	0.00055	0.0013	0.00066	0.00036	0.00016	0.00075
SE (mg a.s./kg bw/day)—ML	0	0.00046	5.01×10^{-5}	8.76×10^{-6}	8.773×10^{-7}	8.26×10^{-6}
TOTAL SE (mg a.s./kg bw/day)	0.00055	0.0018	0.00071	0.00037	0.00016	0.00076
Estimated exposure as % AOEL *	**1.09**	**3.53**	**1.42**	**0.73**	**0.33**	**1.52**
H/Q *	**0.011**	**0.035**	**0.014**	**0.0073**	**0.0033**	**0.015**
	Pesticide B (Tebufenozide)					
	n/a **	**n/a ****	**n/a ****	**4**	**5**	**6**
SE (mg a.s./kg bw/day)—AP	-	-	-	3.06×10^{-5}	0.00011	8.95×10^{-5}
SE (mg a.s./kg bw/day)—ML	-	-	-	2.63×10^{-6}	6.13×10^{-5}	2.51×10^{-5}
TOTAL SE (mg a.s./kg bw/day)	-	-	-	3.30×10^{-5}	0.00017	0.00011
Estimated exposure as % AOEL	-	-	-	**0.41**	**2.15**	**1.43**
H/Q	-	-	-	**0.0041**	**0.021**	**0.014**
	Combined exposure to Pesticide A and B					
	n/a **	**n/a ****	**n/a ****	**4**	**5**	**6**
HI *	-	-	-	**0.011**	**0.025**	**0.029**

* SE: Systemic Exposure, AOEL: Acceptable Operator Exposure Level, H/Q: Hazard Quotient, HI: Hazard Index, ** Mixer loader/Applicators (1), (2) and (3) handled pesticide A (morning), while Mixer loader/Applicators (4), (5) and (6) handled pesticide B (afternoon), and thus have been exposed to pesticide A existing deposits.

For the calculation of the systemic exposure (SE), the dermal penetration factors (Pf) concluded following the EFSA peer review for each a.s. (concentrate and spray dilution) have been considered: (a) Bupirimate: 1.3 and 12% for the concentrate and the dilution, respectively [28]; (b) Tebufenozide: 0.2 and 2.1% for the concentrate and the dilution, respectively [29]. The AOEL value for bupirimate has been set at 0.05 mg/kg b.w./day [30], while for tebufenozide, the respective value is 0.008 mg/kg b.w./day [31].

4. Conclusions

The objective of this field study was to consider the contribution, to total dermal exposure of pesticide operators, of re-entry in greenhouses for conducting a sequential hand-held application, also involving M/L.

The obtained results showed that when re-entering the treated greenhouse in the afternoon for the 2nd pesticide application, the secondary dermal exposure of operator to the 1st pesticide, applied in the morning of the same day, due to transfer of the remaining deposits on the treated crop, accounted for 0.9–18% (average 8%) of the overall dermal exposure for the specific active substance. For the respective M/L task, the secondary dermal exposure to the pesticide handled earlier accounted for 8–16% (average 13%) of the exposure when the actual mixing/loading of this pesticide occurred. Although a higher number of replicates and further studies are needed to verify the above data, the derived values undoubtedly provide an indication that the contamination of an operator due to contact with sprayed crop is a contributing factor to the potential dermal exposure that should not be disregarded.

Moreover, results for the M/L task, although lower in average rate, could support the conclusion that there might be certain cases of significant extra exposure load from the contaminated equipment or incidental contamination related to M/L (splashes, etc.). Thus, best management practices should be embraced to avoid undesired transfer-contamination due to previously applied pesticides, a fact essential also from the operator's safety perspective, considering the incompatibility of some chemicals, the combined exposure, and mixture toxicity.

Irrespective of the tasks examined, the low actual dermal exposure values measured in the present study, along with respective results of previous ones, demonstrated that the cotton coverall, although not impervious certified PPE, but rather standard working clothing, provides a significant level of protection. Hence, its use is considered to be mandatory during PPP application, independent of the hazard class and pesticide labelling.

Author Contributions: Conceptualization, K.M., K.M.K. and C.R.G.; methodology, K.M., A.N.T., K.M.K., C.R.G. and R.G.-E.; software, A.N.T. and K.M.K.; validation, A.N.T. and K.M.K.; formal analysis, A.N.T., K.M.K., C.R.G., R.G.-E., A.N.C. and P.A.; investigation, A.N.T., K.M.K., C.R.G., R.G.-E., A.N.C. and P.A.; resources, K.M.; data curation, K.M.K., A.N.T., C.R.G. and A.N.C.; writing—original draft preparation, K.M.K. and A.N.T.; writing—review and editing, K.M., K.M.K., C.R.G., R.G.-E. and A.N.C.; visualization, K.M.K. and A.N.T.; supervision, K.M.; project administration, K.M.; funding acquisition, K.M., C.R.G. and R.G.-E. All authors have read and agreed to the published version of the manuscript.

Funding: This work was conducted in the frame of the FP7 BROWSE project (Bystander, Resident, Operator and Worker Exposure models for plant protection products); No: 265307, Details on the BROWSE project can be found on the CORDIS website (https://cordis.europa.eu/project/id/265307/reporting) (accessed on 30 March 2022).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Benaki Phytopathological Institute (classified protocol, February 2012).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data generated or analysed during this study are included in this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. EFSA (European Food Safety Authority). Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. *EFSA J.* **2014**, *12*, 55. [CrossRef]
2. Belsey, N.A.; Cordery, S.F.; Bunge, A.L.; Guy, R.H. Assessment of Dermal Exposure to Pesticide Residues during Re-entry. *Environ. Sci. Technol.* **2011**, *45*, 4609–4615. [CrossRef] [PubMed]
3. Toumi, K.; Joly, L.; Vleminckx, C.; Schiffers, B. Exposure of workers to pesticide residues during re-entry activities: A review. *Hum. Ecol. Risk Assess. Int. J.* **2019**, *25*, 2193–2215. [CrossRef]
4. Aprea, M.C.; Bosi, A.; Manara, M.; Mazzocchi, B.; Pompini, A.; Sormani, F.; Lunghini, L.; Sciarra, G. Assessment of exposure to pesticides during mixing/loading and spraying of tomatoes in the open field. *J. Occup. Environ. Hyg.* **2016**, *13*, 476–489. [CrossRef] [PubMed]
5. Yarpuz-Bozdogan, N.; Bozdogan, A.M.; Daglioglu, N.; Erdem, T. Determination of dermal exposure of operator in greenhouse spraying. *AMA Agric. Mech. Asia Afr. Lat. Am.* **2017**, *48*, 33–38.
6. Rincón, V.J.; Páez, F.C.; Sánchez-Hermosilla, J. Potential dermal exposure to operators applying pesticide on greenhouse crops using low-cost equipment. *Sci. Total Environ.* **2018**, *630*, 1181–1187. [CrossRef]
7. Machera, K.; Goumenou, M.; Kapetanakis, E.; Kalamarakis, A.; Glass, R. Determination of potential dermal and inhalation exposure of operators, following spray applications of the fungicide penconazole in vineyards and greenhouses. *Fresenius Environ. Bull.* **2001**, *10*, 464–469.
8. Machera, K.; Goumenou, M.; Kapetanakis, E.; Kalamarakis, A.; Glass, C.R. Determination of Potential Dermal and Inhalation Operator Exposure to Malathion in Greenhouses with the Whole-Body Dosimetry Method. *Ann. Occup. Hyg.* **2003**, *47*, 61–70.
9. Ren, J.X.; Li, Z.K.; Tao, C.J.; Zhang, L.Y.; Zhao, H.F.; Wu, C.C.; She, D.M. Exposure assessment of operators to clothianidin when using knapsack electric sprayers in greenhouses. *Int. J. Environ. Sci. Technol.* **2018**, *16*, 1471–1478. [CrossRef]
10. Tsakirakis, A.; Kasiotis, K.M.; Arapaki, N.; Charistou, A.; Tsatsakis, A.; Glass, C.R.; Machera, K. Determination of operator exposure levels to insecticide during bait applications in olive trees: Study of coverall performance and duration of application. *Int. J. Hyg. Environ. Health* **2011**, *214*, 71–78. [CrossRef] [PubMed]
11. Tsakirakis, A.N.; Kasiotis, K.M.; Charistou, A.N.; Arapaki, N.; Tsatsakis, A.; Tsakalof, A.; Machera, K. Dermal & inhalation exposure of operators during fungicide application in vineyards. Evaluation of coverall performance. *Sci. Total Environ.* **2014**, *470–471*, 282–289.
12. Kasiotis, K.M.; Tsakirakis, A.N.; Glass, C.R.; Charistou, A.N.; Anastassiadou, P.; Gerritsen-Ebben, R.; Machera, K. Assessment of field re-entry exposure to pesticides: A dislodgeable foliar residue study. *Sci. Total Environ.* **2017**, *596–597*, 178–186. [CrossRef] [PubMed]
13. Dong, M.H.; Beauvais, S. Assessment of field re-entry exposure to pesticides: Limitations, uncertainties, and alternatives. *Hum. Ecol. Risk Assess.* **2013**, *19*, 579–600. [CrossRef]
14. Garthwaite, D.; Sinclair, C.J.; Glass, R.; Pote, A.; Trevisan, M.; Sacchettini, G.; Spanoghe, P.; Ngoc, K.D.; Fevery, D.; Machera, K.; et al. *Collection of Pesticide Application Data in View of Performing Environmental Risk Assessments for Pesticides*; EFSA Supporting Publication; EFSA: Parma, Italy, 2015; p. 846E, 246p.
15. Wong, H.L.; Garthwaite, D.G.; Ramwell, C.T.; Brown, C.D. Assessment of exposure of professional agricultural operators to pesticides. *Sci. Total Environ.* **2018**, *619–620*, 874–888. [CrossRef]
16. Son, D.; Zerbo, F.K.B.; Bonzi, S.; Schiffers, B.; Somda, I.; Legrève, A. Assessment of Tomato (*Solanum lycopersicum* L.) Producers' Exposure Level to Pesticides, in Kouka and Toussiana (Burkina Faso). *Int. J. Environ. Res. Public Health* **2018**, *15*, 204. [CrossRef]
17. BfR (Bundesinstitut für Risikobewertung, German Federal Institute for Risk Assessment). *Joint Development of a New Greenhouse Agricultural Operator Exposure Model for Hand-Held Application*; BfR: Berlin, Germany, 2015; 117p. Available online: https://www.bfr.bund.de/cm/350/joint-development-of-a-new-greenhouse-agricultural-operator-exposure-model-for-hand-held-application.pdf (accessed on 12 February 2021).
18. *OECD/GD (97)148*; Guidance Document for the Conduct of Studies of Occupational Exposure to Pesticides during Agricultural Application. OECD Environmental Health and Safety Publications: Paris, France, 1997.
19. Chester, G. Evaluation of agricultural worker exposure to, and absorption of, pesticides. *Ann. Occup. Hyg.* **1993**, *37*, 509–524.
20. WHO. *Field Surveys of Exposure to Pesticides*; Standard Protocol VBC/82.1; WHO: Geneva, Switzerland, 1982.
21. Machera, K.; Tsakirakis, A.; Charistou, A.; Anastasiadou, P.; Glass, C.R. Dermal Exposure of Pesticide Applicators as a Measure of Coverall Performance Under Field Conditions. *Ann. Occup. Hyg.* **2009**, *53*, 573–584.
22. Beránková, M.; Hojerová, J.; Peráčková, Z. Estimated exposure of hands inside the protective gloves used by non-occupational handlers of agricultural pesticides. *J. Expo Sci. Environ. Epidemiol.* **2017**, *27*, 625–631. [CrossRef]
23. Durham, W.F.; Wolfe, H.R. Measurement of the exposure of workers to pesticides. *Bull. World Health Organ.* **1962**, *26*, 75–91.
24. Machera, K.; Kapetanakis, E.; Charistou, A.; Goumenaki, E.; Glass, R.C. Evaluation of potential dermal exposure of pesticide spray operators in greenhouses by use of visible traces. *J. Environ. Sci. Health B* **2002**, *37*, 113–121. [CrossRef]
25. CDC Overtime and Extended Work Shifts: Recent Findings on Illnesses, Injuries, and Health Behaviors. 2004. Available online: https://www.cdc.gov/niosh/docs/2004-143/pdfs/2004-143.pdf (accessed on 20 May 2021).
26. BfR (Bundesinstitut für Risikobewertung, German Federal Institute for Risk Assessment). *Update of the Greenhouse Agricultural Operator Exposure Model—Amendment to Project Report 01/2016*; BfR: Berlin, Germany, 2020; 133p. Available online: https://www.bfr.bund.de/cm/350/revision-of-the-greenhouse-agricultural-operator-exposure-model.pdf (accessed on 20 May 2021).

27. Charistou, A.; Coja, T.; Craig, P.; Hamey, P.; Martin, S.; Sanvido, O.; Chiusolo, A.; Colas, M.; Istace, F. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment of plant protection products. *EFSA J.* **2022**, *20*, 134. [CrossRef]
28. Bupirimate-EFSA. Conclusion on the peer review of the pesticide risk assessment of the active substance bupirimate. *EFSA J.* **2010**, *8*, 1786. [CrossRef]
29. Tebufenozide-EFSA. Conclusion on the peer review of the pesticide risk assessment of the active substance tebufenozide. *EFSA J.* **2010**, *8*, 1871.
30. Bupirimate-AOEL. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as_id=485 (accessed on 10 December 2021).
31. Tebufenozide-AOEL. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/active-substances/?event=as.details&as_id=780 (accessed on 10 December 2021).