SZENT ISTVÁN UNIVERSITY

UNCERTAINTY OF EXPOSURE ASSESSMENT OF CONSUMERS TO PESTICIDE RESIDUES DERIVED FROM FOOD CONSUMED

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PhD School/Program

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1. INTRODUCTION

Pesticides are indispensable components of modern intensive agricultural production which should supply sufficient amount and quality of food for the continuously growing population of the World. The quantity of pesticide residue intake due to food consumption can only be assessed, when data on pesticide residue concentration in food, as well as food consumption data (type, components and quantity) are available.

Presently, based on the guidance of WHO, deterministic methods are used at international level to determine whether the pesticide residue concentration remaining in the edible part of the food, after applying the recommended plant protection technology, have to be considered as a risk from the consumer's point of view. Deterministic models provide simple exposure modelling tools where fixed values of food consumption (such as the average or high-level consumption value) are multiplied by a fixed value of the residue concentration.

To correctly evaluate and communicate the available data regarding food consumption and pesticide residues, as well as the results of food safety risk assessment procedures, the inevitable uncertainty of the calculated pesticide residue exposure due to food consumption has to be assessed, especially in cases when the calculated intake is close to the toxicological reference values (ADI or ARfD) to verify the acceptability of the use pattern of the pesticide. Though, there are many scientific publications available regarding the uncertainty of measured residue values and their distribution, only conceptual guidance documents exist dealing with quantitative uncertainties of exposure to pesticide residues due to food consumption. By identifying the sources of uncertainties, the critical components can potentially be minimalised, the utilization of available resources optimised.

2. OBJECTIVES

1. To investigate the random errors in food portion estimation resulted from the visual perception and conceptualization-memory, applying the EPIC-SOFT food picture series.
2. To identify and quantify if possible, the uncertainty sources of estimation of food consumption data.
3. To identify and quantify, if possible, the uncertainty sources of pesticide residue data applicable for pre-registration pesticide dietary risk assessment.
4. To elaborate a method for calculating the combined uncertainty of the pesticide residue exposure estimated with deterministic method and demonstrating its practical applicability with a detailed worked example, using food consumption data of two days and the bifenthrin pesticide residue results obtained from supervised residue trials.
5. To prioritize the contribution of quantifiable uncertainties of input parameters to the combined uncertainty of the calculated exposure.

3. MATERIALS AND METHODS

3.1. Estimation of portion sizes by perception and memory methods

In the validation of the applicability of EPIC-SOFT (ES) food picture series used in the context of a Hungarian food consumption survey 62 persons participated on a volunteer basis. The study gathered data for exposure assessment, and investigated the random errors in food portion estimation resulted from the visual perception and conceptualization-memory in three age groups (10-17 years adolescents, 18-64 years adults, >64 years elderly).

During the perception part of the study all participants were presented with three different portions of seven foods placed on normal dining plates. The weights of the portions randomly selected from the ES book corresponded (except one) to the weights given in grams in the ES picture book manual. The size of portion on the plate of each food item had to be estimated by each of the participants by choosing the corresponding picture number or an intermediate value on the decimal scale between two pictures. The answers were recorded on the score sheets.

The memory effect was tested during the second phase of the study. For the conceptualization-memory test, the participants served their own portion from the given seven foods. Each participant was asked to take that amounts of the foods that she/he would eat. One-two hours after serving the foods, the participants were asked to estimate, by using the ES picture series, the quantities of foods they took on their plates.

3.2. Statistical evaluation of reported values of portion size estimation

Second order equations could be fitted on the weights of portions and picture numbers of the individual picture series. The intermediate weights between pictures and weights bellow the smallest and above the largest quantities were calculated using these second order equations. The relative differences between the actual (m_k) and estimated (m_b) weight were calculated as:

$$\Delta_m = \frac{m_b - m_k}{m_k} \quad (1)$$

The resulting difference corresponds to the estimation error.
For all foods, the mean of estimated ($\bar{m}_b$) weights and its relative difference ($\bar{\Delta}_m$) from the real value (m_k) were calculated, to enable comparison of the results obtained for various portion sizes:

$$\bar{\Delta}_m = \frac{\bar{m}_b - m_k}{m_k} \quad (2)$$

The relative standard deviations (CV) of the estimated portions were calculated to evaluate the applicability of the pictures series:

$$CV_k = \frac{SD_{ki}}{\bar{m}_i} \quad (3)$$

where SD_{ki} is the standard deviation of the estimated weights of the i^{th} food by the k participants, $\bar{m}_i$ is the average estimated weight of the i^{th} food.

The precision of estimations obtained through the memory study was determined with their average relative difference ($\overline{\Delta}_{irel}$), because each portion served by the participants was different:

$$\Delta_{irel} = \frac{m_s - m_e}{m_s} \quad (4)$$

$$\overline{\Delta}_{irel} = \frac{\sum_{i=1}^{k} \Delta_{irel}}{k} \quad (5)$$

where m_s and m_e are the served and estimated portions of i^{th} food by one of the participants, $\overline{\Delta}_{rel}$ is the average of relative differences.

The spread of the estimated differences was characterized with their relative standard deviation (CV_{ir}) calculated from the average absolute differences applying the basic relationship of range statistics:

$$\Delta_{ira} = \frac{|m_s - m_e|}{m_s} \quad (6)$$

$$CV_{ir} = \frac{\sum_{i=1}^{k} \overline{\Delta}_{ira}}{k \times 1{,}128} \quad (7)$$

The comparison of experimental data and the normal distribution, generated with the same mean and standard deviation as the experimental data, indicated that the estimated portions were far from normal distribution. Therefore, non-parametric tests were applied. The significance of the difference between actual and estimated weights of food items were tested with Wilcoxon Signed Rank Test (WSRT). Kruskal-Wallis H test (KW) was used for investigating the similarity of the distribution pattern and the medians of the portion sizes estimated by the different subgroups (gender and age). Winsorisation was applied to compensate the effect of the potential outliers. Significance of difference between the served and estimated portions was analysed with Wilcoxon Signed Rank Test for paired data (WSRTp).

The results were considered good and the picture applicable if:

(a) the relative difference between estimated and actual weights was within 10%;

(b) the Wilcoxon Signed Rank Test indicated that the estimated average weight did not differ significantly from the known portion size; and

(c) CV of portion sizes was ≤ 0.30.

Where any of the above criteria could not be met, Winsorisation was applied to compensate the effect of potential outliers. From the Winsorised dataset the relative difference between mean and actual value and the CV_w was calculated. The WSRT and KW tests were repeated with the Winsorised data and the applicability of pictures was evaluated based on the latter results. After Winsorisation the results were considered good or acceptable if:

(a) the relative difference between the estimated and true value was <10% and <25%, respectively.

(b) $CV_w \leq 0.30$. If the results did not meet these acceptance criteria, the given picture was considered not applicable for estimation of the portion size of tested food.

3.3. Determination of mass equivalents

During the validation study, it was found that the real masses of visually identical volumes of the foods may differ from the masses of foods presented on the ES pictures. Five foods included in the ES book were prepared according to Hungarian recipes from raw materials available at the national market. Twenty-one volunteers were asked to take from the 5 foods visually identical portions (w_k [g]) to that shown in the EPIC-SOFT picture book., The procedure was repeated three times in random order of the foods. The served portions were weighed (w_h, [g]) to the nearest 0.1 g. The conversion factor was calculated from the average weight ($\overline{w}_h$) of the portions taken repeatedly and the weight (w_k) of the food shown on the relevant ES picture:

$$\rho = \overline{w}_h / w_k \tag{8}$$

The actual weight (w_f) of portion sizes estimated applying the picture book can be calculated as:

$$w_f = w_k \times \rho \tag{9}$$

3.4. Calibration of balances used for body mass measurement purposes

In order to assure comparability of the measured weights and traceability to international standards all balances shall be calibrated. Balances made for professional body mass measurements are usually equipped with self-calibration function. Since these balances are very expensive, I investigated the accuracy, repeatability and reproducibility of digital and analogue commercial bathroom balances, to test whether such balances can be applied for body mass measurement in dietary surveys. The procedure described hereunder can be easily performed in a health centre where a reference balance calibrated to national metrological standard is available.

Four balances were used for the calibration experiment together with one reference balance. Seven participants weighed 3 times independently the body masses of the other members of the team with all balances covering their weighing ranges and recorded the readings on a pre-prepared record sheet. Self-weight measurements were not made.

The average of body mass of all participants ($\overline{w}$) was calculated from the $6 \times 3 = 18$ independent measurements (w_i) performed by 6 team members on each balance:

$$\overline{w} = \frac{1}{jn} \sum_{j=1}^{6} \sum_{i=1}^{n} w_i \tag{10}$$

where w_i is the reading of the one body mass measurement, n is the number of replicate measurements made for one team member on one balance (in this example n=3), j is the number of team members weighted the other team members (j=7-1=6 in our example).

P_1 participant was measured on balance M_1 3-times (w_{P1M1}, w_{P2M2}, w_{P3M3}). From the average ($\overline{w}_{P1M1}$) and standard deviation (SD_{P1M1}), the relative repeatability of the weight measurements of a person performed by 6 team members is calculated as:

$$CV_{r,1,1} = \frac{SD_{P1M1}}{\overline{w}_{P1M1}} \tag{11}$$

Similarly, the typical repeatability of the weight measurements of a person performed by 6 team members on four balances was calculated from the average variance:

$$\overline{SD}_{P1} = \sqrt{\frac{\sum \text{VAR}}{\text{mérlegek száma}}} \qquad \overline{\overline{CV}}_r = \frac{\overline{SD}_{P1}}{\overline{\overline{w}}_{P1}} \tag{12}$$

where $\overline{\overline{w}}_{P1}$ is the average weight of P_1 person weighed on 4 balances.

The reproducibility of the weight measurements of P_1 person performed by 6 team members on 4 balances calculated from P×M×n=6×4×3=72 measurements:

$$CV_{repr}P1' = CV_{repr,P1,M1-6} = \frac{SD_{P1M1-6}}{\overline{w}_{P1M1-6}} \tag{13}$$

The reproducibility of the weight measurements for the whole measuring range (between 45 and 148 kg) for 7 persons on 4 balances calculated from 7x4x18=504 measurements:

$$\overline{\overline{CV}}_{P1-7;M1-4} = \sqrt{\frac{df_{RP1}CV'^2_{reprP1} + df_{RP2}CV'^2_{reprP2} + \cdots + df_{RP7}CV'^2_{reprP7}}{\sum df_{P,M}}} \tag{14}$$

The number of freedom of the standard deviation corresponding to $CV'_{reprP1} \ldots CV'_{reprP7}$ values for $P_1 \ldots P_7$ persons depends on how many balances the weight measurement could be conducted.

3.5. Principles of estimation of quantifiable uncertainty

The exposure is calculated by multiplying the food quantity consumed by the pesticide residue concentration. Calculations of short-term intake from the food consumption within 24 hours recognize four different cases. In the simplest case, the IESTI equation (international estimated short term intake) is:

$$IESTI = \frac{LP \times (HR \text{ or } HR-P)}{bw} \tag{15}$$

where LP is the large portion size, including 97.5th percentile consumption of eaters reported from food consumption surveys, preferably expressed as

consumed food [kg]/body weight[kg] and HR or HR-P is the highest concentration of pesticide residue detected in supervised trial samples or in processed products (HR-P), bw is the body weight.

The long-term daily intakes at international (IEDIs) or national level (NEDI) are derived from the median residues calculated from the results of supervised residue trials (STMR) or processing studies (STMR-P, supervised trial median residue in processed commodity) and the corresponding consumption data:

$$EDI = \sum (STMR_i \text{ (vagy } STMR\text{-}P_i) \times F_i) \quad (16)$$

where $STMR_i$ or $STMR\text{-}P_i$ are the median residues and F_i is the average consumption for the i^{th} commodity.

The first step of the assessment of uncertainties is to identify its various sources and describe the limitation of knowledge available for characterising their effects on the combined uncertainty of the outcome of the study. In our case, it is the exposure of consumer to the bifenthrin residues based on the food consumption reported. The quantifiable uncertainty of dietary intake incorporates the uncertainties of individual parameters. Their combined uncertainty can be calculated based on the general rules of error propagation.
The result (Y) is the sum of measured quantities:

$$Y=C_1P \pm C_2Q \pm C_3R \ldots \quad (17)$$

The random error of the result is calculated as:

$$SD_{(y(x_{P,Q,R}))} = \sqrt{(C_1 \times SD_P)^2 + (C_2 \times SD_Q)^2 + (C_3 \times SD_R)^2} \quad (18)$$

where SD_P, SD_Q, SD_R are the standard deviations of the measurements of P, Q and R; C1, C2 and C3 are constants.
The result is obtained with multiplication or division:

$$Y = \frac{k \times P}{Q \times R} \quad (19)$$

The relative standard deviation (coefficient of variation, CV) of the P value is:

$$CV_p = \frac{SD_p}{P} \quad (20)$$

The calculation of the standard deviation depends on the nature of the distribution of the data. The simplest case is, for instance, the estimation of uncertainty of analytical measurements, which were shown to follow normal distribution. In other cases, the occurrence of events has similar probability such as selecting a recipe for preparing a meal from the list available on the Internet. In this case an estimate for the standard deviation is made from the range (2a) of a constituent of the different recipes. The corresponding standard deviation is calculated as:

$$SD_X = \frac{a}{\sqrt{3}} \quad (21)$$

The relative uncertainty (random error) of the calculated Y value is calculated as:

$$CV_Y = \sqrt{\left(k \times CV_p\right)^2 + CV_Q^2 + CV_R^2} \quad (22)$$

The combined uncertainty of residues (CV_R) comprises the uncertainty of sampling (CV_S), reducing the size of the laboratory sample (sub-sampling, CV_{SS}) sample processing and withdrawing the test portions from the comminuted laboratory (sub-) sample (CV_{Sp}) extraction of test portions and qualitative, quantitative determination of extracted residues (CV_A). The combined uncertainty of measured pesticide residue can be calculated based on the general rules of error propagation:

$$CV_R = \sqrt{CV_S^2 + CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (23)$$

Since the sampling and laboratory determination is separated in place and time, it is appropriate to separate the combined uncertainty related to sampling and the laboratory phase (CV_L). The CV_L incorporates the sub-sampling, samples processing and analysis. The combined uncertainty of the measured residue can be described in a simpler way:

$$CV_R = \sqrt{CV_S^2 + CV_L^2} \quad (24)$$

$$CV_L = \sqrt{CV_{SS}^2 + CV_{Sp}^2 + CV_A^2} \quad (25)$$

The approximate standard deviation of the selected percentile of the residue data population obtained from supervised trials can be calculated with the general equation of standard deviation of binominal distribution as:

$$SD_p = \sqrt{N \times p \times q} \quad (26)$$

where p is the selected percentile, q=1-p and N is the number of data points. In case of median the p=q=0.5. The relationship is accurate for N≥20 values, but provides approximate value for smaller N values. The approximate relative uncertainty of the STMR value can be calculated, assuming normal distribution, from the 95% range of residues ($R_{P0.975}$-$R_{P0.025}$) in the dataset divided by the median value (STMR) as:

$$SD_{STMR} = \frac{R_{P0,975} - R_{P0,025}}{2 \times 1,96} \quad (27)$$

$$CV_{STMR} = \frac{SD_{STMR}}{STMR} \quad (28)$$

The combined relative uncertainty (CV_{comb}) of the STMR value is calculated from the combined uncertainty of residue measurement (CV_R) and relative uncertainty of the STMR value (CV_{STMR}):

$$CV_{komb} = \sqrt{CV_R^2 + CV_{STMR}^2} \quad (29)$$

Because of several factors affect the outcome of processing, the processing factors usually show wide variation. In case of valid study conditions, the occurrence of the processing factors observed for a given pesticide commodity combination have equal probability. Therefore, their standard deviation (SD_{Pf}) is calculated assuming rectangular distribution and calculated from the difference between the maximum and minimum Pf values as:

$$SD_{Pf} = \frac{Pf_{max} - Pf_{min}}{2 \times \sqrt{3}} \quad (30)$$

Each set of processing studies represents a sample of the unknown population of processing factors. The best estimate of their uncertainty, expressed as relative standard deviation is the pooled variances of the relevant sets of processing factors. However, the magnitudes of median processing factors (M_{Pf}) are different, therefore the calculation shall be carried out with the relative standard deviations (CV_{Pf}) using the median processing factor as a robust estimate of the mean value:

$$CV_{Pf} = \frac{SD_{Pf}}{M_{Pf}} \quad (31)$$

where the value of SD_{Pf} is calculated with equation 30 and M_{Pf} is the median of single processing factor values.

Some foods have different recipe variants, where the compounds and their ratio may differ, which can be described with the relative standard deviation (CV_{cu}). The experienced persons preparing the meals regularly do not measure the individual components, but mix them according to their own taste to obtain suitable consistency of the dough or initial mixture of components. The deviation from the written recipes can be over 30%. Therefore, the expectable variability of proportion of ingredients (i), expressed with standard deviation, in composite food was calculated assuming equal probability of applying the recipes taking into account the potential deviations from the written recipe as:

$$SD_{cu} = \frac{1{,}3 \times maxP_i - 0{,}7 \times minP_i}{2 \times \sqrt{3}} \quad (32)$$

The relative standard uncertainty (CV_{cu}) of recipes was calculated from the standard deviation (SD_{cu}) and the median ($\tilde{m}_{Pi}$) of proportions of ingredients (P_i-s):

$$CV_{cu} = \frac{SD_{cui}}{\tilde{m}_{Pi}} \quad (33)$$

The calculations included in published validation studies addressing the effects of memory skills during portion size estimation differs. Gaining information on the variability of weight estimates provides a better basis for the estimation of uncertainty of exposure assessment. In these studies, the relative standard deviations (CV_{di}-s) characterising the relative uncertainty of the

estimated portion sizes due to memory effect were not published. Therefore, the relative standard uncertainty (CV_{di}) of estimated portion size was calculated from the SD_{di} and the mean ($\bar{x}_{di}$) of estimated portion sizes (Pi-s) if available:

$$CV_{di} = \frac{SD_{di}}{\bar{x}_{di}} \quad (34)$$

3.6. Calculation of combined uncertainty of exposure in practice

The known food consumption of a 19-years old boy (192cm, 60kg) reported on two non-consecutive was selected as a basis of the dietary intake calculation. Bifenthrin was selected as model pesticide, since its residue is present in wheat-based foods, fruits and processed products consumed during the 2-day intake survey. Furthermore, relevant data is available to demonstrate the uncertainty. Since the detailed calculation of combined uncertainty is part of the results, it will be discussed under Results.

4. RESULTS

4.1. Evaluation of the results obtained from the picture book validation

Overall, during the validation sessions each of five picture series was evaluated by 62 persons. In the memory test, I obtained answers from 53 participants. The same five foods were evaluated parallel in the perception and conceptualization-memory studies. Based upon the applied acceptance criteria, the estimations were good or acceptable, except in case of the picture of small portion of boiled potato. The direction and proportion of estimation errors in our study was comparable with earlier studies. Differences obtained through both methods provide information on the capability of interviewed persons to recall the portion size of food taken on the plate 1-2 hours earlier. When conceptualization-memory is brought into the equation, errors in estimates increased (up to 61%). Results obtained in this study should be considered as a best-case scenario, since, under the survey conditions the time between eating the food and recalling its quantity is much longer (24-36 hours), which further increase the bias in estimation of portion sizes. The relative standard uncertainty of estimated portion size (CV_{di}) was found between 24-55% in this study.

4.2. Results obtained through the determination of mass equivalents

As part of the validation study the mass equivalents of tested foods were determined. Table 1 summarizes the real mass corresponding to the relevant ES picture, the estimated mass of visually identical portions and the calculated conversion factor per food.

Table 1. Calculated mass equivalent of ES portion sizes

Food	Potato	Creamed spinach	Stew	Spaghetti	Steak
Number of the tested ES portion size	2	4	4	5	2
Real mass of the tested ES portion size (g)	141	243	276	320	132
Average of estimated masses	271.68	265.11	292.72	228.52	129.77
SD	64.68	43.07	94.76	45.14	34.18
CV	0.24	0.16	0.32	0.20	0.26
Median	266.0	271.0	276.0	240.0	111.0
Min.	112.6	161.0	57.0	127.6	89.0
Max.	456.0	319.1	469.7	317.0	203.8
Conversion factor	1.93	1.09	1.06	0.71	0.98

Notes:
ES: EPIC-SOFT;
Min: smallest estimated weight;
Max: largest estimated weight.

The results highlight that the real masses of visually identical portion sizes of the foods may differ from the masses of foods presented on the pictures, which have to be considered when calculating the consumed mass based on portion size estimation using internationally designed food picture booklet during national dietary surveys. This area requires further research.

4.3. Results obtained through the balance calibration

The calibration of the balances was performed by measuring the body masses of the participants, overlapping the total weighing range, assuming that the weights measured with the certified reference balance (M_{ref}) are accurate. The parameters of the linear regression equations were used to calculate the predicted weights (W') from the measured average weights ($\overline{w}$):

$$W' = \frac{\overline{w} - a}{b} \tag{35}$$

The predicted weights and their relative difference from the reference weights before (SSQ_w) and after (SSQ_W) calibration, as well as their ratio ($SSQ_W/SSQ_w=SSQ_{W/w}$) are summarised in Table 2. Comparison of the sum of the square of differences, especially their ratio clearly indicates (SSQW' << SSQw) that the calibration of the balance significantly improves the accuracy of the body mass measurements. The accuracy of the weighing is better than 0.5% and 1% in case of calibrated digital and analogue balances. Consequently, commercial bathroom balances can be used for measuring the body mass of the interviewed persons after calibration for the whole weighing range in dietary surveys.

Table 2. Results of calibration of balances

Code+	Relative difference before calibration*×10+3				Relative difference after calibration *×10+3			
	1	2	3	4	1	2	3	4
I	4.89	7.55	-15.8	-31.3	-2.27	2.2	3.60	-3.37
II	6.29	6.29	-14.5	-25.8	-0.67	5.45	3.37	-3.60
III	4.57	5.96	-1.07	-22.6	-2.21	6.55	5.71	-2.99
IV	6.92	3.70	-20.6	-30.9	0.73	-8.55	-8.73	-2.08
V	6.26	6.68	-13.6	-20.3	0.43	-2.03	-4.50	2.75
VI	6.85	0.92	-2.76	-11.4	1.61	1.12	1.74	0.06
VII	4.06	-2.43		-4.87	-0.63	0.2		-0.4
$SSQ_{w, W}$	0.24	0.2	1.19	3.68	0.01	0.05	0.16	0.16
$SSQ_{W/w}$	0.06	0.23	0.13	0.04				

Notes:
+: Code of participants (I-VII).
* Relative difference between weights measured by the reference balance and tested balances.
1-4: Code of the balances tested.
SSQw: Sum of squares of relative differences of weights before calibration.
SSQW: Sum of squares of relative differences of weights after calibration.
SSQW/SSQw: Ratio of sum of squares of relative differences after and before calibration.

4.4. Calculation of combined uncertainty of bifenthrin exposure due to food consumption

Various potential sources of uncertainties of pesticide residue exposure assessment were identified and quantified.

4.4.1. Calculation of relative uncertainty of pesticide residues in plant commodities and raw food of animal origin

Bifenthrin residues, relevant for the two non-consecutive days' food consumption, reported from supervised trials, the calculated STMR, the 95% percentile range and its relative uncertainty are summarized in Table 3.

For the calculation of exposure, the amount of residues (mg) was considered for each food component based on the results of the supervised trials. However, due to lack of relevant information, the relative uncertainty of expectable pesticide residue content of food of animal origin or components of composite foods could only partly be or could not be quantified.

Table 3. Spread of bifenthrin residues concentration in supervised trials' samples and its relative uncertainty

Crop	Number of trials	Residues [mg/kg]					CV_{STMR}
		Min.	STMR	HR	P0.025	P0.975	
Citrus fruits[1]	36	0.005	0.05	0.05	0.0082	0.05	0.213
Apple			0.1[a]				NA
Banana, pulp	9		0.01	0.01	0	0	
Beans without pod	7			<0.05	0	0	0
Caneberry[2]	5	<0.05	0.29	0.51	0	0.51	0.676
Carrot[3]	10		0.05	0.05			NA
Eggplant	6	<0.05	<0.05	0.1	0	0.1	0.769
Pear			0.1[a]				NA
Peas in pod[4]	6	0.17	0.225	0.49	LOQ	LOQ[b]	0
Maize	25		0	0			0
Mangó[5]	4	0.066	0.14	0.23	LOQ	LOQ[c]	0
Meat (muscle) from mammals			0.07	0.104			NA
Peppers	11	<0.055	0.14	0.31	<0.055	0.24	0.337
Peppers, chili, dried[6]			1.4				0.337[d]
Potato	17		0.05	0.05	0	0	0
Rape seed	6		0.05		0	<0.05	NA
Rape seed oil, edible			0.08				NA
Strawberry	19	0.27	0.46	2.3	0.33	0.59	0.144
Tomato	7	0.03	0.06	0.15	0	0.15	0.638
Wheat[7]	13	0.19	0.25	0.40	0.2	0.28	0.082

Notes:
NA: no data available.
*: At or about the limit of determination.
[a]: STMR or STMR-P.
[b]: Derived as a conservative estimate from the MRL of 0.3 established by the European Union for pome fruits.
[1]: Including lemon, grapefruit and orange.
[2]: Including raspberry, blackberry.
[3]: Rot and tuber vegetable group; residue value is applicable for all crops being in the group.
[4]: No residue is expected in succulent seeds. However, as a conservative estimate, residues equal to LOQ of 0.05 mg/kg were used in the calculations.
[5]: Whole fruit; no residue is in pulp.
[6]: Calculated with the default factor of 10.
[7]: Post-harvest treatment.

4.4.2. Calculation of the relative uncertainty due to pesticide residue concentration in processed food

The relevant processing factors for calculation of dietary exposure based on the model diet are given in Table 4. The uncertainty of the median processing factor, expressed as standard deviation, was calculated directly with equation 21 in cases where the number of processing studies was large (≥22). In case of 1 or 2 studies, based on the evaluation of the variability of processing factors in other studies reported by the JMPR, the standard deviation was calculated

from the range of $1.4P_{fmax}$-$0.6P_{fmin}$ with equation 21. The latter estimate belongs to the uncertainty category reflecting incomplete knowledge.

Table 4. Standard and relative uncertainty of selected processing factors

Product	No. of studies	Min-max P_f values	P_f[1]	STMR-P mg/kg	SD_{Pf}	CV_{Pf}
Wholemeal flour	30	0.29-1.1	0.765	0.19	0.228	0.306
Wholemeal bread	22	0.11-0.97	0.75	0.19	0.248	0.331
White flour	22	0.038-0.52	0.31	0.078	0.139	0.449
White bread	22	0.04-0.31	0.245	0.061	0.078	0.318
Rape seed refined oil	1		1.6	0.08	0.370	0.231
Tomato paste	2	<0.63. <0.71	<0.67	0.04	0.178[3]	0.265[3]
Tomato puree	2	<0.63. <0.71	<0.67	0.04	0.178[3]	0.265[3]
Chilli pepper dry			10	1.4[2]	2.309	0.231[4]

Notes:

[1]: Median value or best estimate.

[2]: Based on default dehydration factor of 10 and STMR for green pepper of 0.14 mg/kg.

[3]: Calculated with worst case assumption taking less than values as real ones (incomplete knowledge or information).

[4]: Calculated from the estimated P_f=10 with the range of $1.4P_{fmax}$-$0.6P_{fmin}$ and equation 21.

4.4.3. Estimation of variability of recipes for preparing food included in the model diet

The type and proportion of ingredients of meals recalled under the same name may differ. I have compared some random recipe variants for the foods consumed by the selected person during two non-consecutive days. The experienced persons preparing the meals regularly do not measure the individual components, but mix them according to their own taste to obtain suitable consistency of the dough or initial mixture of components. The deviation from the written recipes can be over 30%. Therefore, the expectable variability of proportion of ingredients, expressed with standard deviation, in composite food was calculated assuming equal probability taking into account the potential deviations from the written recipe applying equation 33. The relative standard deviation (CV_{cu}) due to recipe variability varies between 0.22 and 1.44.

4.4.4. Estimation of uncertainty of calculated bifenthrin concentrations in home-made foods

To get the required information for demonstration of the estimation of the uncertainty of exposure resulted from the model consumption data, I prepared the composite food items following my own recipes. The mass of raw materials and the end-products were weighted.

The calculation of relative uncertainty of residue concentration (CV_{res}) in ready-to-eat food includes the uncertainty of the laboratory phase (CV_L), the STMR (CV_{STMR}), the uncertainty resulted from recipes (CV_{cu}) and processing factors (CV_{Pf}).

The stepwise method of calculation of residue concentration and its uncertainty is described for pancake hereunder:

i. Calculation of mg residue in the given mass (M_i) of i^{th} ingredient from the STMR values. For eggs the STMR=0, so no residue contribution is calculated. In case of rapeseed oil:

$$R_1=M_1\times STMR;\ 0.18\ kg\times 0.08\ mg/kg=0.0144\ mg.$$

ii. As next step the total residue (mg) derived from all (k) ingredients (rapeseed oil, milk, flour) has to be calculated:

$$R_T[mg]=\sum_{i=1}^{k} R_i \tag{36}$$

0.0144 (rapeseed oil)+0.0196 (milk)+0.0320 (flour)=0.0660 mg.

iii. The total mass (M_T) of 16 pieces fried empty pancakes is 1.34 kg; the concentration of bifenthrin residues (R_c) in empty pancakes is:

$$R_c=\frac{R_T}{M_T}=\frac{0{,}06599}{1{,}34}=0{,}0493\ mg/kg \tag{37}$$

iv. Each pancake is filled with 5g strawberry marmalade. Since the recipe indicated that the home-made marmalade contained 90.7% strawberry, the 5g marmalade is equivalent to 5.5125 g raw strawberry (including cleaning (F_{cl}) and cooking factors (F_{cu})). The bifenthrin contribution from strawberry to the sum of residues is calculated similarly to that steps i and ii (STMR=0.46 mg/kg):

$$0.00551\ kg\times 0.46\ mg/kg=0.0025\ mg.$$

Sixteen pancakes contained 0.0660 mg residues, then one empty pancake contained, on an average, 0.0041 mg residue, and one pancake filled with strawberry marmalade contained 0.0041+0.0025=0.0067 mg bifenthrin.

v. The combined uncertainty of residue concentration (CV_{comb}) comprises of the relative standard deviations of residues (CV_R), the variability deriving from the industrial processing or kitchen operations (CV_{Pf}) and the uncertainty of STMR (CV_{STMR}):

$$CV_{comb}=\sqrt{CV_R^2+CV_{Pf}^2+CV_{STMR}^2} \tag{38}$$

v. The CV_R includes the uncertainty of sampling (CV_{S1}) of raw food item, and the laboratory phase of determination of pesticide residues (CV_L), which consists of the homogenization of laboratory sample, extraction of test portions and qualitative quantitative determination of extracted residues:

$$CV_R=\sqrt{CV_{S1}^2+CV_L^2} \tag{39}$$

The CV_{Pf} includes the variability of industrial or home processing, and analysis of processed product. Note, that it does not include the sampling and analysis of the raw product which is done from a portion taken from the bulk material before processing (CV_R). If the processed product is well

mixed (such as refined oil or milk) the uncertainty of sampling of processed product can be considered negligible compared to the other influencing factors and assumed to be zero. On the other hand, if the processed product is solid it cannot be considered to be well mixed and an additional sampling uncertainty (CV_{S2}), which is usually smaller than the sampling of raw products, shall be accounted for. Consequently, CV_{comb} for processed product should be calculated as:

$$CV_{comb} = \sqrt{CV_R^2 + CV_{Pf}^2 + CV_{STMR}^2 + CV_{S2}^2} \quad (40)$$

Since the variability deriving from the recipes (CV_{cu}) is different for various components of composite food, it has to be taken into account in the calculation of combined uncertainty of residues being in individual components of composite foods. Taking the above influencing factors into account the combined uncertainty of the residue values in processed products can be calculated as:

$$CV_{comb} = \sqrt{CV_{cu}^2 + CV_{Pf}^2 + CV_{STMR}^2 + CV_{S1}^2 + CV_{S2}^2 + CV_L^2} \quad (41)$$

For unprocessed products CV_{Pf} and CV_{S2} are equal to zero. The sampling uncertainty of cereal grains (CV_{S1}=0.2497) and a typical CV_L of 0.15 for supervised trials were taken into account for calculation. In case of flour, which cannot be thoroughly mixed, CV_{S2} is about 0.11. The CV_{Pf} for flour is 0.449, the CV_{cu} is 0.2424. Based on these input data CV_{comb} for white flour:

$$CV_{comb}=(0.082^2+0.449^2+0.2497^2+0.15^2+0.11^2+0.2424^2)^{½}=0.603$$

For obtaining the uncertainty of the residue concentration, first we have to calculate the standard deviation of the sum of residues from the pooled variances of individual residue measurements contributed to the sum of residues ($SD_{Ri}=CV_{comb}\times Ri$; $SD^2=VAR$). The corresponding relative uncertainty of bifenthrin residue (CV_{res}) is 0.4487.

vi. For filled pancake the standard deviations of residues in one empty pancakes (0.0296/16=0.0019 mg) and in strawberry marmalade (0.0009 mg) have to be pooled to obtain 0.0021 mg. Note, since the degrees of freedoms of the two standard deviations are not know, as a first approximation, we assume that they are the same and the pooled SD can be calculated with equation 18. From the pooled SD and the sum of residues (0.0067 mg) we obtain the relative uncertainty of 0.3072 for the 0.0746 mg/kg residue in one piece of filled pancake.

Details are given in Table 5. The principle of calculation of relative uncertainty is the same for other food items and has to be done for each food item separately.

Table 5. Calculation of bifenthrin residue concentration and its uncertainty in pancake filled with strawberry marmalade*

Ingredients	Mass [kg]	STMR or STMR-P [mg/kg]	Contributors to combined uncertainty of residue						CV_{comb}	Bifenthrin			
			CV_{STMR}	CV_{Pf}	CV_{cu}	CV_{S1}	CV_{S2}	CV_L		mg[a]	SD mg	mg/kg[b]	CV_{res}[c]
Eggs	0.14	0								0	0		
Rapeseed oil	0.18	0.08	0	0.23	1.44	0.18	0	0.15	1.48	$1.4x10^{-2}$	$2.1x10^{-2}$		
Milk	0.37	0.05			0.34			0.15	0.37	0.02	0.007		
White flour	0.41	0.08	0.082	0.45	0.24	0.25	0.11	0.15	0.60	$3.2x10^{-2}$	$1.9x10^{-2}$		
M_{Ti}	1.49									$6.6x10^{-2}$♦	$2.9x10^{-2}$♣		
M_T	1.34											$4.9x10^{-2}$	0.05
1 pc empty pancake	0.084									$4.1x10^{-3}$♥	$1.9x10^{-31}$♣		
Strawberry in marmalade	0.0055♠	0.46	0.144			0.27		0.15	0.34	$2.5x10^{-3}$	$8.7x10^{-4}$		
1 pc filled pancake	0.089									$6.7x10^{-3}$	$2.1x10^{-3}$	$7.5x10^{-2}$	0.31

Notes:

* The table shows rounded values, but calculations shown above were made before rounding.

[a]: Calculated from median residues obtained in supervised trials and the mass of ingredients.

[b]: The bifenthrin concentration [mg/kg] is calculated from the sum of residues [mg] and the mass of ready-to-eat (RTE) food.

[c]: Relative uncertainty of residue concentration in RTE.

♦: Sum of residues in raw ingredients.

♣: Pooled standard deviation of residues in raw ingredients.

♥: Residue [mg] in one pancake.

[1]: Standard deviation of residues in one pancake.

♠: Raw strawberry equivalent taking 90.7% fruit in the marmalade.

The daily exposure to bifenthrin is calculated as the sum of the bifenthrin content of food consumed. The combined relative uncertainty of food consumed is calculated from the uncertainty of residues (CV_{res}) and the estimation of the portion of food consumed (CV_{di}).

The daily intakes of bifenthrin residues calculated for the 60kg body mass of the reporting person are 0.0026 mg/kgbw and 0.0028 mg/kgbw for day 1 and day 2, respectively. Assuming that an ordinary bathroom balance was used (±0.5 kg accuracy), the corresponding standard deviation of bodyweight measurement is calculated as:

$$SD=0.5/1.96=0.2551 \text{ kg}$$

with relative uncertainty of:

$$CV_w=0.2551/60=0.0043$$

The combined relative uncertainty of estimated daily residue intake (CV_{EDI}) of the 1st day is calculated with the following equation.

$$CV_{EDI} = \sqrt{CV_{total}^2 + CV_w^2}=(0{,}30042^2+0.0043^2)^{½}= 0.30 \qquad (42)$$

The combined relative uncertainty of estimated daily residue intake of the 2nd day is 0.28. If a precision balance (±0.1 kg accuracy) was used, what is usually applied in dietary surveys, the CV_{EDI} would not change practically, indicating that applying a precision balance would not improve the uncertainty estimate of the daily dietary exposure and their use is not necessary. The expanded combined uncertainties ($U=2\times u$) of the calculated daily exposures to bifenthrin and the upper boundary of the exposure are summarised in Table 6.

Table 6. Daily exposure of the reporting person to bifenthrin*

Day	Bifenthrin[1] (mg)	u[2] (mg)	U[3] (mg)	95%UCL	EDI (mg/kgbw)	95% UCL of EDI (mg/kgbw)
1	0.154	0.0462	0.0925	0.246	$2.57\text{x}10^{-3}$	$4.11\text{x}10^{-3}$
2	0.168	0.0475	0.0950	0.263	$2.81\text{x}10^{-3}$	$4.39\text{x}10^{-3}$

Notes:
* The table shows rounded values, but calculations were made before rounding.
[1]: Bifenthrin residue (mg) in daily food.
[2]: Standard uncertainty.
[3]: Expanded uncertainty.
95% UCL:
Upper 95% confidence limit.

If the calculated combined uncertainty is 30% for day 1 and 28% for day 2, the calculated bifenthrin exposure with extended uncertainty ($U = 2 \times u$) for the 95% confidence interval can be reported for the 1st day: $2.57\text{x}10^{-3}\pm0.77\text{x}10^{-3}$ mg/kgbw, and for the 2nd day: $2.81\text{x}10^{-3}\pm0.59\text{x}10^{-3}$ mg/kgbw. The upper 95% confidence limits of the daily bifenthrin intakes are about 2.4 and 2.3 times lower than the ADI of 0.01 mg/kgbw.

4.5. Contribution of uncertainty sources to the total variance

The uncertainties of parameters influencing the calculated dietary exposure vary at a large extent depending on the components of food consumed, residue levels and the procedures involved in the preparation of the food. Consequently, typical values cannot be given and the uncertainties have to be evaluated case-by-case.

The results indicate that the major contributors to the combined uncertainty of daily residue intake were berry fruits (47%) and apple juice (18%) on the 1st day, blackberry (44%) and pancake (31%) on the 2nd day. The contribution of the uncertainty of individual steps of the intake to the combined uncertainty depends on the particular food item. For instance, the uncertainty blackberry intake comprises of the estimation of the consumption (CV_{di}=0.89), the sampling (CV_S=0.16) the laboratory phase of determination of pesticide residues (CV_L=0.15) and the STMR data (CV_{STMR}=0.68). The uncertainty of pancake intake comprises of the estimation of the residue (CV_{res}=0.31) and consumption (CV_{di}=0.94). The contribution of the consumed mass (CV_{di}) to the total variance in case of blackberry and pancake is 61% and 90%, respectively. In case of composite foods, like pancake, CV_{cu}, CV_{S1}, CV_L, CV_{Pf}, CV_{S2} and CV_{STMR} of the components are calculated as part of CV_{res} at component level.

The ranges of relative uncertainties of the main influencing factors, based on the currently available information, are as follow: recipes of composite foods (CV_{cu}=22.3-144%); amount of food consumed (CV_{di}=29-98%); number of supervised trials providing the basis for the estimation of the supervised trial median residues (CV_{STMR}=8-90%); processing factors (CV_{Pf}=30-50%); sampling of plant materials (CV_S: fresh fruits: 20-30%; sampling processed solid products ~10%; sub-sampling of large crops: 7-21%); analysis of residues in supervised trials (≤15%). However, due to lack of relevant information, the relative uncertainty of expectable pesticide residue content of food of animal origin or components of composite foods could only partly be or could not be quantified.

4.6. New scientific results

1. The applicability of 5 EPIC-SOFT food picture series used in the context of a Hungarian food consumption survey gathering data for exposure assessment was tested, and the random errors in food portion estimation resulted from the visual perception and conceptualization-memory investigated. I characterized the difference between the actual and the estimated portions based on their weights, because it is more accurate compared to judging the applicability of picture series based on the selection of the right, adjacent or distant pictures. Gaining information on the variability of weight estimates provides a better basis for the estimation of uncertainty of exposure assessment. The applicability of the pictures was evaluated after Winsorisation. Differences obtained through both

methods provide information on the capability of interviewed persons to recall the portion size of food taken on the plate 1-2 hours earlier. When conceptualization-memory is brought into the equation, errors in estimates increased (up to 61%). Results presented in this study should be considered as a best-case scenario, since, the time between eating the food and recalling its quantity is much longer (24-36 hours), which further increases the bias in estimation of portion sizes. The relative standard uncertainty of estimated portion size (CV_{di}) was found between 24-55% in this study.

2. I proved that the real masses of visually identical portions of the foods may differ from the masses of foods presented on the pictures, which have to be considered when calculating the consumed mass based on portion size estimation using food picture booklet.

3. I have identified the relative uncertainty sources affecting the value of consumption data and applied mathematical relations to quantify them. I have found that one of the main sources of combined uncertainty of consumer's exposure to pesticide residues is the estimation of the amount of food consumed by recall and the variability of the composition of recipes.

4. The sources of uncertainties related to food consumption survey data, the calculation of supervised trial median residue (STMR) values and processing were identified and quantified by applying mathematical equations. Based on the established relations, it is possible to determine the contribution of residue concentrations to the combined uncertainty of pesticide exposure.

5. I elaborated a procedure to quantify the uncertainties of input parameters of deterministic model to the combined uncertainty of the estimated exposure by applying mathematical equations and, I demonstrated its practical applicability with a detailed worked example, using the bifenthrin pesticide residue results obtained from supervised residue trials and food consumption data of two days. These basic relations are applicable for both, acute and chronical exposure assessments with deterministic model.

6. I determined that the uncertainties of parameters influencing the calculated dietary exposure vary at a large extent depending on the components of food consumed and residue levels. The ranges of relative uncertainties of the main influencing factors, based on the currently available information, are as follow: recipes of composite foods (CV_{cu}=22.3-144%); amount of food consumed (CV_{di}=29-98%); number of supervised trials providing the basis for the estimation of the supervised trial median residues (CV_{STMR}=8-90%); processing factors (CV_{Pf}=30-50%); sampling of plant materials (CV_S: fresh fruits: 20-30%; sampling processed solid products ~10%; sub-

sampling of large crops: 7-21%); analysis of residues in supervised trials (≤15%).

7. I determined that the relative uncertainty due to body mass measurement does not affect essentially the combined uncertainty of the calculated exposure.

5. CONCLUSIONS AND RECOMENDATIONS

1. Applying a well-selected picture book illustrating various portion sizes increases the accuracy of estimating the amount of food consumed. The picture series showing different quantities of food should be adjusted to the consumption patterns of the age group involved in the dietary survey, both in terms of content and illustrated portion sizes.

2. The extent of picture books is limited, therefore preferably such food items should be presented:
   a. which are often consumed by people involved in the survey and their amounts cannot be estimated applying generally available household measures (tablespoons, coffee spoon, cup, coffee cup, etc.);
   b. based on which other foods of similar appearance can be estimated.

3. The weights assigned to the portions illustrated in the EPIC-SOFT picture book apply only to the food presented. The actual weight of foods with visually identical volume, prepared of different raw materials using different recipes may be different.
   In order to promote the most accurate determination of the mass of consumed food, it is appropriate to assign the corresponding masses (g) of different foods belonging to the same picture series.

4. Only a small portion of the persons participating in food consumption surveys have detailed information on the composition of the food consumed. The interviewer should therefore have detailed knowledge of the composition and components of the usually consumed foods, so as to help clarifying the composition of the food consumed asking supporting questions.

5. The errors of portion size estimation grow with time, already after 1-2 hours of delay. Results presented in this study should be considered as a best-case scenario, since, the time between eating the food and recalling its quantity is much longer (24-36 hours) during the surveys, which further increases the bias in estimation of portion sizes.

6. Pesticide exposure calculated from the results of supervised trials is considerably higher than expected for the intended use of the pesticide

given that the trials aim to determine the maximum residue level that is recommended when applying the proposed plant protection technology. However, in practice, not all cultivated areas are treated or not the maximum permitted dose of a given pesticide and the shortest pre-harvest intervals are applied. In cases where exposure calculated on the basis of the results of supervised trials is near at the acceptable daily intake level, it is advisable to complement the results of random monitoring by targeted sampling, to determine realistic consumer exposure and to take appropriate risk management measures.

7. Sources of uncertainties of the calculated pesticide exposure can be divided into two groups. First group includes uncertainties associated with incomplete information such as components and their ratio in ready-to-eat foods, ingredients of composite foods available at the market, residue concentration in the edible part of the product (e.g. meat of banana, cleaned carrot or potato) compared to the concentration in the product analysed, etc. The uncertainties arising from such and similar sources can be reduced by targeted data collection. However, uncertainties of sampling, sample processing or analytical measurements due to the natural variability of pesticide residues practically can't be reduced by applying current instrumentation and requirements of good analytical practice. Minimizing these uncertainties would requires significant cost increase, without resulting in a substantial reduction of the combined uncertainty of the calculated exposure.

8. The basic relations applied for the quantification of identified sources of uncertainty and the mathematical equations are applicable for both, acute and chronic exposure assessments with deterministic model.
   This relatively simple procedure can be used in routine deterministic risk assessment. The future development of an application, such as an Excel Macro supporting the complex calculations, requires further research, and significant data collection. For the most appropriate replacement of the missing information relevant expert judgement is essential.

6. RELATED PUBLICATIONS

Publications in refereed international journals with IF

SZENCZI-CSEH, J., HORVÁTH, ZS., AMBRUS, Á. (2017): Validation of a Food Quantification Picture Book and Portion Sizes Estimation Applying Perception and Memory Methods. *International Journal of Food Sciences and Nutrition*. DOI: 10.1080/09637486.2017.1309521.
IF: 1.504.

AMBRUS, Á., **SZENCZI-CSEH, J**. (2017): Principles of estimation of combined uncertainty of dietary exposure to pesticide residues. *EC Nutrition* 7.5: 228-251.
IF: 1.337.

SZENCZI-CSEH, J., AMBRUS, Á. (2017): Uncertainty of exposure assessment of consumers to pesticide residues derived from food consumed. *Journal of Environment Science and Health, Part B.* DOI: 10.1080/03601234.2017.1331671.
IF: 1.31.

Publications in other refereed international journals

AMBRUS, Á., HORVÁTH, ZS., FARKAS, ZS., DOROGHÁZI, E., **CSEH, J.**, PETROVA, S., DIMITROV, P., DULEVA, V., RANGELOVA, L., CHIKOVA-ISCENER, E., OVASKAINEN, M-L., PAKKALA, H., HEINEMEYER, G., LINDTNER, O., SCHWETER, A., NASKA, A., SEKUŁA, W., GUIOMAR, S., LOPES, C., TORRES, D. (2013) Pilot study in the view of a Pan-European dietary survey - adolescents, adults and elderly, *EFSA Supp. Publ.* EN–508. Vol 10: 1–104. p.

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AMBRUS, Á., HORVÁTH, ZS., **CSEH, J.**, SZEITZNÉ SZABÓ, M. Principles of planning risk-based monitoring programmes. *European Pesticide Residue Workshop*. Vienna, 2012.