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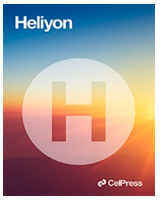

Research article

Assessing malathion residue impact on poultry health, human safety, and production performance

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ABSTRACT

Over the past decade, food safety has become a major concern due to the intensive use of pesticides. Pesticide contamination has been observed in poultry products when seeds are coated with pesticides or when stored products are exposed to pesticides in warehouses. In this experiment, the residue levels of malathion transferred from corn grain to the different parts of the chicken product, its transfer factors (TFs) and the human dietary risk for consumers were evaluated. Growth performance and carcass parameters of the chicken samples were also determined after different doses of malathion exposure. Malathion residues from different parts of chicken meat (breast, thigh, wing, liver and skin) were extracted by the QuEChERS method and analyzed by liquid chromatography-mass spectrophotometry (LC-MS/MS). A deterministic approach was used to calculate the acute and chronic risk assessment. Body weight, feed conversion ratio and feed intake decreased with increasing malathion dose. In addition to reduced feed intake, cold carcass and liver weights of the chicks were also decreased. The highest residues were found in the skin of the chicken followed by the breast, thigh, wing and liver. The TFs of malathion varied between 0.00 and 0.05 according to the different doses applied (4 mg/kg, 8 mg/kg, 16 mg/kg, 32 mg/kg). The chronic exposure assessment (HQ) showed that consumers of all ages and genders consumed 0.008–0.604% of the acceptable daily intake (0.3 mg/kg body weight (bw)/day) of malathion from chicken products. The acute intake assessment (aHQ) of consumers ranged from 0.00015 to 0.0135% of the acute reference dose (0.3 mg/kg bw). In conclusion the results suggest that the risk associated with the malathion residues in chicken meat was found to be low but the residue levels in meat should not be ignored.

1. Introduction

Corn is a versatile and essential crop, with a wide range of uses beyond human consumption. In addition to its culinary applications, corn serves as a crucial component of animal feed, providing a rich source of energy and nutrients. Moreover, the yield obtained is also very high, which means that more digestible energy is produced per unit area than with other cereals. So, the seed is vital with regard to food balance to healthy life for herbivorous animals whose feeds have got only plants parts such as fruits, leaves or grain. It contributes between 15% and 65% to the diet of farm animals in general, but this percentage can increase up to 75% in poultry diets [1].

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Corn seeds with these properties can be considered indispensable for poultry production. Broiler starter diets rely heavily on corn as it provides a majority of their metabolisable energy (65%) and a significant portion of their protein (20%). Livestock producers often choose corn because it is believed to offer consistent and high nutritional qualities [2]. So, corn is more important than other cereals in broilers starter diet.

As one of the main food sources for poultry, corn is exposed to various diseases and pests during growth and storage periods. This situation negatively affects the quality of the seed and reduces the nutritional value. It is therefore very important to prevent yield and quality losses during these periods and various methods are used. Pesticide usage is the most common method to control diseases and pests that cause the losses [3–5]. Pesticide includes a wide variety of insecticides, herbicides, fungicides and other biocides. Though their application is based on selective toxicity such as they cause acute and chronic effects on humans; acute health effects include dizziness, rashes and nausea while chronic health effects could take the form of various asthma, cancer or diabetes [6,7].

One of the pesticides applied to corn is malathion which is one of the commonly used organophosphorous pesticide applied against warehouse pests throughout storage. The history of malathion dates back to the Second World War in the 1940's [8]. It belongs to the group of organophosphorus insecticides; even small doses can cause cumulative toxic effects with continuous exposure [9]. The long-term cumulative toxic effect leads to decrease in acetylcholinesterase enzyme activity, nervous and respiratory disorders [10], enzyme disorders in the liver and various diseases leading to cancer [11]. It is commonly used to protect grains from insects. It is used for empty warehouse disinfection especially to protect grains from *Sitotgraga cereallella*, *Sitophilus oryzae*, *Sitophilus zeamais* (Coleoptera: Curculionidae) which are the main pests of maize also *Ephestia elutella* (Lepidoptera:Pyralidae), *Lasioderma serricorne* (Coleoptera:Anobiidae), *Ephestia cautella* (Lepidoptera:Pyralidae), *Plodia interpunctella* (Lepidoptera:Pyralidae), *Carpophilus* spp., (Coleoptera:Nitidulidae) *Oryzaphilus surinamensis* (Coleoptera: Silvanidae) which are the main pests of tobacco and dried fruits. Although it loses its effect in a short time (2–18 days), frequent spraying can be used to protect the product from warehouse pests in intensive winter conditions [12]. Maize seeds can be contaminated with malathion in warehouse conditions or malathion coated corn seeds can be fed to chickens without waiting for the half-life or degradation of the pesticide and the products obtained (breast, thigh, wing and chicken liver, etc.) can be put on the market. Maxsimum residue limits (MRLs) are set to protect human health from pesticide residues. The MRL is the maximum concentration of the pesticide that may be present in the product and the MRL value of malathion in chicken meat is 0.02 mg/kg. There are some studies on residues of pesticide in poultry products after consumption of contaminated feed [7,13–18]. There are also many methods for calculating risk assessment [6,7,17–20]. To calculate the potential health risks to humans, acceptable daily intake (ADI) and average residue levels in the product are used. The ADI of a pesticide is the amount that can be safely consumed daily over a lifetime, expressed in milligrams per kilogram of body weight [21]. The Hazard Quotient (HQ) was defined as the "The ratio of the potential exposure to a substance and the level at which no adverse effects are expected (calculated as the exposure divided by the appropriate chronic or acute value)" [22]. The long-term risk assessments of the intakes compared to the pesticide toxicological data were performed by calculating the hazard quotient (cHQ), by dividing the estimated daily intake with the relevant acceptable daily intake (ADI) [23]. Estimated daily intake (EDI) is refined as edible portion of each food [24]. The estimated daily intake (EDI) was calculated by multiplying the residue data by the poultry meat consumption (g/day) and then dividing this value by the body weight (kg) of the male or female depending on the age group. The acceptable daily intake (ADI) estimates the amount of a specific chemical (like food additives, pesticide residues, or even medicine) that's generally safe to consume daily throughout your life, without major health risks. To calculate the cHQ value, long-term exposure to pesticide residue is assessed. If the cHQ value is <1, it means that consumption of the food containing the measured level of pesticide residues is not associated with a

Table 1
Ingredients and chemical composition of the experimental diets.

Ingredients (%)	Starter diet	Finisher diet
Corn	55	60
Fish meal	3.5	0
Soybean meal	34.9	32.4
Vegetable oil	3.8	4.8
Dicalcium phosphate	0.65	0.65
Calcium carbonate	1.3	1.3
Salt	0.3	0.3
DL- Methionine	0.2	0.2
Vitamin Premix[a]	0.25	0.25
Mineral Premix[2]	0.1	0.1
Chemical Composition (%)		
Dry Matter	87.5	87.5
Ash	5.9	5.1
Crude protein	21.9	19.2
Ether extract	6.3	7.3
Crude fiber	3.7	3.6
Metabolic Energy (kcal/kg)	3001.4	3103.2

[a] Each 2.5 kg of vitamin premix; 15000000 IU Vit. A, 3000000 IU Vit. D_3, 50000 mg Vit. E, 5000 mg Vit. K_3, 3000 mg Vit. B1, 6000 mg Vit. B2, 5000 mg Vit. B6, 30 mg Vit. B12, 50000 mg Vit. C contains 25000 mg Niacin, 12000 mg Cal.D-Pantothenate, 75 mg D-Biotin, 1000 mg Folic Acid. 2 Each 1 kg mineral premix; 80000 mg Mn, 60000 mg Fe, 60000 mg Zn, 5000 mg Cu, 1000 mg I, 200 mg Co, 150 mg Se, 200000 mg Choline Cloride 60%.

health risk. aHQ or acute short term health risk to the consumer is calculated by ESTI (estimated short term intake) and ARfD (acute reference dose) values. ARfD is an estimate of oral exposure to a pesticide over a short period of time. If aHQ <1, it means that the risk is acceptable [7,25,26]. ESTI was calculated by multiplying the highest level of residue with daily food consumption divided by the body weight. The highest residue concentration was obtained from the chicken feeding experiment. Meat consumption was calculated by taking in to account gender and age. By quantifying exposure and toxicity, risk assessment equations demonstrate the potential severity of harm from pesticides. Excessive pesticide use can contribute to environmental damage and potential health concerns. Sustainable agricultural practices, including organic farming, can benefit both human health and the environment.

Overall, the study aims to comprehensively investigate the impact of malathion application in chicken feed on both the poultry and subsequent human consumers. By evaluating its effects on chicken performance, residue transfer, and associated health risks, the study seeks to contribute valuable information for regulatory decision-making and the establishment of safer practices in pesticide usage in the context of food production.

2. Material and methods

2.1. Birds, diets and experimental design

A total of seventy-five one-day-old Ross 308 broiler chicks were weighed and each chick was randomly placed in an individual pen. The chicks were fed a basal diet based on corn-soybean meal (Table 1) and balanced to meet the nutrient requirements of broilers for the starter (1–21 days) and finisher (21–42 days) periods [27]. Corn seeds that were used in the experiment produced organically. It was also checked that there was no pesticide in the starter diet. For the first 21 days of the experiment, all chicks were fed only the starter diet (malathion-free). On day 21, the chicks were weighed and randomly divided into 5 groups of 15 chicks each. Finisher diets were fed to the chicks between 21 and 42 days. Five treatment groups received the following diets: control diet, 4 mg/kg malathion diet (M4), 8 mg/kg (M8), 16 mg/kg (M16) and 32 mg/kg (M32) for 21–42 days. Malathion (Nivathion 25 WP) was applied to the corn grains used in the ration according to the determined doses and these corns were used to prepare experimental diets. The dosage was determined according to the pesticide's active ingredient percentage and the required amount of pesticide was weighed into the feed and mixed immediately before the experiment. The pesticide was thoroughly homogenized into the food and distributed evenly. The temperature of the rearing house was set at 32 °C for the first three days, then decreased by 3 °C each week to 21 °C, where it remained until the end of the experiment. The lighting schedule was 24 h per day for the first three days, and then decreased to 23 h per day afterwards until the experiment was over. During the experiment, feed and water were given ad-libitum to animals. The chemical composition of the diets shown in Table 1 was determined based on the methods of the Association of Official Analytical Chemists [28]. The body weight and feed intake were measured weekly for each chick. Body weight gain was determined by taking the difference in body weight between weeks. Feed conversion ratio (FCR) was calculated by dividing feed intake by the body weight gain. All chicks were weighed and slaughtered at the end of the 42-d study. Warm carcass weights were determined after removal of the head and internal organs. After warm carcasses being kept +4 °C for 24 h, cold carcass weights were determined. Carcass yield was calculated as the ratio of carcass weight to final body weight.

2.2. Analytical methods for malathion residues

2.2.1. Sample preparation and analysis

Extraction procedures for chicken samples (liver, leather, thigh, breast, and wing) were performed according to the QuEChERS method [29,30]. Each sample was homogenized by grinding. 8 g of homogenized meat samples and 10 ml of acetonitrile:citric acid (99:1 vv:ratio) were put in to a 50 ml Teflon centrifuge tube. The solution was shaken for 30 s with ultra-turrax, IKA, Staufen, Germany. The solution was centrifuged at 4000 rpm for 5 min. The supernatant of the extraction prepared from meat samples was then passed through SPE columns. The homogenized sample was mixed with sodyum sulphate (15 g) and shaken in a flask containing a mixture of cyclohexane:ethyl acetate mixture (1:1 v/v). Sodyum sulphate (10–20g) was added to the extracts and the sample was concentrated to 2 ml using a rotary evaporator. A glass column was filled with glass wool and 10 g Florisil with 5 g sodium sulphate. The column was flushed with a mixture of cyclohexane and ethyl acetate mixture (10 ml). The extract was eluted with 40 ml of cyclohexane:ethyl acetate. A rotary evaporator was used to elute the solution for 2 ml in cyclohexane: acetate mixture (9:1 v/v) and the upper layer of the samples was filtered through 0,25-μm PTFE filter and stored at −20 °C in the deep freezer until analysis.

2.2.2. Analysis

European Commission's analytical method validation criteria SANTE-11312/2021 was performed for the detection of pesticide residues [31]. Pesticides were analyzed using Shimadzu 8040 LC-MS/MS. LC flowrate was 0.4 ml/min, injection volume was 20 μl. Eluent A consisted of water, eluent B of methanol, both eluents 5 mM ammonium formate. Chromatographic separation was achieved using gradient elution with Inertsil column ODS-4 (50 cm × 2.1 mm x 3 μl) at 40 C. The gradient elution programme was as follows: 0-0,01 min 5% B, 0.01–3.50 min 95% B, 3.51–5.50 min 95% B and 5.51–8.00 5% B.

For the quality control and method validation section Limit of Quantification (LOQ) and Limit of Detection (LOD) values of malathion were detected. LOQ is the minimum concentration of the analyte that can be quantified with acceptable accuracy and precision [29,32]). LOD means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods [32]. LOD of malathion was detected 3.04 ng/g and LOQ was detected 10.13 ng/g.

Statistical analysis: For all data set, to confirm the normality of data distribution and homoscedasticity before variance analysis,

Shapiro -Wilk normality test and Durbin-Watson statistic were used. One way analysis of variance (ANOVA) according to the General Linear Model (GLM) procedure was performed to evaluate the difference between treatments in the SPSS 18 package programme. Differences between means were determined using the LSD multiple comparison test. Statistical differences between the means were determined at the $P < 0.05$ level.

2.2.3. Extrapolating pesticide risk assessment

Exposure assessment is crucial to determine the dietary risk of malathion present in poultry meat. The quantification model is easy to understand, popular among experts around the world and accepted by regulatory agencies [33]. Dietary risk assessment uses two types of data to estimate the amount of pesticide that people are exposed to. One of them is meat consumption and the other one is pesticide residue data. There are chronic (long-term) and acute (short-term) exposure models to calculate the dietary risk. In chronic dietary exposure assessment, the median residue concentration in the tissues of the target species is usually used to estimate the exposure. The estimated daily intake (EDI) was calculated by multiplying the residue data by the poultry meat consumption (g/day) and then dividing this value by the body weight (kg) of the male or female depending on the age group. The hazard quantification value was assessed by comparing the EDI with the acceptable daily intake value (ADI). The ADI value for malathion (0,3 mg/kg body weight) was based on a 2004 JMPR report for malathion [34]. In the current study, the average daily per capita consumption of chicken meat was used to calculate the EDI value as follows;

$$EDI = (Poultry\ meat\ consumption\ x\ Average\ residue\ in\ poultry\ matrixes) / Average\ body\ weight$$

2.2.4. The hazard quotient (HQ) was calculated by dividing EDI value by ADI value as following

$$cHQ = (EDI\ /ADI)\ x\ 100\%$$

The acute intake assessment is calculated using the estimated short-term intake (ESTI), which is the highest daily consumption estimate multiplied by the highest residue concentration obtained from the chicken feeding study [35]. The hazard quotient (aHQ) is then calculated as follows:

$$aHQ = \left(\frac{ESTI}{ARfD}\right) x\ 100\%$$

3. Results and discussion

The broiler performance data of the malathion treated corn fed chickens and the control group are shown in Table 2. At the beginning of the experiment, the body weights of the animals were not found significantly different, indicates that the experiment was started homogeneously. At the end of the experiment, the highest body weight was observed in the control group. However, as the malathion concentration increased, there was a significant decrease in the body weight of the animals. Thus, it was found that body weight decreased significantly with increasing malathion concentration. In parallel with the body weights, there was a decrease in the body weight gain in the malathion groups, but significant body weight loss occurred in the 16 mg/kg and 32 mg/kg groups.

There were significant differences in feed intake between the control and different treatment groups (with increasing malathion concentration) from three weeks to six weeks (end of experiment) (Table 2). Feed intake of the groups decreased with increasing malathion concentration, similar to body weight and body weight gain. The maximum feed intake was obtained from the control group (1997.59 g). In particular, the data from the group treated at 16 mg/kg concentration showed a significant decrease (1538.32 g). This group was also statistically different from the others. In poultry species, increases in growth rate have been maintained throughout the life cycle by adjustments in feed intake and energy expenditure [36]. Disturbances in the balance between feed intake and energy expenditure have been reported to result in severe growth disorders [37,38]. In the light of the information on feed intake obtained from the study, it can be said that increasing doses of malathion (especially high doses) have a negative effect on the development of poultry.

While there was no statistically significant difference in feed conversion ratios between groups, the best ratio was observed in the control group and the worst ratios were observed in the 16 mg/kg and 32 mg/kg groups. When the mortality rate (not shown in the

Table 2
Effects of malathion on broiler performance[a].

Groups	Body Weight		Body Weight Gain	Feed Intake	Feed Conversion Ratio
	0–21 days	21–42 days	21–42 days	21–42 days	21–42 days
Control	684.29 ± 47.60	1957.62 ± 156.68^{a}	1273 ± 129.63^{a}	1997.59 ± 237.07^{a}	1.58 ± 0.20
4 mg/kg	684.22 ± 71.38	1483.00 ± 178.83^{b}	808.16 ± 121.74^{b}	1989.01 ± 152.99^{a}	2.51 ± 0.44
8 mg/kg	684.00 ± 54.59	1244.19 ± 131.63^{c}	560.19 ± 102.21^{c}	1805.85 ± 249.51^{ab}	3.38 ± 1.05
16 mg/kg	684.08 ± 53.11	670.46 ± 54.92^{d}	−26.45 ± 39.26^{d}	1538.32 ± 349.38^{c}	37.36 ± 146.09
32 mg/kg	684.21 ± 49.56	624.23 ± 52.62^{d}	−77.03 ± 22.14^{d}	1627.03 ± 208.54^{b}	−22.30 ± 5.80
P value	0.992	0.0001	0.0001	0.005	0.608

[a] Mean ± standard deviation. The difference between the means carrying different letters in the same column is statistically significant ($p < 0.05$).

table) was 0% in the control group, it was 20%, 25%, 40% and 60% in the 4 mg/kg, 8 mg/kg, 16 mg/kg and 32 mg/kg groups, respectively. When the information on feed intake and feed conversion is evaluated together, it can be said that increasing doses of malathion both reduced feed consumption and prevented feed conversion. As a result, body weight and body weight gain were also reduced. Even high doses resulted in high mortality rates. High mortality is also an indication that the high dose (32 mg/kg) has serious effects.

Slaughter and carcass parameters and liver weights of chickens fed different doses of malathion are shown in Table 3. The highest cold carcass weight was observed in the control group. In parallel with body weights, cold carcass weights decreased with increasing malathion dose. There was no statistically significant difference in liver weights between the control group and the 4 mg/kg group, while malathion levels increased in the other groups. In parallel with the experiment results [39] mentioned that malathion contamination in feed decreased the weight of broilers, total protein, and globulin. Additionally [40], indicated that thiram that is a dimethyldithiocarbamate pesticide had an adverse effect on bones and platelets of the chicken. Moreover, the experiment finding was confirmed by reduced weight gains of caged white Leghorn pullets by consuming malathion and carbaryl in the feed [41].

The chicken products from the control group did not contain any malathion residues. Malathion accumulated mainly in the skin of the chickens. In particular, at a dose of 16 mg/kg, 2185.968 μg/kg of malathion accumulated in the skin. As shown in Table 4 malathion residues in the thigh ranged from 108.34 to 485.11 μg/kg. Malathion residues in thigh and breast show a linear dose-response relationship, meaning that the effects are directly proportional to the residue level, which is consistent with the findings of [13,17]. Cyromazine residues increased in association with increased dose. Apparent linear dose response effects were detected between cyromazine residues in chicken products [17]. It was also mentioned that there was an increase in cyromazine residues in eggs as apparent dose-response relationship [13]. Chicken diets containing between 4 mg/kg and 32 mg/kg malathion resulted in malathion residue levels ranging from 82.17 to 1065 μg/kg for breast samples and 103.92–680 μg/kg for wing samples. The median levels of malathion in meat were 137 μg/kg at 4 mg/kg dose, 242 μg/kg at 8 mg/kg and 784 and 648 μg/kg at 16 and 32 mg/kg respectively. Malathion residues were highest in the skin of the chicken, followed by the breast, thigh, wing and liver, significantly higher cyromazine residues were found in the liver, compared to other parts of the chicken [17,42]. The median values of malathion were higher than the European Union (EU) MRL (0.02 mg/kg) at all doses except in the liver; only at the 4 mg/kg and 8 mg/kg doses did the median values of malathion in the liver exceed the EU MRL. A similar study in the same country showed that the concentration of malathion ranged from 2 to 52 μg/kg in chicken meat and from 10 to 56 μg/kg in chicken liver and that 26.4% of the meat samples and 41.2% of the liver samples exceeded the MRLs for malathion [7].

3.1. Exposure and risk assessment

In the current study, different levels of malathion were added to the diet of chickens for 42 days to assess the potential risks of malathion exposure. In Table 5, the EDI values for males in the age group 3–12 years varied from $2{,}5 \times 10^{-5}$ to $9{,}2 \times 10^{-5}$ mg/kg b.w./day using the average malathion residue from this study. The maximum EDI of 9.2×10^{-5} was lower than the established ADI for malathion (0.3 mg/kg b.w./day), indicating a low dietary exposure to malathion from poultry products. According to the results of the hazard quotient value, it was calculated as $17{,}4 \times 10^{-5}$ for the 4 mg/kg dose and increased to 30.7×10^{-5} for the other doses. For females the EDI increased to 20.2×10^{-5} and then decreased to 16.7×10^{-5} at the 32 mg/kg dose. The HQ value was also reduced from 67.3×10^{-5} to 55.5×10^{-5} at the 32 mg/kg dose. This reduction may be related to the fact that the chickens don't consume diets containing high levels of pesticides. Between 18 and 45 years of age, the EDI value for males increased to 19.2×10^{-5} at 16 mg/kg, as did the HQ value to 64×10^{-5} at 16 mg/kg, then both the EDI and HQ values decreased at 32 mg/kg. In females, the EDI decreased from 181.3×10^{-5} to 31.6×10^{-5} with increasing dose. The HQ value also decreased from 604.4×10^{-5} to 105.5×10^{-5}. When the male was older then 45, the EDI value increased to 16.7×10^{-5}. The HQ value also increased to 5.6×10^{-5} although both the EDI and the HQ value were reduced at a dose of 32 mg/kg. When the female was older then 45, the EDI value was reduced to 13.7×10^{-5} at 16 mg/kg and 11.3×10^{-5} at 32 mg/kg. The HQ value was also increased to 45.8×10^{-5} at 16 mg/kg and decreased to 37.8×10^{-5} at 32 mg/kg (Table 5). As mentioned above, EDI values were found between 2.4×10^{-5} and 181.3×10^{-5}, while in one of the studies EDI values of OPs varied between 108×10^{-5} and 197×10^{-4} [24]. In a study conducted in Jordan, EDI values were varied between 1×10^{-6} mg/kg and 9×10^{-5} mg/kg bw/day for the local chicken meat, 2×10^{-7} mg/kg bw/day and 3×10^{-5} mg/kg bw/day for the imported chicken meat. While the EDI value of the local chicken meat (9×10^{-5} mg/kg bw/day) was found to be higher than that of the imported chicken meat (3×10^{-5} mg/kg bw/day) [7]. The results of the risk assessment were supported by the study conducted according to the existing biomonitoring data on OPs. Malathion diazinon and parathion were found to be safe in line with the current study results, while phorate and dimethoate were found to be hazardous to human health. The median HQ values for children ranged

Table 3
Effects of different malathion doses on carcass parameters and liver weights.

Groups	Final Body Weight	Cold Carcass	Liver Weight
Control	2144.70 ± 229.09^{a}	1590.03 ± 185.92^{a}	41.17 ± 7.96^{a}
4 mg/kg	1711.86 ± 138.73^{b}	1234.67 ± 105.62^{b}	42.69 ± 9.26^{a}
8 mg/kg	1576.53 ± 129.81^{b}	1137.06 ± 97.59^{b}	31.80 ± 9.25^{b}
16 mg/kg	676.09 ± 199.49^{c}	440.78 ± 147.93^{c}	20.14 ± 7.69^{c}
32 mg/kg	573.82 ± 178.87^{c}	418.74 ± 160.42^{c}	16.05 ± 3.55^{c}
P value	0.0001	0.0001	0.0001

Table 4
Amount of residues measured in meat samples (μg/kg).

Groups	Liver	Skin	Thigh	Breast	Wing
Control	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
4 mg/kg	0.70 ± 0.76^{ab}	389.33 ± 42.19^{d}	108.34 ± 11.22^{d}	82.17 ± 4.32^{c}	103.92 ± 4.51^{cd}
8 mg/kg	1.42 ± 1.55^{a}	711.98 ± 188.58^{c}	235.43 ± 68.27^{c}	95.22 ± 18.04^{c}	163.81 ± 13.69^{bc}
16 mg/kg	0.01 ± 0.00^{b}	2185.97 ± 163.74^{a}	424.57 ± 79.50^{b}	630.79 ± 89.10^{b}	680.34 ± 202.31^{a}
32 mg/kg	0.01 ± 0.00^{b}	1411.58 ± 103.91^{b}	485.11 ± 22.10^{a}	1065.64 ± 27.68^{a}	275.82 ± 100.08^{b}
P value	0.012	0.0001	0.0001	0.0001	0.0001

Table 5
Acute risk assessment evaluated by EDI and HQ by age group and gender.

Age Group	Gender	Dosage	Body Weight	Poultry meat consumption (g/day)	EDI	HQ
3–12	Male	4	24.9	9.5	5.2×10^{-5}	0.0174
		8			9.2×10^{-5}	0.0307
		16			2.9×10^{-5}	0.0307
		32			2.5×10^{-5}	0.0307
3–12	Female	4	24.1	6.2	3.5×10^{-5}	0.0117
		8			6.2×10^{-5}	0.0207
		16			20.2×10^{-5}	0.0673
		32			16.7×10^{-5}	0.0555
18–45	Male	4	65	15.9	3.3×10^{-5}	0.0112
		8			5.9×10^{-5}	0.0197
		16			19.2×10^{-5}	0.064
		32			15.8×10^{-5}	0.0528
18–45	Female	4	56	13.9	181.3×10^{-5}	0.6046
		8			102.7×10^{-5}	0.3425
		16			31.6×10^{-5}	0.1055
		32			38.3×10^{-5}	0.1278
>45	Male	4	63	13.4	2.9×10^{-5}	0.01
		8			5.1×10^{-5}	0.017
		16			16.7×10^{-5}	0.056
		32			13.8×10^{-5}	0.046
>45	Female	4	56.1	9.8	2.4×10^{-5}	0.008
		8			4.2×10^{-5}	0.0141
		16			13.7×10^{-5}	0.0458
		32			11.3×10^{-5}	0.0378

from 0.016 to 0.618 and for the general population from 0.008 to 0.206. The results of the HQ values in the young population were close to the results of the adult HQ values in the current study. This reflects the differences in cultural diet or habits [24]. Similarly, in another study, HQ values ranged from 1×10^{-5} and 0.2654 when organophosphorus pesticide residues were assessed for chicken meat. Since all the HQ values were less than 1, it means that the amount of residues was not associated with health risk for adult Jordanians for each pesticide tested [7].

Acute exposures were evaluated to assess the pesticide risks lead to consumers (Table 6). The ESTI values estimated from the highest levels of malathion in chicken meat ranged from 3 10^{-6} to 34 10^{-6} mg/kg b.w./day. The aHQ values from chicken consumption ranged from 0.00015 to 0.0135% for males and females of different age groups. The aHQs of adolescents and adults older than 45 have the same range of aHQ values (2.4–20.2%). The aHQs of adults (18–45 years) were significantly higher than those of adolescents (3–12 years old), whereas [17] found that the aHQs of adolescents were significantly higher than those of adults.

Overall, the acute dietary risks posed by the highest malathion residues in chicken meat were found to be safe for the consumers. Other dietary routes such as fruits, water etc. should be considered to assess the overall dietary exposure. Furthermore, modelling can be used to evaluate the cumulative dietary risk assessment to improve the risk assessment process.

Transfer factors (TFs) from dosing experiments are commonly used to assess the risk of transfer of residues from feed to poultry tissues. Transfer factors (TFs) are the ratio of the pesticide concentration in tissues (liver, skin, thigh, breast, and wing) to the pesticide concentration in the diet. TFs were calculated using data from the chicken products used in this study. The results showed that the difference between the transfer factors of different parts depended on the potential accumulation of malathion in different chicken matrices. The TFs varied between the different chicken products and groups, but the overall range was between 0.00 and 0.05. Skin had the highest transfer factors, followed by breast, wing, thigh and liver (Fig. 1). In contrast to the current study the transfer factor of cyromazine in chicken products were found higher in the liver compared to meat and ranged from 0,007 to 0,002 [17]. The way a pesticide is absorbed and metabolized by the chicken also affects how it accumulates in different tissues. For example, some pesticides are absorbed through the digestive system, while others are absorbed through the skin or feathers. Once absorbed, pesticides are metabolized by the chicken's liver and other organs. The metabolites of some pesticides are more toxic than the parent compound, while the metabolites of other pesticides are less toxic. Malathion is a cholinesterase inhibitor and activated by metabolic oxidative desulfuration to the corresponding oxon. It is non systemic insecticide and acaricide with contact, stomach, and respiratory action.

Table 6
Acute risk assessment evaluated by ESTI and ARfD.

Age Group	Gender	Dosage	Body Weight	Poultry meat consumption (g/day)	Highest concentration of malathion residues in meat	ESTI	aHQ
3–12	Male	4	24.9	9.5	0	5×10^{-6}	0.00025
		8	24.9	9.5	0	11×10^{-6}	0.00055
		16	24.9	9.5	1	34×10^{-6}	0.0017
		32	24.9	9.5	1	27×10^{-6}	0.0135
3–12	Female	4	24.1	6.2	0	4×10^{-6}	0.0002
		8	24.1	6.2	0	8×10^{-6}	0.0004
		16	24.1	6.2	1	22×10^{-6}	0.0011
		32	24.1	6.2	1	17×10^{-6}	0.00085
18–45	Male	4	65	15.9	0	4×10^{-6}	0.0002
		8	65	15.9	0	7×10^{-6}	0.00035
		16	65	15.9	1	22×10^{-6}	0.0011
		32	65	15.9	1	17×10^{-6}	0.00085
18–45	Female	4	56	13.9	0	3×10^{-6}	0.00015
		8	56	13.9	0	7×10^{-6}	0.00035
		16	56	13.9	1	22×10^{-6}	0.0011
		32	56	13.9	1	17×10^{-6}	0.00085
>45	Male	4	63	13.4	0	3×10^{-6}	0.00015
		8	63	13.4	0	6×10^{-6}	0.0003
		16	63	13.4	1	18×10^{-6}	0.0009
		32	63	13.4	1	14×10^{-6}	0.0007
>45	Female	4	56.1	9.8	0	3×10^{-6}	0.00015
		8	56.1	9.8	0	5×10^{-6}	0.00025
		16	56.1	9.8	1	16×10^{-6}	0.0008
		32	56.1	9.8	1	12×10^{-6}	0.0006

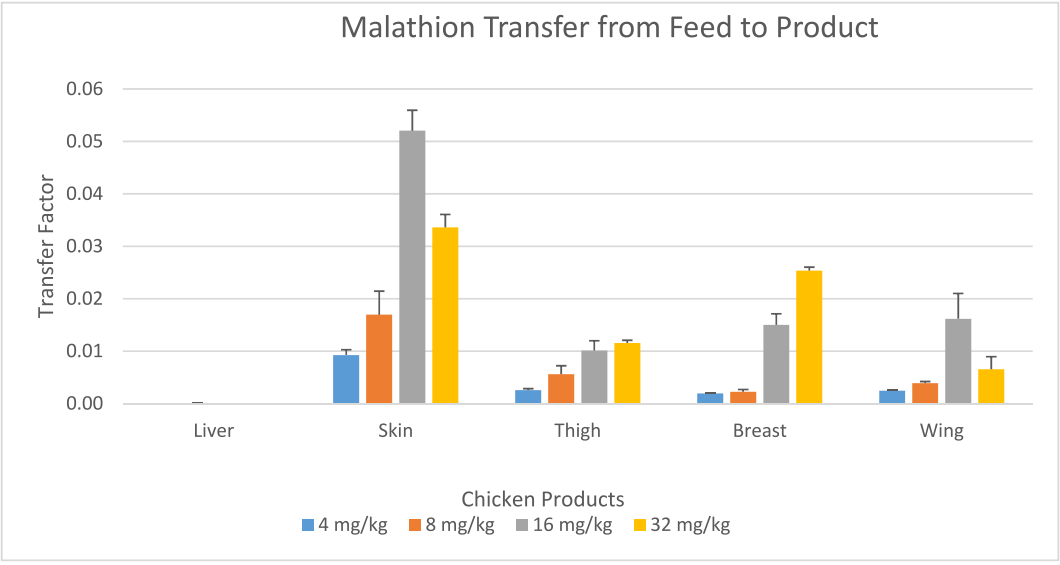

Fig. 1. Transfer factors (TFs) of malathion in different chicken products (P<0.05).

Comprehensive degradation pathways from the parent compound malathion to a variety of experimental observed degradation products. These data corroborate experimental observations that several degradation pathways (ester hydrolysis and elimination) compete and that the final products can therefore be influenced by environmental factors such as temperature. In addition, the products resulting from any of the initial degradation pathways may further degrade under the same conditions to compounds that are also reported to be toxic [43]. In another study it was reported that TFs for all chicken parts (kidney, liver, muscle, fat, egg) ranged from 0.00 to 2.15 Following the feedings rates of 0.031 and 0.103 mg/kg in the diet of chickens for 42 days, it was found that liver had lower residue levels (range of 0.06–0.67) when compared to fipronil residues in egg (range of 0.96–1.16) and in fat (0.64–2.03) [44]. The results of the current study showed that the transfer of malathion from chicken feed to the different parts of the chicken was low compared to fipronil and cyromazine residues.

4. Conclusion

This study provides compelling evidence for a dose-dependent accumulation of malathion residues in chicken products, with skin showing the highest levels and liver demonstrating a remarkable efficiency in the elimination of malathion residues. This differential distribution pattern suggests tissue-specific affinities, with skin acting as a primary reservoir for malathion accumulation. In particular, increasing doses of malathion adversely affect the growth performance of chickens, as evidenced by reduced body weight gain and impaired feed conversion. However, the potential risks of malathion exposure through poultry consumption, even at high doses, are considered negligible. The current study was limited to only 4 pesticide doses and one pesticide furthermore broiler number and kind of broiler would affect the calculation of risk assessment results. Further research is warranted to elucidate the potential transfer of malathion to egg constituents, as this information is crucial to establish comprehensive risk assessments associated with the consumption of eggs from malathion-treated hens. In addition to minimize the risks to people and the environment, good agricultural practices including integrated pest management and organic farming should be incorporated with informing and educating the farmers.

Data availability

No data were used for the research described in this article.

Ethics statements

All procedures and experiments were approved by the Animal Ethics Committee of Adnan Menderes University (64583101/2015/129).

CRediT authorship contribution statement

Yakup Onur Koca: Resources, Project administration, Data curation. **Ahmet Önder Üstündağ:** Resources, Formal analysis, Data curation. **Melis Yalcin:** Writing – original draft, Data curation. **Cafer Turgut:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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