Residues of Fenitrothion in chick embryos following exposure of fertile eggs to this organophosphorus insecticide

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SUMMARY

The purpose of the study was to investigate whether the broadly applied insecticide fenitrothion was able to penetrate the avian eggshell following exposure to Sumithion 50 EC (50 % fenitrothion) by immersion. Residues were quantified in yolk and embryonic samples, and two widely used routes of exposure (injection and immersion) were compared in terms of residues.

In the first part of the experiment, fertile hen’s eggs (Shaver Starcross 288) were immersed in a 0.33% aqueous solution of Sumithion 50 EC on day 0 or day 12 of incubation, while in the second part of the experiment eggs were injected with 0.1 ml of water/DMSO solution (9:1, v/v) containing 0.15 mg fenitrothion having the same exposure time. Following day 0 exposure, eggs were incubated until day 12, and eggs treated on day 12 were incubated until day 19. For gas chromatographic analysis, eggs exposed to the insecticide on day 0 were sampled on days 1, 3, 6, 9 and 12, while from eggs treated on day 12 samples were taken on days 13, 15 and 19.

We demonstrated that fenitrothion was able to cross the eggshell after external exposure and contaminate the developing embryo with a penetration rate that increased after the mobilisation of Ca from the eggshell. The fenitrothion residues measured in the samples indicated that the embryos were contaminated by the chemical for a longer time after external exposure than after administration of the insecticide by injection. Moreover, the data suggest that fenitrothion appearing at distinct embryonic developmental stages after exposure by immersion and injection might have different toxic effects on the embryo. These findings suggest that external exposure cannot be simulated by the injection method; however, the latter is also a relevant technique for egg treatment in toxicology studies.

KEY-WORDS : fenitrothion - chicken embryo - pesticide residue - organophosphorus insecticide.

RÉSUMÉ

Résidus de Fénitrothion dans des embryons de poulet après exposition des œufs à cet insecticide organophosphoré. Par T. VARGA, J.-P. CRAVEDI, I. FŰZESI et L. VÁRNAGY.

Notre étude a consisté à analyser la pénétration d’un insecticide, le feni- trothion, dans l’œuf d’oiseaux lors d’une exposition (immersion) à un pro- duit phytosanitaire, le Sumithion 50 EC, qui contient 50 % de fenitrothion. Les résidus furent quantifiés dans différents échantillons d’œufs et d’em- bryons. De plus, deux méthodes d’exposition largement utilisées dans ce domaine (injection et immersion) ont été comparées sur la base de leur inci- dence sur les niveaux résiduels.

Dans la première partie de l’expérimentation, des œufs fertiles de poules (Shaver Starcross 288) furent immergés dans une solution aqueuse de Sumithion 50 EC (0,33 %) au jour 0 ou au jour 12 de la période d’incuba- tion. Dans la deuxième partie, 0,1 ml d’une solution eau/DMSO (9:1,v/v) contenant 0,15 mg de fenitrothion fut injecté dans les œufs suivant les mêmes temps d’exposition. Après un traitement à jour 0, les œufs furent incubés jusqu’au jour 12 et les œufs traités à jour 12 furent, quant à eux, incubés jusqu’au jour 19. Pour l’analyse en chromatographie en phase gazeuse, les œufs furent échantillonnés au jour 1, 3, 6, 9, 12 après une expo- sition à jour 0 et au jour 13, 15, 19 après traitement à jour 12.

Nous avons pu montrer que le fenitrothion pouvait traverser la coquille après une exposition par contact et ainsi contaminer l’embryon avec un taux de pénétration croissant après la mobilisation du calcium de la coquille. Les taux de résidus de fenitrothion dans les échantillons démontrent que les embryons restent plus longtemps contaminés par la substance active dans le cas d’une exposition de la coquille que dans le cas d’une injection. De plus, les résultats suggèrent que le fenitrothion, qui apparaît à des périodes de développement embryonnaire différentes après immersion et injection, puisse avoir un impact différent sur l’embryon. Ces observations montrent qu’une exposition externe ne peut pas être modélisée par la méthode d’in- jection bien que cette dernière soit très utilisée dans le traitement d’œufs lors d’études toxicologiques.

MOTS-CLÉS : fenitrothion - embryon de poulet - résidus de pesticide - insecticide organophosphoré.
Pesticide contamination of the environment is a problem of global importance and data on the fate of pesticides in living organisms are useful in evaluating their safety to non-target organisms. Several studies on the direct exposure of avian eggs to environmental contaminants have demonstrated that xenobiotics can cross the shell and its membranes and are subsequently taken up by the embryo [6, 8]. The transfer and distribution of the chemical appear to be dependent on the nature of the vehicle, the lipophilicity of the contaminant and the lipid content of the tissues [3]. The presence of pollutants in the developing avian egg has been shown to result in decreased hatchability and increased neonatal death [5].

Fenitrothion (O,O-dimethyl O-[3-methyl-4-nitrophenyl]-phosphorothioate) is a broad-spectrum organophosphorus insecticide which has been used extensively to control Acrididae, wheat bugs, Blattodea, Coleoptera, etc. Spray application of an insecticide containing a 0.2 % solution of fenitrothion (Owadofos) on fertile eggs of ring-necked pheasants was shown to result in paralysis and a 20 % decrease of the hatching rate [7].

The objective of the present study was to quantify the egg-shell penetration of fenitrothion in chicken eggs immersed in aqueous solutions of commercial fenitrothion (Sumithion 50 EC). Residue analyses were performed at different developmental stages and the results were compared with those obtained in an egg injection study.

Material and methods

Fertile hen’s eggs (Shaver Starcross 288), obtained from Bölly Agricultural Company Ltd. (Hungary), were incubated (starting day of incubation = day 0) in an atmosphere of 55-60 % relative humidity and a temperature of 37.8 °C. The eggs were turned every 8 hours.

Two types of experiments were conducted. In the experiment of the first type, one group of eggs was injected on day 0 and then incubated until day 12, whereas a second group was injected on day 12 and incubated until day 19. Injection was performed into the air space through a small hole previously made in the shell. Immediately after injection, the holes were sealed with paraffin. The injected dose was 0.1 ml of water/DMSO solution (9:1, v/v) containing 0.15 mg fenitrothion (CIL Cluzeau, France).

In the experiment of the second type, eggs were immersed into a 0.33 % emulsion of Sumithion 50 EC (Sumitomo, Japan) containing 50 % fenitrothion, and kept in that emulsion for 30 min. One group was immersed on day 0 while another group was immersed under similar conditions on day 12.

Analysis of fenitrothion residues was performed on eggs sampled on days 1, 3, 6, 9 and 12 for groups treated on day 0 and on eggs sampled on days 13, 15 and 19 for those treated on day 12. Residues were determined in the yolk (days 1 and 3) and in live embryos (days 6, 9, 12 and days 13, 15, 19). The metabolites of fenitrothion were not measured during the study.

The yolk was isolated from the egg, weighed and homogenized with 20 g quartz sand and 20 g anhydrous sodium sulphate. Then it was extracted once with 125 ml and twice with 50 ml acetonitrile. The extracts were filtered and then pooled with 500 ml sodium sulphate solution (25 g/1000 ml deionized water). The mixture was agitated for 2-3 min in a separating funnel with 50 ml chloroform and then allowed to separate (agitation was repeated twice more). The chloroform phase was then collected in a 250 ml round-bottomed flask, evaporated to dryness, and the residue was dissolved in 2 ml n-hexane. This fraction was analyzed by gas chromatography (GC) after clean up by elution with 75 ml hexane/acetone (13:3, v/v) after a first wash with 25 ml n-hexane on a column containing 15 g Florisil (60-100 mesh, Fluka, Switzerland). Florisil was activated by heating at 130 °C overnight. The eluate was evaporated to dryness and dissolved in 1-2 ml n-hexane.

The embryos were excised from the eggs, weighed and homogenized 3 times in 25 ml acetonitrile with an Ultra-Turrax homogenizer (Janke & Kundel, Germany). The homogenate was centrifuged (800 g, 10 min), and the supernatant was treated as indicated above for the yolk extract [2].

For GC analysis, a Packard instrument (model 428) was used with an NPD selective detector. It was packed with 1.5 % SP 2250+1.95 % SP 2401 on GasChrom Q 100-120 mesh, ID coiled Pyrex glass column (1.8 m x 3 mm). The operating conditions were as follow: column-oven temperature 200 °C; injection port temperature 260 °C; detector temperature 260 °C. The flow rate of the carrier gases 20 ml/min for N₂, 5 ml/min for H₂, and 50 ml/min for air. The detection limit (MDQ) was 0.02 ng. The limit of determination (LOD) was < 1 ng/g (above 5 g sample) and < 5 ng/g (below 5 g sample). The recovery rate of the method after spiking with 2 µg fenitrothion to control embryo sample was 96 % as compared to 99 % for untreated eggs.

Results

After injection or immersion on day 0, the egg yolk rather than the embryo was sampled for extraction on days 1 and 3 of incubation (DI). The egg yolk plays an important physiological role in embryonic development, and at these early stages the low weight of the embryo impairs the accuracy of the extraction procedure. Moreover, the yolk was sampled because only a low amount of fenitrothion could be extracted from the albumen after injection on day 0 (data not shown). This is due to the lipophilicity of fenitrothion and to the high lipid content (63 % of the dry weight) of the yolk.

The results concerning exposure to fenitrothion on day 0 of incubation are presented in Table I. The concentration of fenitrothion in the yolk was about 15-fold higher in injected eggs than in those treated by immersion. The limited number of samples (3 eggs per point) resulted in relatively high standard deviation values for fenitrothion residues. Residues of fenitrothion could be detected at DI 6 and then, after DI 9, the detection limit was reached (5 ng/g). On DI 6, fenitrothion residues could not be found in the embryo after immersion treatment (below LOD), but it was still present on DI 9. The residues of fenitrothion was < 5 ng/g on DI 12. This finding supports our hypothesis that fenitrothion can cross the egg-shell.
In the experiment using injection of eggs on day 12 (Table II), a residue level of 111 ng/g fenitrothion was found in the DI 13 embryonic extract. The residue level decreased on the subsequent sampling days, and it was even below the detection limit on DI 15. In the immersion study, fenitrothion was on measurable levels at each sampling day throughout the post-immersion period. From DI 13 until the last sampling day (DI 19), the average active ingredient level gradually decreased from 0.399 µg to 0.1016 µg per embryo.

**Discussion**

The penetration of pesticides through the eggshell after application by immersion or by spraying has been reported for DDT, organophosphates and 2,4-D in different species [1, 11, 12]. Our results obtained in the immersion study suggest that the active ingredient can penetrate the eggshell after both day 0 and day 12 exposures. Indeed, after both immersion applications, fenitrothion was still at a detectable level in the embryonic samples because of its presence on the egg surface and its continuous penetration. The presence of adjuvant in the commercial formulation might explain this last observation.

Furthermore, our results are consistent with the data of other authors [5] concerning the importance of the chorioallantoic membrane in the uptake of active ingredients in contact with the eggshell after immersion. Indeed, after exposure on day 0 by the immersion method, no traces of residues were found in the embryo on DI 6 while detectable amounts of fenitrothion were present on DI 9. This finding suggests that the chorioallantoic membrane, which is already well developed at DI 9 and fully enclosed by DI 12 in chicken embryos, takes up the pesticide and helps to transfer into the embryo. Our findings are confirmed by the results obtained after exposure on day 12 that showed fenitrothion residues at each sampling day. The chorioallantoic membrane should immediately transfer the pesticide from the shell surface to the embryo. In contrast, the detected amount of residues present in the yolk was low when the injection method was used.

The chick embryo has an active mixed-function oxidase system (MFO) what is highly inducible by 3,4,3,4-tetrachlorobiphenyl (TCB) as early as DI 5 and reaches adult levels of induction by DI 7 [4]. As MFO is known to play a major role in fenitrothion biotransformation [9] the disappearance of residues from the eggs could be due to the chick embryonic MFO system. However, other enzymes such as esterases and transferases could be involved in this phenomenon since they are reported to participate to organophosphorus pesticide metabolism in vertebrates [10].

A comparison of the two methods of treatment suggests that the embryo is exposed to the pesticide for a longer time when the immersion method is used. Moreover, depending on the method, the chemical is present at different stages of embryonic development.

Both methods are used in toxicology for multiple reasons. Immersion, i.e. the method that intends to mimic the expo-

### Table I. — Residues of Fenitrothion in yolk (day 1 and 3) or in embryos (day 6, 9 and 12) following treatment of the eggs with this insecticide. Eggs were injected (0.15 mg fenitrothion/egg) or immersed (0.33 % Sumithion 50 EC / 0.165 % fenitrothion) on day 0.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sampling days</th>
<th>1</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection</td>
<td>Sample Weight (g)</td>
<td>20.08 ± 1.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.89 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Residues (ng/g)</td>
<td>333 ± 151</td>
<td>624 ± 148</td>
<td>&lt;5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>Immersion</td>
<td>Sample Weight (g)</td>
<td>21.96 ± 1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.85 ± 3.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.94 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.05 ± 0.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Residues (ng/g)</td>
<td>21 ± 1</td>
<td>41 ± 25</td>
<td>&lt;5</td>
<td>16 ± 7</td>
<td>&lt;5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> yolk ; <sup>b</sup> embryo ; values are the mean ± SD from 3 samples ; <sup>c</sup> embryo samples correspond to 3 pooled embryos
ND: Not Determined ; * conly one sample > 5 ng/g.
sure associated with agricultural practice, allows us to assess the potential hazard posed by chemicals to avian embryos while the injection method is widely used for evaluating the risk of embryotoxicity and teratogenic potency of drugs.

From the point of view of environmental contamination, the injection method does not reflect field exposure because, as we observed, fenitrothion residues did not appear at the same stage of embryonic development as after immersion treatment. This is why the injection method cannot replace the immersion technique in modelling external exposure.

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References


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Table II. — Residues of Fenitrothion in embryos (day 13, 15 and 19) following treatment of the eggs with this insecticide. Eggs were injected (0.15 mg fenitrothion/egg) or immersed (0.33 % Sumithion 50 EC / 0.165 % fenitrothion) on day 12.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sampling days</th>
<th>13</th>
<th>15</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection</td>
<td></td>
<td>Sample</td>
<td>Residues</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weight (g)</td>
<td>(ng/g)</td>
<td></td>
</tr>
<tr>
<td>Injection</td>
<td>4.17 ± 0.60</td>
<td>&lt;5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immersion</td>
<td>4.19 ± 1.64</td>
<td>8.56 ± 0.58</td>
<td>23.46 ± 0.94</td>
<td></td>
</tr>
<tr>
<td>Immersion</td>
<td>111 ± 61</td>
<td>25 ± 8</td>
<td>4 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

values are the mean ± SD from 3 samples
ND: Not Determined