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# Untersuchungen zur Bestimmung der geografischen Herkunft von Rindstrockenfleisch mittels NIR-Spektroskopie*

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Im Rahmen eines Projektes mit dem Ziel, geeignete Methoden zum Nachweis der Fleischauthentizität zu erarbeiten, wurde versucht, sowohl den Verarbeitungsort von Trockenfleisch als auch die Herkunft des dafür eingesetzten Rindfleisches mittels Nahinfrarot-(NIR)Messungen zu bestimmen. Allgemein ist die NIR-Spektroskopie ein schnelles und kostengünstiges Untersuchungsverfahren, das eine geringe Probenaufbereitung verlangt. Es erlaubt, eine grosse Anzahl Proben in relativ kurzer Zeit zu untersuchen. Daher wäre es ein ideales Verfahren für stichprobenartige, routinemässige Untersuchungen im Rahmen einer Überprüfung der korrekten Deklaration beispielsweise von Fleischprodukten, die mit der Herkunftsbezeichnung «Geschützte Geografische Angabe» (z.B. Bündnerfleisch, Walliser Trockenfleisch) versehen sind und damit die in den jeweiligen Pflichtenheften festgelegten Anforderungen an das verwendete Rohmaterial und den Produktionsort erfüllen müssen.

Für die Untersuchungen stand Trockenfleisch zur Verfügung, das aus schweizerischem, brasilianischem, kanadischem, US-amerikanischem und australischem Rindsstotzen in verschiedenen Ländern hergestellt wurde (Tabelle 1). Die Lagerung erfolgte vakuum-eingeschweisst bei 2,5 °C.

*Poster präsentiert an der 118. Jahresversammlung der SGLUC vom 14./15. September 2006

Tabelle 1
**Trockenfleischproben sowie Herkunft des Rohfleisches und Produktionsland**

| *Produktbezeichnung* | *Anzahl Proben* | *Herkunft des Rohfleisches* | *Produktionsland* |
|---|---|---|---|
| Bündnerfleisch | 4 | Brasilien | Schweiz |
| Bündnerfleisch | 4 | Schweiz | Schweiz |
| Bündnerfleisch | 2 | Kanada | Kanada |
| Bündnerfleisch | 2 | USA | USA |
| Walliser Trockenfleisch | 3 | Schweiz | Schweiz |
| Rinderrohschinken | 2 | Brasilien | Österreich |
| Grisciutto | 4 | Australien | Australien |
| Bresaola | 1 | Brasilien | Schweiz |
| Bresaola | 1 | Schweiz | Schweiz |

Aus der Mitte jedes Stückes wurden etwa 100 g Probenmaterial mit einem Büchi Mixer B-400 (Büchi Labortechnik AG, Flawil, CH) mit Keramikmessern homogenisiert, in Petrischalen gefüllt und mit einem Büchi NIRLab N-200 Spektrometer (Büchi Labortechnik AG, Flawil, CH) gemessen. Für jede Probe wurden 3 Messungen mit je 64 Scans in einem Wellenlängenbereich zwischen 10000 $cm^{-1}$ und 4000 $cm^{-1}$ mit einer Auflösung von 4 $cm^{-1}$ aufgenommen, wobei die Glaspetrischale um sich selbst rotierte.

Die Auswertung der NIR-Spektren erfolgte nach einer Datenreduktion (Hauptkomponentenanalyse) mit Linearer Diskriminanzanalyse und Kreuzvalidierung (Jackknife).

Die Ergebnisse (Tabelle 2) zeigen im Durchschnitt eine zu 78 % korrekte Klassifikation aller Proben im Bezug auf die Herkunft des Rohfleisches. Bezüglich des Verarbeitungsortes wurden 96 % der Proben korrekt zugeordnet. Trockenfleisch-

Tabelle 2
**Klassifikationsmatrizen (jackknifed) gruppiert nach Herkunft des Rohfleisches und des Verarbeitungsortes**

| *Herkunft des Rohfleisches* | *Australien* | *Brasilien* | *Schweiz* | *Kanada* | *USA* | *% korrekt* |
|---|---|---|---|---|---|---|
| Australien | 12 | 0 | 0 | 0 | 0 | 100 |
| Brasilien | 0 | 15 | 5 | 1 | 0 | 71 |
| Schweiz | 1 | 7 | 15 | 1 | 0 | 63 |
| Kanada | 0 | 0 | 0 | 6 | 0 | 100 |
| USA | 0 | 0 | 0 | 0 | 6 | 100 |
| Total | 13 | 22 | 20 | 8 | 6 | 78 |
| *Verarbeitungsort* | *Österreich* | *Australien* | *Schweiz* | *Kanada* | *USA* | *% korrekt* |
| Österreich | 6 | 0 | 0 | 0 | 0 | 100 |
| Australien | 0 | 12 | 0 | 0 | 0 | 100 |
| Schweiz | 0 | 1 | 36 | 2 | 0 | 92 |
| Kanada | 0 | 0 | 0 | 6 | 0 | 100 |
| USA | 0 | 0 | 0 | 0 | 6 | 100 |
| Total | 6 | 13 | 36 | 8 | 6 | 96 |

proben aus australischem, kanadischem und US-amerikanischem Rohfleisch konnten zu 100 % korrekt klassifiziert werden, ebenso die Verarbeitungsländer Österreich, Australien, Kanada und USA.

Die Ergebnisse zeigen, dass eine Unterscheidung der Herkünfte mittels NIR-Analysen möglich sein könnte. Besonders die Auswertung in Bezug auf den Herstellungsort des Trockenfleisches gibt Hinweise auf eine gute Unterscheidbarkeit. Um statistisch besser gesicherte Klassifizierungsraten zu erhalten, müssen die Ergebnisse jedoch noch mit einer grösseren Zahl von Proben überprüft werden.


## Kurzzusammenfassung

23 Rindstrockenfleischproben aus verschiedenen Ländern wurden mit NIR-Spektroskopie untersucht, um zu ermitteln, ob die geografische Herkunft des Rohfleisches und der Verarbeitungsort mit dieser Methode bestimmbar sein könnten. Dabei konnten die Rohfleischherkünfte durchschnittlich zu 78 % und die Verarbeitungsorte durchschnittlich zu 96 % korrekt zugeordnet werden. Eine Überprüfung dieser ersten Hinweise mit mehr Proben ist aber notwendig, um die Brauchbarkeit der Methode zu verifizieren.

## Résumé

23 échantillons de viande séchée de bœuf de différents pays ont été analysés par spectroscopie NIR afin de déterminer le potentiel de cette technique pour la détermination de l'origine de la viande ainsi que celle de la méthode de fabrication utilisée. L'application de cette technique a permis d'identifier l'origine de la viande dans 78 % des cas et l'origine de la méthode de fabrication dans 96 % des cas. Cette technique doit encore être testée sur un nombre plus important d'échantillons avant de pouvoir tirer des conclusions sur son applicabilité.

## Short Summary "Investigations on the determination of the geographic origin of dried beef using NIR spectroscopy"

23 dried beef samples originating from different countries were analysed using NIR spectroscopy in order to investigate its potential of determining the geographic origin of the raw meat and the place of processing. Thereby the geographic origin of the raw meat could be determined at an average of 78 % and the place of processing at an average of 96 %. However, a verification of these first indications with a higher amount of samples is necessary to verify the applicability of this method.



## Key words

dried beef, meat, NIR, authenticity, geographic origin



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# Monitoring of DDTs in fishes of the Lake Maggiore*

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*In memory of Michele Ceschi*

## Introduction

Persistent organochlorine pollutants (POPs) such as DDT, 1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane (pp-DDT, Figure 1), are well known for their long half-life in the environment. These substances are cause of growing concern, because many POPs are toxic on biota and are able to bioaccumulate and biomagnify in the food chains of various ecosystems (1, 2). Some ninety countries including Switzer-

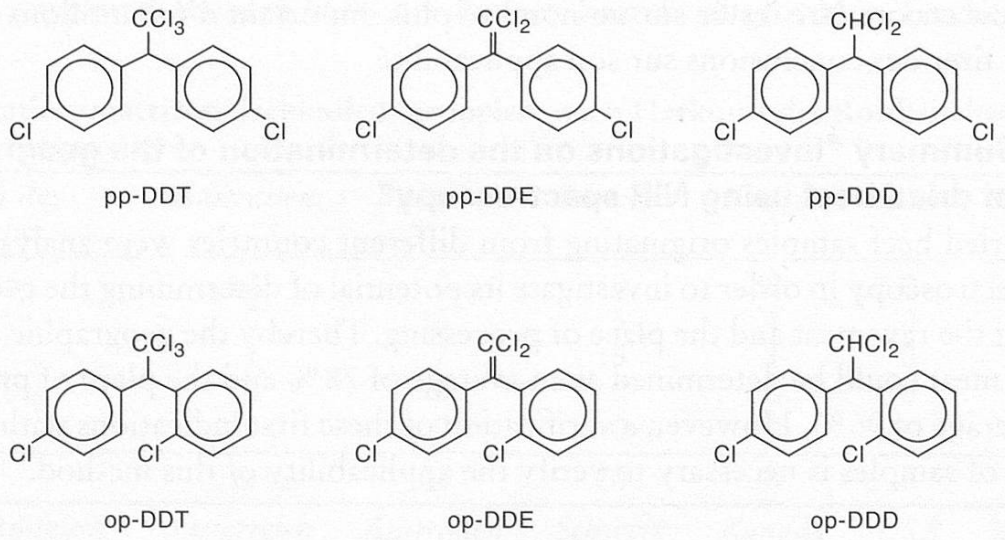


Figure 1 **Structures of DDT and its major homologues and metabolites: pp-DDT, op-DDT, pp-DDE, op-DDE, pp-DDD and op-DDD**

*Poster presented at the 118th annual conference of the Swiss Society of Food and Environmental Chemistry on 14./15. September 2006

land signed in 2001 the Stockholm Convention, which aims to eliminate or reduce the release of persistent organic pollutants in the environment.

In 1996, our laboratory reported very high levels of DDT in different fish species captured in the Swiss part of the Lake Maggiore (3). Subsequent investigations revealed a generalized contamination of the whole lake and in different environmental compartments caused by a chemical plant which discharged DDT over several years into the stream Marmazza, a tributary of the river Toce (Pallanza bay, Italy, Figure 2) (4). As a consequence, the commerce for human consumption of the two most contaminated fish species (twaite shad, Alosa fallax lacustris and char, Salvelinus alpinus) has been banned (5, 6). While for these two fatty species the mean amount of total DDT (sum of DDT homologues and metabolites, $DDT_{TOT}$) turned out to exceed the limit value fixed by the Swiss ordinance on foreign substances and constituents in foods (1 mg/kg edible part), other species revealed a mean contamination below the limit value (7).

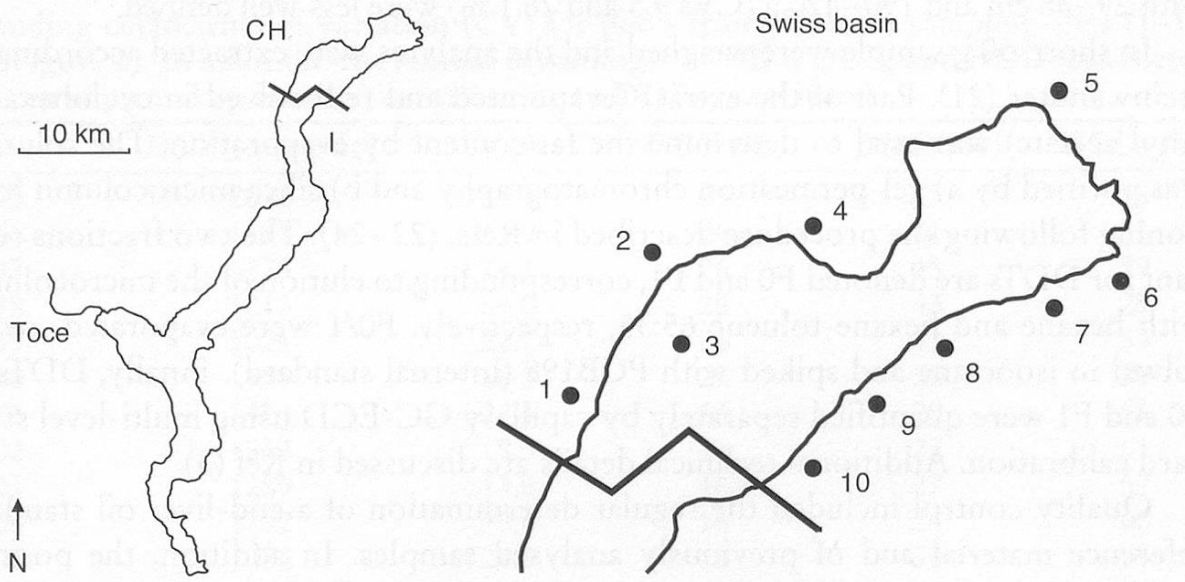


Figure 2 **Sketch of the lake Maggiore and closest locations of origin of the fishes considered in the present work (Swiss basin, see Experimental part): Brissago 1, Ronco sopra Ascona 2, Isole di Brissago 3, Ascona 4, Tenero 5, Magadino 6, Vira Gambarogno 7, San Nazzaro 8, Gerra Gambarogno 9 and Ranzo 10. River Toce, one of the main input sources of DDTs in the lake, is indicated**

Since 1996, we have been monitoring systematically the level of pp-DDT, pp-DDD, pp-DDE, op-DDT, op-DDD, and op-DDE (Figure 1) in two planktonivore fish species: twaite shad (Alosa fallax lacustris) and whitefish (Coregonus macrophtalmus). The present study aims to provide an updated assessment of the heavy DDT contamination in the fishes of the lake Maggiore. For this purpose, we discuss in particular the annual trends observed for the mean concentration of $DDT_{TOT}$ and

the percentage of DDT and its metabolites (DDT%, DDD% and DDE%, calculated as sum of pp and op isomers) contributing to the total amount. The data presented here complement various other studies reporting DDTs in tributary rivers and lacustrine sediments, lake's water, rain, aquatic indicator organisms including fishes (Italian basin), molluscs, and bird eggs (4, 8–18).

## Experimental

All fishes considered in the present work, excepting those forming the samples of 1995, 1996, and 1998 (3, 19, 20), were captured in the pelagic zones of 10 different Swiss locations (Tenero, Magadino, Ascona, Brissago, Vira Gambarogno, Isole di Brissago, Ronco sopra Ascona, Gerra Gambarogno, San Nazzaro, Ranzo, Figure 2) and during weeks 15–20 of every considered year. The edible parts of five middle-size fishes from a single location were homogenized to form one pooled sample. Efforts were taken to provide year-to-year comparable samples with respect to size and age of the fishes considered. This worked best for shads with typical lengths and gross weights of the order of 29–32 cm and 190–225 g (CVs 3.1 and 6.8 %). In contrast, the whitefish samples with 29–38 cm and 190–420 g (CVs 9.5 and 26.1 %) were less well defined.

In short, 50 g sample were weighed and the analytes were extracted according to Steinwandter (21). Part of the extract (evaporated and redissolved in cyclohexane-ethyl acetate) was used to determine the fat content by evaporation. The solution was purified by a) gel-permeation chromatography and b) silica microcolumn fractioning following the procedure described in Refs. (22–24). The two fractions relevant for DDTs are denoted F0 and F1, corresponding to elution of the microcolumn with hexane and hexane-toluene 65:35, respectively. F0/1 were evaporated, redissolved in isooctane and spiked with PCB198 (internal standard). Finally, DDTs in F0 and F1 were quantified separately by capillary GC-ECD using multi-level standard calibration. Additional technical details are discussed in Ref (3).

Quality control included the regular determination of a cod-liver oil standard reference material and of previously analysed samples. In addition, the positive results obtained in a proficiency test of 2006 for the quantification of pp-DDE, pp-DDT, pp-DDD, and other OC pesticide and PCB residues in a cod-liver oil sample demonstrated good selectivity, precision, sensitivity, and accuracy of the method employed. Recoveries of DDTs and limit of quantifications are of the order of 85–110 % and <1 µg/kg, respectively. In general, the proficiency test results obtained for residues of 20–40 µg/kg show suitable standard deviations for repeatabilities and reproducibilities ($s_r$~14 % and $s_R$~26 %).

The sampling strategy for 1995, 1996, and 1998 deviates somewhat from the parameters described above (number of fishes considered, and/or the geographical origin, and/or the week of capture, and/or the average size of the fishes) (3, 19, 20). Without going into further details (additional information are available upon request), it is pointed out that the results obtained for these samples may be regarded as less appropriate in ascertaining the trends discussed below.

## Results and discussion

Tables 1 and 2 summarize the annual monitoring results for shads and whitefishes during the last 11 years (1995–2006). As concentration differences measured for fishes captured in different locations (1–10, see Figure 2) are not systematic, only averaged data are considered. The evolutions of the mean $DDT_{TOT}$ concentration and of the related percentages DDT%, DDD% and DDE% observed in the monitoring period are illustrated in Figure 3. The data for shads (Figure 3a) are selected to guide the following discussion dealing with the annual evolution of DDTs registered in both shads and whitefishes. This choice is motivated by the larger concentrations in shads and the possibility to evaluate year-to-year fairly comparable pooled samples (see Experimental part). The rather general discussion presented below appears to hold also for whitefishes (Figure 3b), although the corresponding results are affected by larger standard deviations which obscure somewhat the same general trends.

Table 1
**Mean annual concentrations of $DDT_{TOT}$ for twaite shad samples along with corresponding coefficients of variation (CV) for pools from different sampling sites (1–10, see Figure 2). In addition, the annual percentages of DDT, DDE, and DDD (calculated as sum of pp and op isomers) contributing to $DDT_{TOT}$ are listed**

| *year* | *$DDT_{TOT}$ (µg/kg)* | *CV (%)* | *DDTs (%)* | *DDEs (%)* | *DDDs (%)* |
|---|---|---|---|---|---|
| 1995 | 2042 | 32.1 | 29 | 33 | 38 |
| 1996 | 1611 | 34.0 | 26 | 41 | 33 |
| 1997 | 1375 | 24.1 | 26 | 45 | 29 |
| 1998 | 1013 | 64.0 | 28 | 43 | 29 |
| 1999 | 1021 | 22.7 | 26 | 41 | 33 |
| 2000 | 959 | 18.5 | 23 | 38 | 39 |
| 2001 | 1404 | 22.1 | 16 | 29 | 56 |
| 2002 | 1862 | 8.5 | 16 | 23 | 61 |
| 2003 | 1289 | 13.6 | 16 | 27 | 57 |
| 2004 | 1792 | 29.0 | 16 | 24 | 60 |
| 2005 | 1074 | 24.5 | 16 | 30 | 54 |
| 2006 | 603 | 18.5 | 12 | 37 | 51 |

Table 2
**Mean annual concentrations of $DDT_{TOT}$ for whitefish samples along with corresponding coefficients of variation (CV) for pools from different sampling sites (1–10, see Figure 2). In addition, the annual percentages of DDT, DDE, and DDD (calculated as sum of pp and op isomers) contributing to $DDT_{TOT}$ are listed**

| *year* | *$DDT_{TOT}$ (µg/kg)* | *CV (%)* | *DDTs (%)* | *DDEs (%)* | *DDDs (%)* |
|---|---|---|---|---|---|
| 1996 | 425 | 27.5 | 26 | 43 | 32 |
| 1997 | 282 | 15.7 | 25 | 48 | 27 |
| 1998 | 189 | 36.5 | 24 | 42 | 34 |
| 1999 | 227 | 41.9 | 24 | 41 | 35 |
| 2000 | 220 | 20.1 | 24 | 33 | 43 |
| 2001 | 226 | 30.9 | 12 | 24 | 64 |
| 2002 | 309 | 30.9 | 14 | 26 | 60 |
| 2003 | 151 | 12.7 | 15 | 30 | 55 |
| 2004 | 164 | 20.3 | 13 | 29 | 58 |
| 2005 | 101 | 23.4 | 12 | 30 | 58 |
| 2006 | 95 | 38.1 | 9 | 33 | 58 |

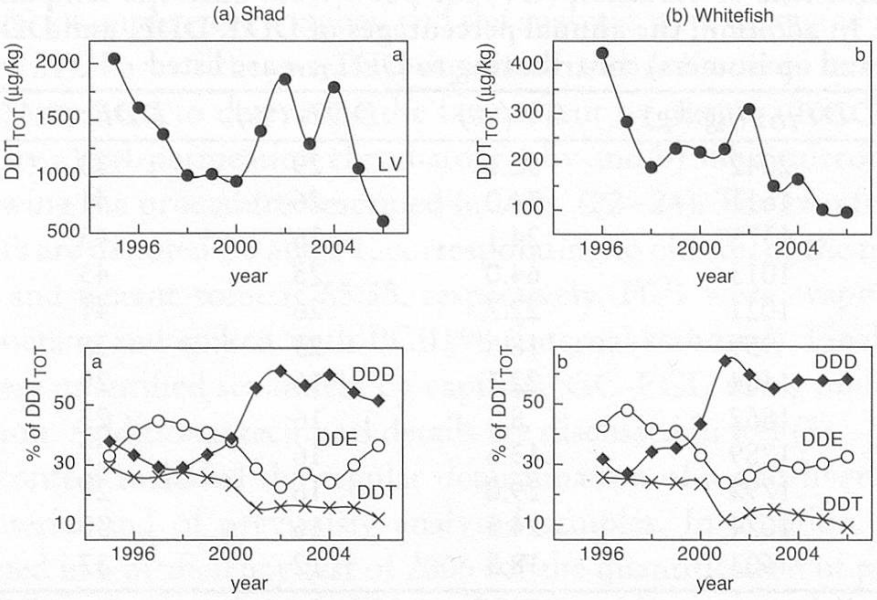


Figure 3 **Annual evolution of the mean $DDT_{TOT}$ concentration (filled circles) and of DDT% (crosses), DDD% (filled diamonds) and DDE% (open circles) observed in the monitoring period for shad (a) and whitefish (b) samples. Experimental data points are fitted to spline functions. The limit value fixed by the Swiss ordinance on foreign substances and constituents in foods, LV, is indicated**

The temporal trend observed for $DDT_{TOT}$ illustrates, after an initial decrease in the period 1995–2000 (with the only exception of 1999), heavy fluctuating results between 2000 and 2005 (Figure 3a). Similar sharp maxima of insecticide concentrations have been registered in related monitoring programmes (investigating the lake's water column, lacustrine sediments, and indicator organisms) and interpreted

as a consequence of flood events (such as in October 2000 and November 2002) in which DDTs have been transported in significant amounts from contaminated soil and river sediments (in particular via the river Toce) to the lake (8–11). Our results are fully consistent with the same interpretation. As pelagic fishes of the lake Maggiore preserve the accumulated DDTs during their life time (18), the effects of floods on the $DDT_{TOT}$ contamination of these fishes is significant on a middle-term time scale (e.g. few years).

The chemical plant identified as the source of pollution in the lake Maggiore environment stopped the production of DDT in June 1996. Since then DDT% experienced, in first approximation and with the only exception of 1998, a slow and monotonic annual reduction. As DDT in the environment is primary transformed to DDD and DDE (25), this observation is in line with a DDT contamination which is getting "older" in time and allows us to exclude recent pollutions by large amounts of "fresh" DDT.

It is interesting to evaluate the curves describing the temporal trends of DDD% and DDE% with the curve of $DDT_{TOT}$. This comparison may shed some additional light on the irregular trends of $DDT_{TOT}$ revealed between 2000 and 2005. Apparently, annual increments in the concentration of $DDT_{TOT}$ are related to an increase of DDD% and to a decrease of DDE% (Figure 3a). This observation suggests that either contaminated lacustrine sediments and/or contaminated sediment materials transported under reducing (e.g. anoxic) conditions play a crucial (indirect) contribution in polluting the two investigated fish species to date. In fact, DDD is the homologue which is usually prevailing in lacustrine sediments (in other compartments, such as river sediments and rain, DDT and/or DDE are typically predominant) (8–11). Moreover, contaminated flooded soils are known to promote microbial and/or abiotic DDT reduction to DDD (25–27). Related monitoring results on molluscs come similarly to the conclusion that lacustrine sediments play a relevant role in determining the $DDT_{TOT}$ contamination in selected aquatic organisms of the lake Maggiore (16).

For the first time in 2006 all spring samples investigated by our laboratory reveal contaminations below the limit value fixed by the Swiss ordinance on foreign substances and constituents in foods (1 mg/kg edible part). As compared to the oldest data (Tables 1 and 2), $DDT_{TOT}$ decreased by 70 % and 78 % in shads and whitefishes, respectively. Assuming an exponential decay these total reductions translate into half-lifes of 6.3 and 4.6 years, which is reasonable as compared to similar monitoring results obtained for other lakes (28). However, as discussed above, floods appear to have a significant middle-term impact on the level of $DDT_{TOT}$. These findings are actually not surprising, because a satisfactory remediation of the most polluted sites (such as contaminated sediments of the stream Marmazza) is not achieved yet. Considering these possible and large middle-term fluctuations, special care must be taken before allowing again human consumption of the two fatty species Alosa fallax lacustris and Salvelinus alpinus.

In conclusion, annual monitoring results for the concentration of DDTs in two different fish species of the lake Maggiore show that the heavy DDT pollution revealed in 1996 is slowly reducing. Of course, the present discussion is rather rough and should be extended considering, for example, lake productivity effects and possible shifts in the annual plankton community composition or other fish species with complementary dietary habits (29). The results for shads and whitefishes show often higher $DDT_{TOT}$ concentrations as those reported in Refs (8–11) for comparable monitoring periods in the time span 2001–2005 (Italian basin). These differences may be well explained by the significant longer and heavier fishes considered in the present investigation. Consequently, this study provides additional data which may be used as experimental references for testing challenging models applied to rationalise the DDT contamination of lake Maggiore (18, 30).

## Acknowledgments

Most of the data were obtained under the direction of Ing. Michele Ceschi and Ing. Mario Jäggli. The present study is dedicated in particular to Ing. Michele Ceschi which, unfortunately, passed away in 2006. We are grateful to Morenos Macchi and Bernardo Hubrecht for the reliable execution of all the analyses, and to Mauro Bordoni for helpful discussions.

## Summary

This study reports annual monitoring results for the concentration of pp-DDT, pp-DDD, pp-DDE, op-DDT, op-DDD, and op-DDE in two fish species of the lake Maggiore (Swiss basin). Shads (Alosa fallax lacustris) and whitefishes (Coregonus macrophtalmus) have been captured in spring and provide year-to-year rather comparable samples with respect to size and age of the fishes considered. The temporal trends registered suggest that the DDT pollution, revealed in 1996 and originating from a chemical plant in the Italian territory, is slowly reducing. However, flood events appear to be important in releasing contaminated materials to the lake and in determining large middle-term fluctuations of DDTs in fishes. These findings are actually not surprising, because a satisfactory remediation of the most polluted sites (such as sediments of the stream Marmazza, a tributary of the Toce river) is not achieved yet.

## Zusammenfassung

Diese Arbeit berichtet über jährliche Rückstandsbestimmungen von pp-DDT, pp-DDD, pp-DDE, op-DDT, op-DDD, und op-DDE in zwei Fischsorten des Lago Maggiore (schweizerisches Becken). Agone- (Alosa fallax lacustris) und Felchenproben (Coregonus macrophtalmus) wurden jeweils im Frühling erhoben und gewährleisteten jährlich vergleichbare Fische bezüglich der Grösse und das Alter. Die zeitliche Entwicklung der Resultate deutet auf eine Abnahme der DDT Kontamination, die im 1996 erstmals entdeckt wurde und auf einen Chemiebetrieb in

Italien zurückzuführen ist. Trotzdem scheinen Überschwemmungen in der Lage zu sein, mit DDT verunreinigtes Material in den See zu transportieren und damit bedeutsame mittelfristige Konzentrationsschwankungen von DDTs in Fischen zu verursachen. Da eine definitive Sanierung der am meisten verunreinigten Standorte (z.B. Sedimente des Baches Marmazza, ein Nebenfluss vom Fluss Toce) noch nicht realisiert wurde, sind diese Beobachtungen nicht überraschend.


## Résumé

Ce travail reporte les résultats de plusieurs déterminations annuelles de pp-DDT, pp-DDD, pp-DDE, op-DDT, op-DDD, et op-DDE dans deux espèces de poisson du lac Maggiore (bassin suisse). Des exemplaires de alose (Alosa fallax lacustris) et bondelle (Coregonus macrophtalmus) ont eté capturés au printemps et assure des échantillons plutôt comparables par rapport à l'âge et au poids des poissons considérés. L'évolution des résultats indique que la contamination de DDT, remarquée pour la première fois en 1996 et due à une usine de produits chimiques dans le territoire italien, se réduit lentement. Malgré ça, des évènements alluviales semble important pour la délivrance de matériaux contaminés dans le lac et pour des fluctuations des niveaux de DDTs dans les poissons à moyen terme. Ces résultats ne sont pas très surprenants en considérant qu'un assainissement des endroits plus pollués (comme les sédiments du torrent Marmazza, un affluent de la rivière Toce) n'a pas encore eté réalisé.



## Key words

Lake Maggiore, POPs, DDT, fish, monitoring trends


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# Low level determination of fipronil and its metabolites in milk and milk products by GC-ECD or GC-MS*

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## Introduction

Fipronil is a phenylpyrazole insecticide that kills by contact and ingestion, acting as a potent blocker of the GABA-regulated chloride channel. It helps in the control of multiple species of insects on a broad range of crops by foliar, soil or seed treatment (1).

The use of fipronil is nowadays controversial as it is suspected to kill bees and be toxic to humans. Furthermore one of its photodegradation products (fipronil-desulfinyl) shows higher toxicity than fipronil and one of the major mammalian metabolites, fipronil sulfone, shows lipophilic behavior, tending thus to persist in relevant fatty matrices.

The EU commission decisions 2003/14/EC set the maximum residue level (MRL) at 0.004 mg/kg for fipronil considered as the sum of fipronil and fipronil-desulfinyl for infant formulae and follow-on formulae (Annex X) (2). It is very likely that this limit will further decrease in the future. Furthermore Codex has set an MRL for milk and milk products of 0.02 mg/kg.

Considering the problem of fipronil in milk, the major lipophilic mammalian metabolite, i.e. fipronil sulfone, was also included in the analytical procedure.

Thus, a gas chromatographic method for the trace level determination of fipronil, fipronil desulfinyl and fipronil sulfone in milk, milk powders including infant formulae has been developed.

This method is based on the direct elution procedure described in a CEN method for the analysis of organochlorine pesticides (EN-1528, Method D, column chromatography on partially deactivated Florisil, small-scale procedure (3)). Following inter-laboratory tests in Europe, this method has been recognized as simple

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and reliable method; consequently it was accepted as official method at national (Switzerland, Germany) and European level (European CEN EN-1528-3, Method D/MSDA Chapter 3.2.3 (4)/Kapitel 35 LMBG – 00.00-38/3, Methode S9 (5)).

However, the eluting mixture proposed in the CEN method was adapted in order to replace dichloromethane by a less toxic solvent (Eluting mixture I now consists in petroleum ether-ethyl acetate 992:8 v/v). Furthermore a second more polar elution mixture has been used to eluate fipronil and its metabolite/degradation products (Elution mixture II consists in petroleum ether-ethyl acetate 7:3 v/v). As small portions of fat being co-eluted in eluate II, a dispersive solid phase extraction has been added.

Adequate precision and sensitivity have been obtained: in the low µg/kg range (0.625 –2 µg/kg) the recoveries ranged from 82.7 to 100.9 % with good repeatability and intermediate reproducibility.

It is worth noting that two other metabolites, fipronil carboxamide and fipronil sulfide, may also be analysed by this method in the low µg/kg range. Some analytical information are given for these two compounds even though a full validation has not been conducted on them.

The same procedure has been evaluated for the determination of about seventy different pesticides including organochlorine (at 2.5 µg/kg), organophosphorous (at 50 µg/kg) and pyrethroids (at 50 µg/kg) with good recoveries for most of them (eluate I and II injected on GC-ECD and GC-FPD). The distribution between eluate I and eluate II has been established.

## Experimental

### *Chemicals*

Fipronil desulfinyl, fipronil sulfide and fipronil sulfone were supplied by Accu-Standard (New Haven, USA). All other pesticide standards were from Dr. Ehrensdorfer (Augsburg, Germany). Acetone, acetonitrile, cyclohexane, ethyl acetate, Florisil (0.15–0.25 mm), octadecyl (C18) 40 µm preparative LC packing, petroleum ether and water were purchased at analytical grade.

### *Special glassware*

*Chromatographic tube,* 8 mm i.d., 20 cm long, with a glass wool plug and a glass stopcock and at least 100 mL reservoir at the upper end.

### *Preparation of reagents and solutions*

*Activated Florisil* is obtained after calcination (12 hours at 500 °C). It is stored in a tightly closed container and stored in a desiccator.

*Deactivated Florisil 3 % water* is obtained after thoroughly homogeneisation of activated Florisil with water in a 97:3 w/w ratio. It has to be stabilised for at least 12 hours before use. It is stored in a tightly closed container and stored in a desiccator.

### *Food samples*

Pasteurised milk and infant formulae were purchased in selected retails outlets in Switzerland.

### *Sample preparation*

Reconstituted or liquid milk sample (2 g) is thoroughly mixed with activated Florisil (5 g) in a 100 mL Erlenmeyer and let for adsorption (10 min).

Meanwhile the chromatographic column with its glass plug is rinsed with petroleum ether, filled with petroleum ether (20 mL) and deactivated Florisil (3 g) is slowly added. When the Florisil has settled down, a few mL of the petroleum ether are allowed to percolate to pack the sorbent and the sample preparation is added; after sedimentation, the Florisil layer should lay slightly under the petroleum ether. The petroleum ether is eluted until it reaches the sorbent bed (typically 1–2 mL); a petroleum ether-ethyl acetate mixture 992:8 (v/v) (50 mL) is carefully added, eluted at about 5 mL/min and collected in a 100 mL round flask (do not let the sorbent dry).

This first eluate corresponds to the one obtained after the elution with eluting mixture petroleum ether-dichloromethane 4:1 (v/v) and contains most apolar organochlorine pesticides. It may be further processed as described in the EN 1528 method.

Then a cyclohexane-ethyl acetate mixture 7:3 (v/v) (50 mL) is carefully added, eluted at about 5 mL/min and collected in a 100 mL round flask.

This second eluate contains fipronil and its metabolites/degradation products, as well as more polar pesticides like organophosphorous or pyrethroids pesticides.

The second eluate is then concentrated (~0.5 mL) under vacuum (40 °C, 200 mbar) and the residual solvent gently evaporated under nitrogen to a droplet. Acetonitrile (1 mL) is added and the fatty residue is liquefied for a few seconds in a 40 °C water bath, sonicated (15 sec), and supplemented with octadecyl powder (150 mg). The extract is then gently swirled, transferred into a conical vial and let for sedimentation, the clear supernatant being finally transferred into a GC vial.

### *Determination*

The determination of fipronil and its metabolites/degradation products is carried out either by GC-ECD or GC-MS, in the last case ideally using negative chemical ionization. Only GC-MS/NCI conditions are given hereunder.

The GC-MS/NCI system used encompassed an Agilent GC 6890 coupled to an Agilent mass spectrometer HP 5973 (Agilent, USA) working in SIM mode. The analytical parameters are presented in Table 1 whereas the retention times as well as the relevant ions for fipronil and its metabolites/degradation products are given in Table 2. The confirmation of the identification should be done in accordance with the EU Commission Decision 2002/657/EC (6).

Table 1
**GC-MS/NCI parameters**

| | |
|---|---|
| Capillary column | DB-5MS, length 30 m, ID 0.25 mm, film thickness 0.025 mm |
| Carrier gas | Helium at 18 psi[1] (constant pressure) |
| Injection | 0.002 mL Pulsed splitless mode, 250 °C, 1 min purge activation time |
| Purge flow | 25 mL/min |
| Temperature program | 70 °C for 2 min, 70 °C → 150 °C at 25 °C/min, 150 °C → 200 °C at 3 °C/min, 200 °C → 280 °C at 8 °C/min, 10 min at 280 °C |
| Transfer line temperature | 250 °C |
| Source temperature | 150 °C |
| Solvent delay | 13 min |
| Detector off | 28 min |
| Acquisition mode | Selected Ion Monitoring (SIM), NCI |
| % Methane gas | 40 |

[1]These GC parameters are based on the Agilent method for the multi-determination of pesticides (7) using the Retention Time Locking system.

Table 2
**GC-MS/NCI retention time and relevant ions used for Fipronil and its metabolites/ degradation products**

| *Compound* | *Retention time (min)* | *Quantifier (m/z)* | *1st qualifier (m/z)* | *2nd qualifier (m/z)* |
|---|---|---|---|---|
| Fipronil desulfinyl | 14.8 | 352 | 354 | 316 |
| Fipronil | 18.9 | 384 | 366 | 331 |
| Fipronil sulfone | 22.2 | 416 | 383 | 347 |
| Fipronil sulfide | 18.4 | 410 | 412 | 384 |
| Fipronil carboxamide | 25.9 | 384 | 386 | 350 |

Considering the quantitation, a matrix-matched calibration point corresponding to a 0.004 mg/kg contamination level (sum of fipronil, fipronil desulfinyl and fipronil sulfone at each 0.0013 mg/kg) is done with each series (e.g. 0.19 mL of a blank sample extract is spiked with 0.01 mL of a 50 ng/mL mix solution). Highly contaminated samples should be quantified with the appropriate matrix-matched calibration point.

### *Method performance*

The linearity of fipronil, fipronil desulfinyl and fipronil sulfone by GC-MS/NCI has been evaluated at a low level in order to cover the EU and Codex MRL (corresponding to an absolute quantity injected of 0.25 to 6 pg), as well as at higher level (absolute quantity injected of 20 to 400 pg). Typical correlation coefficients ranged from r=0.995 to 1.000, whereas the residue analysis as well as the ratio "signal to concentration" indicated that the calibration curve follows a linear model in both low and high concentration level.

An experimental plan encompassing recoveries on two different samples (a UHT milk and an infant formula) at three different levels (0.5×MRL/1×MRL/1.5×MRL,

MRL expressed as the sum of fipronil, desulfinyl, fipronil and fipronil sulfone equally represented) conducted in triplicate, and repeated on three different days by three different persons allowed the calculation of the following parameters (see Table 3 and Table 4):

- two different decision limits, $CC_{\alpha 0}$ and $CC_{\alpha 4}$, the first being the concentration of analyte that can be differentiated from a 0 mg/kg concentration with a false positive risk set at 1 % (it correspond to the limit of detection) and the second being the concentration of the sum of the three analytes that can be differentiated from a 0.004 mg/kg concentration with a false positive risk set at 5 %;
- two different detection capabilities, $CC_{\beta 0}$ and $CC_{\beta 4}$, the first being the concentration of analyte that can be differentiated from its $CC_{\alpha 0}$ concentration with a false negative risk set at 5 % (it corresponds to the limit of quantitation) and the second being the concentration of the sum of the three analytes that can be differentiated from its respective $CC_{\alpha 4}$ concentrations with a false positive risk set at 5 %;
- the trueness;
- the repeatability (r) and intermediate reproducibility (iR);
- the expanded measurement uncertainty (U) considering both SD(iR) and the uncertainty on the recovery.

All these parameters show that the method is fit for purpose.

Table 3
**Decision limits and detection capabilities (GC-MS/NCI data)**

| *Compounds* | *$CC_{\alpha 0}$ UHT milk µg/kg* | *$CC_{\alpha 0}$ infant formula µg/kg* |
|---|---|---|
| Fipronil | 0.12 | 0.10 |
| Fipronil desulfinyl | 0.18 | 0.15 |
| Fipronil sulfone | 0.23 | 0.22 |
| *Compounds* | *$CC_{\beta 0}$ UHT milk µg/kg* | *$CC_{\beta 0}$ infant formula µg/kg* |
| Fipronil | 0.32 | 0.18 |
| Fipronil desulfinyl | 0.27 | 0.26 |
| Fipronil sulfone | 0.31 | 0.37 |
| *Compounds* | *$CC_{\alpha 4}$ UHT milk µg/kg* | *$CC_{\alpha 4}$ infant formula µg/kg* |
| Sum of 3 fipronil compounds | 4.20 | 4.19 |
| *Compounds* | *$CC_{\beta 4}$ UHT milk µg/kg* | *$CC_{\beta 4}$ infant formula µg/kg* |
| Sum of 3 fipronil compounds | 4.40 | 4.38 |

Table 4
**Performance of the method for a UHT milk and an infant formula (I.F.), data from GC-MS/NCI**

| *Sample (n=9)* | *Median µg/kg* | *Recovery iR basis %* | *Fipronil desulfinyl SD(r) µg/kg* | *SD(iR) µg/kg* | *U ±µg/kg* |
|---|---|---|---|---|---|
| UHT Milk 0.625 µg/kg | 0.605 | 96.8 | 0.032 | 0.048 | ±0.103 |
| UHT Milk 1.250 µg/kg | 1.190 | 95.2 | 0.068 | 0.100 | ±0.217 |
| UHT Milk 1.875 µg/kg | 1.805 | 96.3 | 0.121 | 0.336 | ±0.711 |
| I.F. 0.625 µg/kg | 0.610 | 97.6 | 0.026 | 0.038 | ±0.085 |
| I.F. 1.250 µg/kg | 1.230 | 98.4 | 0.054 | 0.049 | ±0.103 |
| I.F. 1.875 µg/kg | 1.843 | 98.3 | 0.090 | 0.103 | ±0.219 |
| *Sample (n=9)* | *Median µg/kg* | *Recovery iR basis %* | *Fipronil SD(r) µg/kg* | *SD(iR) µg/kg* | *U ±µg/kg* |
| UHT Milk 0.625 µg/kg | 0.595 | 95.2 | 0.046 | 0.108 | ±0.232 |
| UHT Milk 1.250 µg/kg | 1.170 | 93.6 | 0.038 | 0.139 | ±0.312 |
| UHT Milk 1.875 µg/kg | 1.730 | 92.3 | 0.057 | 0.257 | ±0.571 |
| I.F. 0.625 µg/kg | 0.630 | 100.8 | 0.038 | 0.067 | ±0.137 |
| I.F. 1.250 µg/kg | 1.245 | 99.6 | 0.056 | 0.058 | ±0.118 |
| I.F. 1.875 µg/kg | 1.783 | 95.1 | 0.065 | 0.097 | ±0.226 |
| *Sample (n=9)* | *Median µg/kg* | *Recovery iR basis %* | *Fipronil sulfone SD(r) µg/kg* | *SD(iR) µg/kg* | *U ±µg/kg* |
| UHT Milk 0.625 µg/kg | 0.590 | 94.4 | 0.068 | 0.171 | ±0.369 |
| UHT Milk 1.250 µg/kg | 1.105 | 88.4 | 0.094 | 0.261 | ±0.573 |
| UHT Milk 1.875 µg/kg | 1.695 | 90.4 | 0.082 | 0.329 | ±0.743 |
| I.F. 0.625 µg/kg | 0.605 | 96.8 | 0.030 | 0.070 | ±0.149 |
| I.F. 1.250 µg/kg | 1.210 | 96.8 | 0.061 | 0.113 | ±0.251 |
| I.F. 1.875 µg/kg | 1.680 | 89.6 | 0.129 | 0.152 | ±0.403 |

### *Applicability of the modified EN 1528 method to other GC amenable pesticides*

The modified EN 1528 method has been evaluated for about seventy pesticides in UHT milk on a repeatability basis (n=7) on a GC-ECD. A third eluate consisting of ethyl acetate (50 mL) has been added to identify late eluting compounds. The results (see Table 5) show that this modified method offers similar analytical performances for organochlorine pesticides as the standard method while offering two decisive advantages, i.e. the use of non-halogened solvent and the extension to more polar analytes, especially organophosphorous and pyrethoids pesticides.

Looking more in detail for some specific problematic cases, it may be observed that

- some very polar pesticides are not totally eluted with the petroleum ether-ethyl acetate 7:3 (v/v) mixture (e.g. fenamiphos);
- some pesticides may be oxidized during the analysis (e.g. fenamiphos);

- some pesticides may be problematic as such (e.g. disulfoton);
- very volatile pesticides may be lost if not enough care is taken or no keeper is used (e.g. dichlorvos).

Table 5
**Recoveries (n=7) for different pesticides as well as their elution distribution (GC-ECD data)**

| *Pesticide* | *Spike level µg/kg* | *Distribution** *Eluate I* | *Eluate II* | *Recovery %* | *CV %* |
|---|---|---|---|---|---|
| Aldrine | 2.5 | 5 | 0 | 93 | 9.2 |
| Allethrine bio | 50 | 0 | 5 | 104 | 5.0 |
| Azinphos ethyl | 50 | 0 | 5 | 115 | 6.0 |
| Azinphos methyl | 50 | 0 | 5 | 143 | 5.0 |
| Bifenthrine | 50 | 4 | 2 | 103 | 11.0 |
| Bromophos ethyl | 50 | 4 | 2 | 102 | 3.9 |
| Chlordan alpha | 2.5 | 5 | 0 | 85 | 2.7 |
| Chlordan gamma | 2.5 | 5 | 0 | 96 | 1.8 |
| Chlorpyriphos ethyl | 50 | 4 | 2 | 95 | 4.1 |
| Chlorpyriphos methyl | 50 | 4 | 2 | 97 | 5.8 |
| Coumaphos | 50 | 0 | 5 | 104 | 5.2 |
| Cyfluthrine | 50 | 2 | 4 | 70 | 7.2 |
| Cyhalothrine lambda | 25 | 2 | 4 | 125 | 3.9 |
| Cypermethrine | 50 | 2 | 4 | 70 | 7.8 |
| DDE o,p' | 2.5 | 5 | 0 | 94 | 2.7 |
| DDE p,p' | 2.5 | 5 | 0 | 97 | 6.2 |
| DDT o,p' | 2.5 | 5 | 0 | 100 | 7.9 |
| DDT p,p' | 2.5 | 5 | 0 | 97 | 10.7 |
| Deltamethrine | 50 | 2 | 4 | 50 | 8.4 |
| Diazinon | 50 | 0 | 5 | 106 | 7.3 |
| Dichlorvos | 50 | 4 | 2 | 20 | 35.1 |
| Dieldrine | 2.5 | 5 | 0 | 97 | 3.6 |
| Disulfoton | 50 | 3 | 3 | 9 | 33.2 |
| Endosulfan alpha | 2.5 | 5 | 0 | 94 | 2.4 |
| Endosulfan beta | 2.5 | 5 | 0 | 95 | 3.7 |
| Endosulfan sulfate | 2.5 | 0 | 5 | 90 | 4.4 |
| Endrine | 2.5 | 5 | 0 | 101 | 7.8 |
| Ethion | 25 | 4 | 2 | 99 | 4.3 |
| Ethoprophos | 50 | 0 | 5 | 103 | 4.4 |
| Etrimphos | 50 | 1 | 5 | 109 | 7.4 |
| Fenamiphos | 50 | 0 | 2** | 44*** | 10.6 |
| Fenamiphos sulfoxide | 100 | 0 | 2** | 76 | 9.7 |
| Fenamiphos sulfone | 200 | 0 | 5** | 110 | 10.8 |
| Fenchlorphos | 50 | 4 | 2 | 99 | 4.8 |
| Fenitrothion | 50 | 0 | 5 | 119 | 6.3 |
| Fenpropathrine | 50 | 4 | 2 | 105 | 9.8 |
| Fenvalerate | 50 | 2 | 4 | 53 | 13.3 |
| Flucithrinate | 50 | 0 | 5 | 67 | 9.6 |
| Fonophos | 50 | 2 | 4 | 82 | 6.8 |
| HCB | 2.5 | 5 | 0 | 80 | 7.7 |
| HCH alpha | 2.5 | 5 | 0 | 92 | 4.7 |
| HCH beta | 2.5 | 5 | 0 | 95 | 2.7 |

| *Pesticide* | *Spike level* | *Distribution** | | *Recovery %* | *CV %* |
|---|---|---|---|---|---|
| | *µg/kg* | *Eluate I* | *Eluate II* | | |
| HCH delta | 2.5 | 5 | 0 | 82 | 7.6 |
| HCH epsilon | 2.5 | 5 | 0 | 99 | 2.5 |
| HCH gamma | 2.5 | 5 | 0 | 93 | 3.4 |
| Hept Epox | 2.5 | 5 | 0 | 98 | 3.3 |
| Hept Epox-trans | 2.5 | 5 | 0 | 95 | 5.3 |
| Heptachlore | 2.5 | 5 | 0 | 95 | 4.2 |
| Iodofenphos | 50 | 4 | 2 | 106 | 4.9 |
| Isodrine | 2.5 | 5 | 0 | 97 | 4.0 |
| Malathion | 50 | 0 | 5 | 117 | 6.7 |
| Methacriphos | 50 | 1 | 5 | 114 | 6.5 |
| Methidathion | 50 | 0 | 5 | 123 | 7.1 |
| Methoxychlore | 2.5 | 5 | 0 | 91 | 10.2 |
| Mirex | 2.5 | 5 | 0 | 89 | 6.4 |
| Oxychlordane | 2.5 | 5 | 0 | 96 | 3.1 |
| Parathion ethyl | 50 | 2 | 4 | 111 | 6.5 |
| Parathion methyl | 50 | 0 | 5 | 126 | 8.1 |
| PCB 101 | 2.5 | 5 | 0 | 94 | 4.0 |
| PCB 138 | 2.5 | 5 | 0 | 92 | 7.2 |
| PCB 153 | 2.5 | 5 | 0 | 100 | 8.8 |
| PCB 180 | 2.5 | 5 | 0 | 90 | 7.6 |
| PCB 28 | 2.5 | 5 | 0 | 93 | 3.9 |
| PCB 52 | 2.5 | 5 | 0 | 95 | 2.6 |
| Permethrine | 2.5 | 4 | 2 | 110 | 3.6 |
| Phenthoate | 25 | 1 | 5 | 112 | 5.9 |
| Phosalon | 50 | 0 | 5 | 115 | 6.2 |
| Pirimiphos methyl | 50 | 3 | 3 | 105 | 4.4 |
| Profenophos | 50 | 0 | 5 | 100 | 7.0 |
| Tau-Fluvalinate | 50 | 0 | 5 | 67 | 11.9 |
| TDE o,p' | 2.5 | 5 | 0 | 96 | 3.3 |
| TDE p,p' | 2.5 | 5 | 0 | 95 | 3.3 |
| Tetrachlorvinphos | 100 | 0 | 5 | 117 | 5.6 |
| Tetradifon | 2.5 | 5 | 0 | 106 | 14.6 |
| Tetramethrine | 100 | 0 | 5 | 99 | 6.4 |
| Triazophos | 50 | 0 | 4** | 127 | 5.1 |

* 1 = recovery < 10 %/2 = recovery 10–30 %/3 = recovery 30–60 %/4 = recovery 60–90 %/5 = recovery > 90%.

** The rest of the residue is found in Eluate 3 (100 % ethyl acetate), the recovery is given for the sum of eluate 2 and 3.

*** The pesticide is oxidized in sulfone during the analysis.

## Conclusion

The modified EN 1528 method presented here is a valuable tool for the low level determination of fipronil and its metabolites/degradation products in milk and infant formula. Moreover it offers a broader screening range, including more polar pesticides like organophosphorous and pyrethroids ones without complicating significantly the method.

## Summary

Fipronil is a phenylpyrazole pesticide, which has a maximum residue level in infant formulae and follow-on formulae set by the EU commission decisions 2003/14/EC at 0.004 mg/kg (expressed as the sum of fipronil and fipronil-desulfinyl) (2). Furthermore Codex has proposed an maximum residue limit for milk and milk products of 0.020 mg/kg.

A GC-MS/NCI method has been developed based on the CEN method EN-1528 (Method D, column chromatography on partially deactivated Florisil, small-scale procedure (3)) to allow for the low level determination of fipronil, fipronil sulfone (the major mammalian metabolite) and fipronil-desulfinyl (its main photodegradation products) in milk or in milk powder including infant formulae and follow-on formulae.

This adaptation of the official EN-1528 method offers decisive advantages as it avoids the use of halogened solvents while broadening the screening range, allowing more polar compounds, such as organophosphorous or pyrethroid pesticides, to be analysed.

## Zusammenfassung

Fipronil ist ein Pestizid basierend auf Phenylpyrazol. Der maximal akzeptierte Rückstandsgehalt in Säuglings- und Kindernährmitteln wurde von der EU Kommission (2003/14/EC) auf 0.004 mg/kg gesetzt (ausgedrückt als Summe von Fipronil und Fipronil-Desulfinyl (2). Zusätzlich wurde im Codex der Grenzwert für Rückstände in Milch und Milchprodukten auf 0.020 mg/kg gesetzt.

Eine GC-MS/NCI Methode wurde entwickelt, basierend auf der CEN Methode EN-1528 (Methode D, Säulenchromatographie mit teilweise deaktiviertem Florisil, mini-Methode (3)). Diese Methode ermöglicht die Bestimmung von Fipronil, Fipronilsulfone (der meist vorkommende Metabolit in Säugetieren) und Fipronil-desulfinyl (sein photoreduzierendes Haupt-Produkt) in Milch oder in Milchpulver, sowie in Säuglings- und Kindernährmitteln.

Die Anpassung der offiziellen EN-1528 Methode hat die entscheidenden Vorteile, dass keine halogenierten Lösungsmitteln gebraucht werden und gleichzeitig die Unterschungsmöglichkeiten grösser werden, d.h., dass mehr polare Substanzen (wie die Organophosphor- oder Pyrethroid-Pestizide) analysiert werden können.

## Résumé

Le Fipronil est un pesticide de la famille des phenylpyrazoles, dont la limite maximale de résidus a été fixée à 0.004 mg/kg (somme de fipronil et fipronil desulfinyl) par la décision européenne 2003/14/EC (2). Le Codex a de plus proposé une limite maximale de résidus à 0.020 mg/kg pour le lait et les produits laitiers.

Une méthode d'analyse par GC-MS/NCI a été développée, basée sur la méthode officielle européenne EN-1528 (Méthode D, chromatographie sur colonne de florisil partiellement désactivé, méthode miniaturisée (3)), permettant l'analyse

du fipronil, du fipronil sulfone (le métabolite principal chez les mammifères) ainsi que du fipronil desulfinyl (le produit principal de photodégradation) dans le lait et les poudre de lait, formules infantiles inclues.

Cette adaptation de la méthode officielle EN-1528 offres deux avantages décisifs dans le sens où elle exclut l'usage de solvants halogénés tout en élargissant le domaine d'application de la méthode en couvrant des composés plus polaires, tels que les pesticides organo-phosphorés et les pyréthroides.

**Key words**
fipronil, milk, infant formulae, EN-1528

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