

**UNIVERSITA' CATTOLICA DEL SACRO CUORE
PIACENZA**

Scuola di Dottorato per il Sistema Agro-alimentare

Doctoral School on the Agro-Food System

cycle XXII

S.S.D: AGR 15, AGR 13

**Consumers' exposure assessment of pesticide
residues in food: current status and future
perspectives in Lombardy**

Candidate: Alessandro Marino CHIODINI

Matr. n.: 3580176

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Abstract

The presented thesis describes the results of the pesticide monitoring programme in Lombardy Region from 1996 to 2008 and analyses the data gathered to calculate consumer exposure assessment with different approaches. A total of 9387 samples were analysed and the number of irregular samples was equal to 1%. The number of samples without residues was 69% and the number of samples with residues within the MRL was 30%. A further step to understand the exposure of consumers to residue of pesticides was obtained with the use of a deterministic approach developed by EFSA (PRIMo Model). It was found that among the detected irregular samples, only 31 might cause harm to the health of the consumer. An additional step was constituted by the use of one probabilistic method (Creme Software) to calculate the cumulative exposure of pesticides for the consumers. As a first step, residues of Chlorprofam were plotted in the software on samples of potato. In addition, samples containing residues of Organophosphates were also plotted along with the Italian consumption data. In both the case studies, the probabilistic acute cumulative assessment indicated that the intake, for adults and toddlers was below the set toxicological endpoint.

Introduction

Pesticides are designed to control biological organisms. The same chemical properties that control pests may also harm humans thus there may be a risk to human health if people are exposed to pesticides. For pesticides there are three main routes of exposure into the human body – dermal exposure, inhalation and ingestion.

The aim of this thesis is: to review the legislation framework adopted by the European Union to evaluate the risk from dietary pesticide exposure before and after the placing of plant protection products in the market (chapters 1 and 2). In addition, the monitoring of pesticide residues in plants of organic origin is described in chapter 3. In order to focus on the dietary exposure assessment, current methods highlighting their pros and cons in terms of transparency and communication to consumers and risk managers were analysed. Finally two series of case studies are reported in chapters 4 and 5 to illustrate the application of those methods using data of the monitoring programme of pesticides performed in Lombardy Region from 1996 to 2008.

In particular, the first chapter of this thesis will introduce the Directive 91/414/EEC concerning the placing of plant protection products in the market and Regulation 396/2005/EEC with reference to maximum residue levels of pesticides in or on food and feed of plant and animal origin, amending Council Directive 91/414/EEC. In order to give a more comprehensive overview the European Monitoring of Pesticide Residues in Products of Plant Origin is reported in a dedicated section with particular reference to the Italian Control Plan of Residues of Plant Protection Product in foods of Plant Origin regulated by the Decree of the Ministry of Health of 23 December 1992.

The second chapter provides a summary of the data gathered by the 12 local health units where the food samples were collected and analysed in the past years in Lombardy.

A sub-section of the database regards samples of foodstuffs coming from biological agriculture, which are analysed in the third chapter of the

thesis. In particular, this work was previously published in the *Journal of Environmental Science* and reported here for the sake of completeness.

In the fourth chapter of the thesis, the EFSA PRIMO Model was used to obtain acute and chronic dietary exposure assessment for the Italian population who had consumed foodstuffs analyzed for pesticide contamination in Lombardy from 1996-2008.

The fifth chapter refined the exposure assessment process by using the probabilistic software (Creme Software) considering three data sets. The first case study was conducted on one active substance (Chlorpropham) with one commodity (potato). The second case study consisted of an assessment of the impact, on the health of consumers, of the single active substance (Chlorpyrifos) combined with all the food commodities (where it was detected). A third level of aggregation was assessed considering the cumulative effects of Organophosphates looking at a common mechanism of action (inhibition of Acetylcholine esterase).

Results from the previous chapters were merged in a concluding section where the different aspects of monitoring programme of pesticides in Lombardy and the assessments of the exposure on the consumers were illustrated. In addition, some recommendations were given on how to implement the strategy of sampling and how to interpret the results obtained from the various models of exposure.

European Pesticide Legislation

Pesticides are used to protect crops before and after harvest from infestation by pests and plant diseases. A possible consequence of their use may be the presence of pesticide residues in the treated products.

It is necessary to ensure that such residues should not be found in food or feed at levels presenting unacceptable risks to humans. Maximum Residue Levels (MRLs) were therefore set by the European Commission to protect consumers from exposure to unacceptable levels of pesticides residues in food and feed (91/414/EEC).

In the European Union (EU), as from 1st September 2008, a new legislative framework Regulation (EC) No 396/2005 of the European Parliament on pesticide residues is applicable. This Regulation completes the harmonisation and simplification of pesticide MRLs, whilst ensuring better consumer protection throughout the EU. With the new rules, MRLs undergo a common EU assessment to make sure that all classes of consumers, including the vulnerable ones, like babies and children, are sufficiently protected (396/2005/EEC).

Until 1st September 2008, the legislation for pesticide residues was a shared responsibility of the Commission and the Member States. Since 1976, more than 45.000 Community MRLs have been set for various commodities for 245 pesticides on cereals (Directive 86/362/EEC), foodstuff of animal origin (Directive 86/363/EEC), fruit and vegetables and other plant products (Directive 76/895/EEC and Directive 90/642/EEC). For the tens of thousands of pesticide/commodity combinations for which no Community MRLs existed, Member States could set MRLs at national level to facilitate trade and to protect the health of their consumers.

However, safety of consumers in one country does not necessarily mean that all consumers in the EU are protected because food consumption patterns differ from one Member State to another. Nowadays food and feed circulate freely in the EU internal market, and therefore it is indispensable to assure that all EU consumers are equally protected from the exposure to unacceptable levels of pesticides in their food.

Regulation (EC) 91/414

The evaluation, marketing and use of pesticides (herbicides, insecticides, fungicides etc.) in plant protection in the Community are regulated under Council Directive 91/414/EEC. This Directive lays out a comprehensive risk assessment and authorisation procedure for active substances and products containing these substances. Each active substance has to be proven safe in terms of human health, including residues in the food chain, animal health and the environment, in order to be allowed to be marketed. It is the responsibility of industry to provide the data showing that a substance can be used safely with respect to human health and the environment.

The first step of the evaluation process involves a Rapporteur Member State, which transmits its preliminary conclusions on the substance to the European Food Safety Authority. A scientific risk assessment involving the European Food Safety Authority is then carried out, followed by risk management steps carried out by the Commission with the assistance of the Member States within the Standing Committee on the Food Chain and Animal Health.

If the evaluation shows that the substance has no harmful effect on human or animal health and that it has no unacceptable influence on the environment, the substance can be approved.

A EU list of approved active substances (Annex I to Directive 91/414/EEC) is established, and Member States may authorise only plant protection products containing active substances included in this list.

Regulation (EC) 396/2005

Regulation (EC) 396/2005 envisages a full harmonization for all pesticide MRLs and replaces the previous legislation concerning MRLs. Based on this legislation; the European Commission is taking forward a food standards programme, which strives to achieve the harmonization of the

existing pesticides on the market, which could potentially be present as residues on food. According to this Regulation the harmonized Maximum residue Level (MRLs) should be based on existing national provisions in place in EU Member States.

The European Commission compiled a list of the national MRLs for non-harmonized substances. As many of these pesticides are no longer used in agriculture either within or outside the EU, the Commission considered it appropriate to set MRLs for these essentially obsolete compounds at the lowest possible level. The Commission will therefore propose to set MRLs for around 660 obsolete pesticides at the limit of determination, which is the lowest level surveillance laboratories can achieve in monitoring analysis.

For around 240 remaining compounds, which are still in use either in or outside the EU, Member States have established specific national MRLs. These are now subject to the European harmonisation programme, which in the first instance involves establishing temporary European level MRLs for these substances. The temporary MRLs will be subject to a detailed scientific assessment leading to the establishment of final EU MRLs following a comprehensive assessment of the active substances.

As from 1st September 2008, Regulation (EC) 396/2005 of the European Parliament and of the Council on Maximum Residue Levels (MRLs) of pesticides in products of plant and animal origin defines a new fully harmonised set of rules for pesticide residues. This regulation simplifies the existing legislation by harmonising pesticide MRLs and making them directly applicable (396/2005/EEC) and progressively would amend the Council Directive 91/414/EEC.

The Regulation (EC) 396/2005 is composed by the main text, where definitions and scope are set; in addition four technical annexes specify the MRLs and the products to which they apply.

Annex I is the list of products to which the MRLs apply, it has been established by Commission Regulation (EC) No 178/2006. It contains 315 products, including fruits, vegetables, spices, cereals, animal products (178/2006/EEC).

Annex II is the list of EU definitive MRLs and it consolidates the existing EU legislation before 1 September 2008. It specifies MRLs for 245 pesticides.

Annex III is the list of the so-called EU temporary MRLs. It is the result of the harmonisation process as it lists pesticides for which, before 1 September 2008, MRLs were only set at national level.

Annex IV is the list of pesticides for which no MRLs are needed because of their low risk.

Annex V will contain the list of pesticides for which a default limit other than 0,01 mg/kg will apply. This Annex has not been published yet.

Annex VI will contain the list of conversion factors of MRLs for processed commodities. This Annex has not been published yet.

Annex VII contains a list of pesticides used as fumigants for which the Member States are allowed to apply special derogations before the products are placed on the market.

If a pesticide is not included in any of the above mentioned Annexes the default MRL of 0,01 mg/kg applies, as stated in the article 18(1b) of Regulation (EC) 396/2005.

Settings of MRL

All decision-making in this area has to be science-based and a consumer intake assessment has to be carried out by the European Food Safety Authority before concluding on the safety of an MRL.

European MRLs for pesticide residues are set to protect the consumers and to make trade possible in products containing residues. MRLs are set based on authorised uses of plant protection products pursuant to Directive 91/414/EEC (91/414/EEC).

Applicants for an MRL have to submit data on the level of residues resulting from the specified agricultural use and on the toxicology of the pesticide.

The level of the MRL is determined by 'supervised trials'. From the toxicological information an Acceptable Daily Intake (ADI) and an Acute Reference Dose (ARfD) are derived.

The acceptable daily intake (ADI) reflects the chronic toxicity. It is the estimate of the amount of a substance in food, expressed on a body-weight

basis that can be ingested daily over a lifetime without appreciable health risk to the consumer.

The acute reference dose (ARfD) reflects the acute toxicity. It is the estimate of the amount of a substance in food, expressed on a body-weight basis that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer.

To determine whether an MRL is acceptable, the intake of residues through all food that may be treated with that pesticide is calculated and compared with the ADI and the ARfD, for long and short term intake and for all available models of European consumer groups.

In case that the MRL requested is not safe, the lowest limit of analytical determination (LOD) is set as the MRL. The LOD is also set for crops on which there are no uses of the pesticide and when uses do not leave any detectable residues. The default LOD in the EU legislation is 0,01 mg/kg.

The European Food Safety Authority (EFSA) is responsible for the risk assessment and evaluates each intended new MRL.

Based on the EFSA's opinion, the Commission can issue a Regulation to establish a new MRL or to amend or remove an existing MRL.

Sustainable Use Of Pesticides

The existing European policies and legislation on pesticides scarcely address the actual use phase of the pesticides life-cycle, e.g. the temporary storage of pesticides at farm level, the management/calibration of application equipment, the protection of operators, the preparation of the spraying solution and the application itself. As a result of the misuse of pesticides including overuses, the percentage of food and feed samples in which residues of pesticides exceed maximum regulatory limits has not decreased over the last ten years (EFSA 2008).

A proposal for a framework directive would make it mandatory for all Member States to establish national action plans involving all the relevant stakeholders in the process. They would also have to create a system of awareness raising and training of all professional users. Compulsory inspection of existing application equipment would be introduced and aerial

spraying would be prohibited (derogations would be granted in situations where there are no viable alternatives or where it has clear advantages in terms of reduced impacts on health and the environment in comparison to land-based application).

Member States would designate areas of significantly reduced or zero pesticide use. Safe conditions would be established for storage and handling of pesticides and their packaging and remnants.

Member States would also have to create the necessary conditions for implementing Integrated Pest Management (IPM), which would become mandatory as of 2014. In the context of IPM, the EU would draw up crop-specific standards, the implementation of which would be voluntary. Finally, a set of harmonised indicators and substitution of pesticides with alternative products would be developed to measure progress in implementing the Strategy (SSLRC 1997).

European Official Control Plan

The concept of the EU Reference Laboratories (EURLs) and National Reference Laboratories (NRLs) was laid down in the EC Regulation 882/2004 of the European Parliament and of the Council. The overall objective of the EURLs and NRLs is to improve the quality, accuracy and comparability of the results at official control laboratories (882/2004/EEC).

According to Article 32 of EC Regulation 882/2004 the EURLs are responsible for:

- providing NRLs with details of analytical methods, including reference methods
- organisation of Proficiency Tests
- development and validation of new analytical methods
- organisation of workshops & training of laboratories in the Members States
- providing scientific and technical assistance to the Commission, e.g. for the establishment of co-ordinated programmes
- collaborating with laboratories responsible for analysing feed and food in third countries

- development of the analytical control guidelines

The main tasks of the EURLs for residues of pesticides can be summarized as follows:

- to promote research, development and validation of new analytical methods;
- to inform NRLs about new advances in methods and equipment;
- to assist NRLs and official control laboratories by:
 - helping them to implement quality assurance systems,
 - providing them technical advice,
 - organising training courses,
 - organising comparative tests;
- to act as arbiter in analytical disputes between Member States
- to provide the Commission with technical and scientific advice and prepare annual reports;
- to help the Commission in creating guidelines and monitoring programs
- to establish a network between EURLs-NRLs-official control laboratories
- to assist the harmonisation process by increasing the current analytical scope through EU in quantity and quality of the results.

Rapid Alert System for Food and Feed (RASFF)

The RASFF was put in place to provide food and feed control authorities with an effective tool to exchange information about measures taken responding to serious risks detected in relation to food or feed. This exchange of information helps Member States to act more rapidly and in a coordinated manner in response to a health threat caused by food or feed. Its effectiveness is ensured by keeping its structure simple: it consists essentially of clearly identified contact points in the Commission, European food safety Authority (EFSA) and at national level in member countries, exchanging information in a clear and structured way by means of templates.

The legal basis of the RASFF is Regulation (EC) 178/2002. Article 50 of this Regulation establishes the rapid alert system for food and feed as a network involving the Member States, the Commission as member and manager of the system and the EFSA (178/2002/EEC). Also the European Economic Area (EEA) countries: Norway, Liechtenstein and Iceland, are longstanding members of the RASFF.

Whenever a member of the network has any information relating to the existence of a serious direct or indirect risk to human health deriving from food or feed, this information is immediately notified to the Commission under the RASFF. The Commission immediately transmits this information to the members of the network. Article 50.3 of the Regulation lays down additional criteria for when a RASFF notification is required.

Without prejudice to other Community legislation, the Member States shall immediately notify the Commission under the rapid alert system of:

- any measure they adopt which is aimed at restricting the placing on the market or forcing the withdrawal from the market or the recall of food or feed in order to protect human health and requiring rapid action
- any recommendation or agreement with professional operators which is aimed, on a voluntary or obligatory basis, at preventing, limiting or imposing specific conditions on the placing on the market or the eventual use of food or feed on account of a serious risk to human health requiring rapid action
- any rejection, related to a direct or indirect risk to human health, of a batch, container or cargo of food or feed by a competent authority at a border post within the European Union.

The system differentiates between 'market' notifications and 'border rejections'. Market notifications are about products found on the Community territory for which a health risk was reported. Products that are subject of a border rejection never entered the Community and were sent back to the country of origin, destroyed or give another destination.

These notifications report on health risks identified in products that are placed on the market in the notifying country. The notifying country reports on the risks it has identified, the product and its traceability and the measures it has taken. According to the seriousness of the risks identified and the

distribution of the product on the market, the market notification is classified after evaluation by the Commission Services as alert notification or information notification before the Commission transmits it to all network members.

An 'alert notification' or 'alert' is sent when a food or a feed presenting a serious risk is on the market or when rapid action is required. Alerts are triggered by the member of the network that detects the problem and has initiated the relevant measures, such as withdrawal/recall. The notification aims at giving all the members of the network the information to verify whether the concerned product is on their market, so that they can take the necessary measures.

Products subject to an alert notification have been withdrawn or are in the process of being withdrawn from the market. The Member States have their own mechanisms to carry out such actions, including the provision of detailed information through the media if necessary.

An 'information notification' concerns a food or a feed on the market of the notifying country for which a risk has been identified that does not require rapid action, e.g. because the food or feed has not reached the market or is no longer on the market (of other member countries than the notifying country). A 'border rejection notification' concerns a food or a feed that was refused entry into the Community for reason of a health risk.

A 'news notification' concerns any type of information related to the safety of food or feed which has not been communicated as an alert, information or border rejection notification, but which is judged interesting for the food and feed control authorities in the Member States.

News notifications are often made based on information picked up in the media or forwarded by colleagues in food or feed authorities in Member States, third countries, EC delegations or international organisations, after having been verified with the Member States concerned.

As far as market and border rejection notifications are concerned, two types of notifications are identified:

- an 'original notification' is a notification • referring to one or more consignments of a food or a feed that were not previously notified to the RASFF

- a 'follow-up notification' is a notification, which is transmitted as a follow-up to an original notification.

An original notification sent by a member of the RASFF system can be rejected from transmission through the RASFF system, after evaluation by the Commission, if the criteria for notification are not met or if the information transmitted is insufficient. The notifying country is informed of the intention not to transmit the information through the RASFF system and is invited to provide additional information allowing the Commission to reconsider the intended rejection. In the other event the notifying country agrees with the rejection. A notification that was transmitted through the RASFF system can be withdrawn by the Commission at the request of the notifying country if the information, upon which the measures taken are based, turns out to be unfounded or if the transmission of the notification was made erroneously.

SCHEMATIC REPRESENTATION OF THE INFORMATION FLOW OF THE RASFF

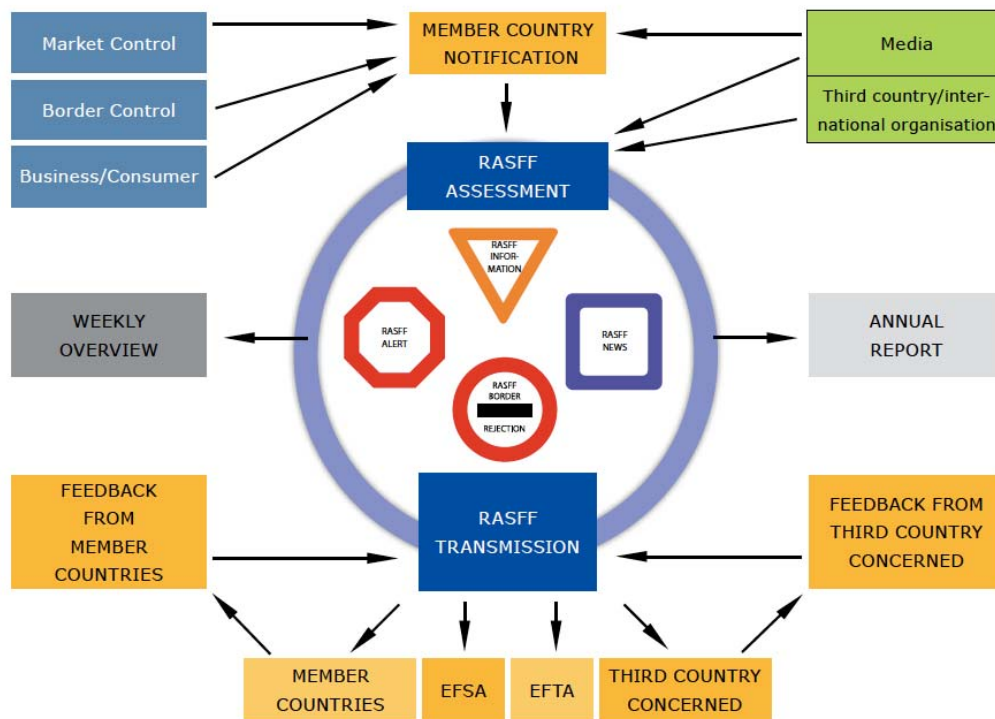


Figure 1: Schematic representation of the information flows of the Rapid Alert System for Food and Feed (http://ec.europa.eu/food/food/rapidalert/about_rasff_en.htm).

Italian Official Control Plan

The Decree of the Italian Ministry of Health of 23 December 1992 transposing Directive 90/642/EEC on the maximum residue limits for active substances in medical devices and food permissible, provided the minimum requirements to the Regions and Autonomous Provinces of Trento and Bolzano to program the controls on residues of active substances by the local health units (90/642/EEC).

The decree contains tables showing the number of samples in each Region and Autonomous Province matrix for the following foods: vegetables, fruits, cereals, wine, oil, meat, dairy and eggs. They are divided into separate tables samples to be collected for food products within the region or province and those for food from outside the Region or Autonomous Province of reference. The Departments of States / Provinces shall use the Prevention Departments of ASL (Local Health Department) for collecting food samples, which are analyzed by laboratories (ARPA, IZS). The latter shall send the results on residues of plant protection products, directly and via the Web, the Ministry - Directorate General of Food Security and Nutrition.

Regional planning is made taking into account the minimum value indicated by the Directive 90/642/EEC and the data production and consumption of fruits and vegetables (90/642/EEC). In particular, it contained the details of the number of samples expected in the Region or Autonomous

Province, and the number of laboratories that sent data over the Web data analysis for the detection of pesticide residues, the minimal total number of samples Fruit set by the National Plan for Pesticides Residues is equal to 4370, including 2361 and 2009 fruit and vegetable.

The recommended sampling points are for crop production: the corporate and cooperative collection centres for products coming within the Region or Autonomous Province, the general markets specialised, non-specialised wholesale stores, hypermarkets and supermarkets for products from the outside the Region or Autonomous Province.

For products of animal origin: the slaughter plants, the company collection centres, shopping centres for products from within the Region or

Autonomous Province, the general markets specialized, non-specialized wholesale deposits, the hypermarkets, supermarkets and merchants for various products from outside the region or autonomous province.

Following the entry into force of Regulation 396/2005 on harmonized maximum residue limits in food the European Food Safety Authority (EFSA) has introduced new procedures for data collection.

New modes of transmission are described in the note of 16 June 2010 (Note 16 June 2010) of the Italian Ministry of Health, where data collection have to be sent by the reference laboratories to the Ministry of Health in eXtensible Markup Language (XML) format; integrated in the new flows of health information system (NSIS).

Current Scenario in Italy

The official controls on pesticide residues in food is one of the most important public health priority in food safety and has the aim of ensuring a high level of consumer protection.

The Italian Ministry of Health - Department of Veterinary Public Health, Nutrition and Food Safety coordinates and establishes programs of official controls on foodstuff.

These are part of a coordinated program of official control provided by the European Union on domestic food production and import activities, to investigate the presence of food matrices where the maximum permitted levels is eventually overcome.

In order to implement this program refers to the Ministerial Decree of 23 December 1992 (90/642/EEC) laying down the annual plans of control on residues of plant protection products, and Regulation 882/2004/EEC on official controls (882/2004/EEC). For the method of sampling refers to the Ministerial Decree dated 23 July 2003 (2004/44/EEC).

The aforementioned Ministerial Decree (1992) includes a detailed program of implementation of controls within the region and autonomous provinces, indicating the minimum number and type of samples for analysis. The distribution of samples for each Region and Autonomous Province is calculated from data on consumption and production of food involved

(90/642/EEC).

Regulation 882/2004/EEC lays down general principles instead of performing official controls to verify compliance with the law, establishing the characteristics required of the official control laboratories, procedures, activities, methods and techniques to make controls. The analyses for the detection of residues of plant protection products are performed by the laboratories, Regional Agencies for Environmental Protection/Prevention (ARPA) and Zooprohylactic Institutes (IZS) (882/2004/EEC).

Laboratories should be accredited and the analytical methods they use must be validated. Furthermore, these laboratories need to report the results of analysis to the Ministry. The data are also used for official testing by the Institute of Health to obtain an estimate dietary intake of pesticide residues in the diet in Italy.

Monitoring of Pesticide Residues in Products of Plant Origin in Lombardy (Italy)

The official controls on pesticide residues in food are one of most important health priorities in food safety, in Italy, and it has the aim of ensuring a high level of consumer protection. The Italian Ministry of Health – Department for Health Veterinary Public, Nutrition and Food Safety – DG Food Safety and Nutrition (D.G.S.A.N), coordinates and defines programs in Italy official control on food, including for the annual plans on pesticide residues in food. These latter are part of a coordinated program of official control provided by the European Union on food production domestic and import activities, see the actual presence of maximum permitted residues in foodstuffs.

To implement this program refers to the Decree of the Italian Ministry of Health of 23 December 1992 (23 December 1992), which defines the annual plans of control on residues of plant protection product, and Regulation 882/2004/EEC (882/2004/EEC) provides a detailed program of implementation of controls within the Regions and Provinces autonomous, with an indication of the other the minimum number and type of samples to be analysed. In this respect it is useful to mention that the minimum number of samples, to be analysed, is 434 (plant origin) exclusively for Lombardy. In addition, the distribution of samples for each Region and Autonomous Province is calculated from data on consumption and on the production of food concerned.

The EC Regulation 882/2004/EEC (882/2004/EEC) lays down general criteria for hand the checks formal verification of compliance by establishing the characteristics that must own laboratories for the official control, procedures, activities, methods and techniques to carry out checks.

The analyses for the detection of pesticide residues are carried out by official control laboratories (Regional Agency for Environmental Protection / Local Prevention laboratories and Zooprophyllactic Experimental Institutes). Under Regulation 882/2004/EEC (882/2004/EEC) have been set up National Reference Laboratories, which are coordinated by the EU Community Reference Laboratory.

Laboratories should be accredited and analysis methods they used are validated. Also they shall report the results of analysis to the Ministry of Health (63/2002/EEC). In addition, the Italian Institute of Health (ISS) also uses the data of the official control plan for an estimation of the dietary intake of pesticide residues of plant in comparison with the Italian diet.

The data described and analysed in this section refers to the official monitoring plan of pesticide residues, for samples from Lombardy from 1996 to 2008; this plan has to the main objectives of:

- Assess the risk to public health arising the degree of contamination of food
- Distinguish the performance of controls on residues plant protection products in food of vegetable origin carried out in Italy by all Departments central and territorial health

The nature of this summary report provides a framework both general and detailed on the results and provides guidance on future actions to undertake to improve and further strengthen the Italian control system of residues of plant protection products, to ensure adequate levels of food safety.

Introduction

The Decree of the Minister of Health of 23 December 1992, transposing Council Directive 90/642 (90/642/EEC), relating to maximum residue limits for active substances in medical devices tolerated in and on foodstuffs, provided the minimum requirements to the all Italian Regions, including Lombardy to program the controls on residues of active substances by ASL.

The decree contains tables showing the number of samples in each region for the following matrices foods: vegetables, fruits, cereals, wine, oil, meat, dairy products and eggs. In the following sections of the thesis, food products of plant origin would be exclusively considered.

Local Health Units, in Lombardy, are in charge for collecting food samples, which are analysed by official laboratories. These shall send the

results of residue plant protection products, directly via web, to the Italian Ministry of Health.

Regional planning takes account of the minimum value indicated by the Council Directive 90/642/EEC (90/642/EEC) and the data production and consumption of fruit and vegetables.

In addition, for foodstuffs of plant origin sampling points are recommended as like collection centres and cooperative company for products from within the region, the general markets specialised, non-specialised stores wholesale hypermarkets and supermarkets for products coming from outside the region.

For the sampling method it refers to the Ministerial Decree dated 23 July 2003 (23 July 2003) implementation of Council Directive 2002/63/EEC (2002/63/EEC).

The maximum residue levels of active substances of plant protection products, with its conventional classification and ranges security, which must elapse between the last treatment and harvest for food or stored home use, are reported in staff in the Decree of the Italian Minister of Health of 27 August 2004 (24 August 2004) and its subsequent amendments.

Further efforts were intensified by regional government to bring the laboratories, carrying out analysis for the official control of food products, to the general criteria of testing laboratories, of Lombardy, which will all be credited from 1st January 2010.

Materials and methods

The data were reported by Local Health Unit of Lombardy (ASL) in appropriate paper forms provided by the Prevention Unit, Department of Health of the Lombardy Region, which were sent for processing.

The collection form of analytical results indicated:

- Outcome of the analysis (regular, irregular)
- Origin of the sample (Lombardy, Italy (excluding Lombardy, European State, Non-European States and Unknown).

Regular samples are defined as those with residue of pesticide below the legislative limit (MRL) and they were accompanied by the name of the

active ingredient found and the relative amount expressed in mg/kg, while irregular ones were characterised by the name of the active ingredient with the value found above the MRL.

The data from monitoring campaigns conducted by the Lombardy Region since 1996, were collected by the International Centre of Pesticide Safety and Health Risk Prevention (ICPS) in an Access database. The data are available, by year and food matrix, in one section of the web sites of ICPS in collaboration with the General Health Directorate of Lombardy Region (<http://www.icps.it/residui.asp>).

The data was extracted from access database in order to show the cluster elaboration and summary statistics for Region, as such, and disaggregated by ASL. In addition the number of samples analysed, the number of active inquiry, the number of samples overcoming of the MRL, name of the active ingredients found also divided by functional class and chemical class.

The distribution of the active ingredients found in food matrices for functional class and chemistry class was designed to allow comparison with similar tables drawn from the database Fitoweb (<http://www.icps.it/fitoweb290/>) on the centralised collection of sales data as stated in the Lombardy Regional Council of 25 November 2002 No. 11225.

The allocation of functional class and the class of chemicals was carried out using the database built for "Pestidoc" currently available on the website (<http://www.icps.it/ITALIANO/Pestidoc/index.htm>).

For active substances that were found with a level of residual greater than the maximum allowed, by law, was performed an investigation into the status of their assessment in European or international level. For this the database built by the European Commission, D.G. SANCO with the purpose of providing a useful search tool for all the MRLs, as set in the European Directive 396/2005/EEC (396/2005/EEC), was consulted. It is freely available at http://ec.europa.eu/sanco_pesticides/public/index.cfm and contains information on active substances and their placing on the market according to the Council Directive 91/414/EEC (91/414/EEC) and MRL according to the Commission Regulation 396/2005 (396/2005/EEC), with updates on 05/08/2010.

In addition, it should be noted that the excess of an MRL does not pose itself a risk in terms of chronic exposure, if the excess is sporadic. The situation is different from the risk assessment acute exposure (food consumed in a meal or within 24 hours). In this case, the occurrence of an overrun could potentially cause damage to health when the intake calculation would results in an overrun of the reference value of concern.

Results

The Italian Decree 23 December 1992 (23 December 1992) in Annex 1, Section 6 anticipates sending the results of analysis performed by the official laboratories of each Italian region, by a web-based tool to Ministry of Health, in the manner prescribed by the Decree of the Minister of Health 22 April 1991 (22 April 1991).

The transmission of analytical results for each year must be completed by 31 March of the following, in order to transmit the EFSA within the deadline 31 August the following year.

In accordance with Regulation (EC) No 396/2005, the task of preparing the Annual Report on Pesticide Residues has been transferred to the European Food Safety Authority (EFSA), therefore please see the EFSA website <http://www.efsa.europa.eu/en/prapertopics/topic/pesticides.htm> for reports subsequent to 2006. Annual Reports prior to 2006 are available at the website of the European Commission, DG SANCO, at http://ec.europa.eu/food/fvo/specialreports/pesticides_index_en.htm.

European Overview

As mentioned in the first chapter of the thesis each European country has to produce an annual report on pesticide residues detected in food commodities. Therefore, comparing the total number of analysed samples, as mentioned in the Annual Report on Pesticide Residues prepared by EFSA; the total number of samples taken in the context of the national programmes in 2008 was 70.143. Italy had analysed 6788 samples, which are the second highest results, Germany and France, first and third in the ranking, had

analysed 15683 and 6383, respectively. These results highlight that almost each country had analysed a number of samples much higher compared with those requested, by law, in the European Regulation 396/2005 (396/2005/EEC). In order to give a complete set of information in figure 2 it is shown that the number of analysed samples (red bar) compared with the respective population (blue bar), expressed in million of inhabitants.

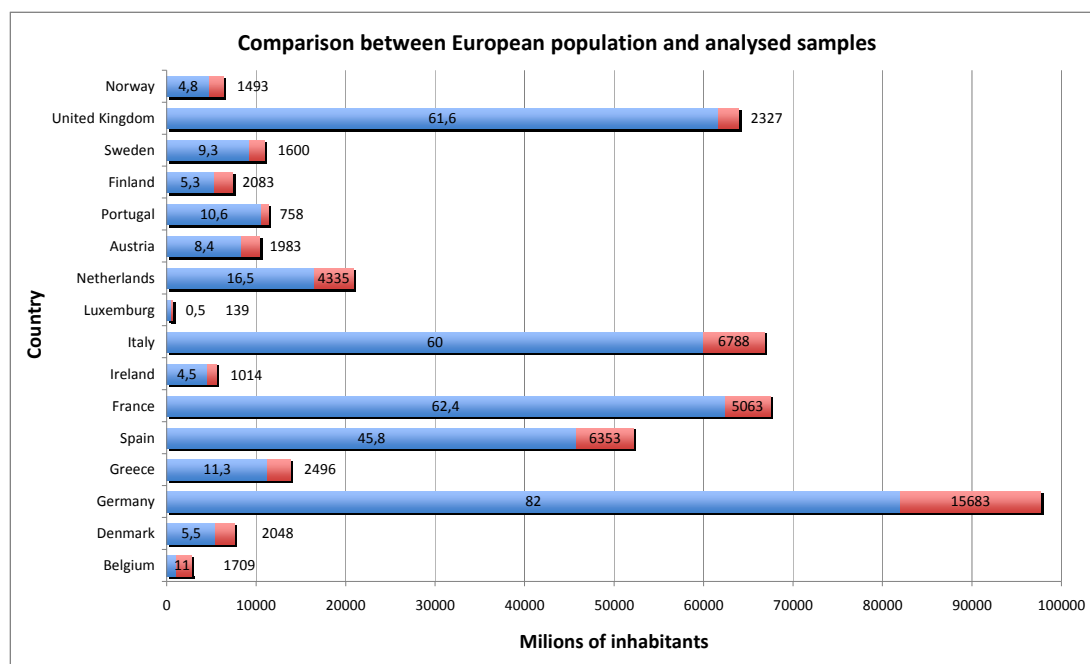


Figure 2: Population of different European countries, expressed in million of inhabitants (from Eurostat, updated on 1st January 2009), compared with the total number of samples analysed, for the detection of pesticide residues.

Data processing

The data analysis carried out on plant protection products from 1996 to 2008 were prepared by official laboratories and collected by the Lombardy Region, DG Health, Prevention Unit.

Investigations conducted by the laboratories considered products of vegetable origin: fruits, vegetables, cereals, wine, oil, processed products and baby food.

The same data, with particular reference to the results of the European Coordinated Programme, will be the subject of the report to the European Union. The objectives are intended primarily to verify the results at regional level (Lombardy) that, together with other Italian Regions, will lead to the national control plan in terms of the outcome of tests carried out at the end

of an accurate assessment of risk to public health arising from the degree of contamination of foodstuffs. In particular, the development focused on the following aspects:

- size of the sampling
- food matrices analysed
- summary of results
- incidence of residual
- irregularities
- active substances used

During the years taken into consideration 15 official control laboratories, under the supervision of the Prevention Units of the Lombardy Region collected and analysed the food sample. Due to the implementation of the official control plan of the Lombardy Region, during the period from 1996 to 2001 the official laboratories were only 12; therefore new laboratories were created and some of the remaining were renamed. The details are shown in the Table 1.

Table 1: Official laboratories, named as the capital of the various districts of the Lombardy Region.

| | Official Laboratory | | | | | | | | | | | | | |
|-----------|---------------------|---------|------|---------|-------|------|---------|-----------|----------|----------|--------|-------|---------|----------------|
| 1996-2001 | Bergamo | Brescia | Como | Cremona | - | - | Mantova | Parabiago | Varese | - | Milano | Pavia | - | - |
| 2002-2008 | Bergamo | Brescia | Como | Cremona | Lecco | Lodi | Mantova | Milano 1 | Milano 2 | Milano 3 | Milano | Pavia | Sondrio | Vallecarnonica |

During the twelve years of official control plan, for the Lombardy Region, a total number of 9837 samples were collected and analysed. The average is about 722 samples per year, which is much higher than the number of 434 samples suggested by the Decree of the Italian Ministry of Health of 23 December 1992 (23 December 2002). Only the year 1998 the number of collected samples was below the recommended value.

In accordance to the above-mentioned Regulation, from 1992 it was introduced the mandatory sampling of foodstuffs obtained from organic farming (column 'Organic' in table 2). The two main categories of sampled products were fruits and vegetables with 4266 and 3179 samples, respectively. In addition the category 'Other' contains a series of food products not referred to in any of the other categories. Spices, herbs, processed products and herbal infusions compose this food category.

Table 2: General results of the official control plan of the Lombardy Region of pesticide residues in food from 1996 to 2008.

| | N. Tot. Sample | Organic | Fruit | Vegetable | Cereal | Animal Origin | Other |
|------------|----------------|------------|-------------|-------------|------------|---------------|-------------|
| 1996 | 1001 | - | 550 | 243 | 96 | 44 | 68 |
| 1997 | 714 | - | 283 | 277 | 68 | 39 | 47 |
| 1998 | 284 | - | 86 | 115 | 45 | 21 | 17 |
| 1999 | 488 | - | 175 | 210 | 36 | 28 | 39 |
| 2000 | 502 | - | 222 | 181 | 61 | 23 | 15 |
| 2001 | 683 | - | 297 | 221 | 82 | 17 | 66 |
| 2002 | 756 | 36 | 311 | 291 | 79 | 2 | 109 |
| 2003 | 587 | 14 | 274 | 197 | 52 | - | 78 |
| 2004 | 773 | 75 | 355 | 298 | 47 | - | 148 |
| 2005 | 1051 | 83 | 428 | 377 | 53 | - | 276 |
| 2006 | 951 | 72 | 432 | 302 | 40 | - | 249 |
| 2007 | 880 | 71 | 513 | 240 | 14 | - | 184 |
| 2008 | 717 | 100 | 340 | 227 | 18 | - | 232 |
| Tot | 9387 | 451 | 4266 | 3179 | 691 | 174 | 1528 |

In order to go more in dept in the analysis of the food matrices used for the official plan of monitoring of pesticide residues in food, all the collected samples were sub-divided in food classes, as described in the European Pesticides Database, from the DG SANCO website (http://ec.europa.eu/sanco_pesticides/public/index.cfm).

As shown in Table 3 the class of fruits, vegetables and processed products are those food clusters with the majority of collected samples.

Table 3: Official control plan of the Lombardy Region of pesticide residues in food, the results are disaggregated by food matrices.

| | 1996 | 1997 | 1998 | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 |
|--------------------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|-------------|-------------|------------|------------|
| Animal Origin | 44 | 39 | 21 | 28 | 23 | 17 | 2 | - | - | - | - | - | - |
| Berry and Small Fruit | 52 | 54 | 12 | 11 | 26 | 66 | 42 | 44 | 52 | 69 | 50 | 61 | 33 |
| Brassica Vegetable | 4 | 12 | 1 | 9 | 3 | 10 | 10 | 9 | 14 | 15 | 10 | 42 | 14 |
| Bulb Vegetable | 1 | 12 | - | 3 | 7 | 1 | 8 | 2 | 6 | 9 | 6 | - | 10 |
| Cereal | 96 | 68 | 45 | 36 | 61 | 82 | 79 | 52 | 47 | 53 | 40 | 14 | 18 |
| Citrus Fruit | 91 | 48 | - | 25 | 56 | 45 | 62 | 52 | 69 | 114 | 124 | 79 | 106 |
| Fruiting Vegetable | 82 | 107 | 44 | 75 | 37 | 84 | 79 | 54 | 88 | 121 | 75 | 98 | 78 |
| Fungi | - | 1 | - | 1 | - | 2 | - | - | 4 | 3 | 3 | 2 | - |
| Herb | - | - | - | - | - | - | - | - | - | 1 | 3 | - | - |
| Herbal Infusion | 2 | 15 | - | 12 | - | 1 | - | 1 | 2 | 5 | 4 | 1 | - |
| Leaf Vegetable | 62 | 83 | 38 | 65 | 52 | 39 | 81 | 18 | 75 | 75 | 78 | 108 | 33 |
| Legume Vegetable | 19 | 8 | 6 | 4 | 7 | 7 | 12 | 12 | 8 | 15 | 16 | 74 | 12 |
| Miscellaneous Fruit | 34 | 36 | 9 | 31 | 22 | 47 | 51 | 30 | 49 | 30 | 61 | 53 | 39 |
| Oilseed | - | - | - | - | - | - | 6 | 1 | 3 | - | - | 3 | - |
| Pome Fruit | 323 | 65 | 17 | 61 | 64 | 89 | 84 | 92 | 119 | 137 | 141 | 137 | 111 |
| Processed Product | 59 | 31 | 17 | 26 | 14 | 60 | 96 | 77 | 143 | 261 | 203 | 154 | 227 |
| Root and Tuber Vegetable | 59 | 48 | 25 | 48 | 72 | 71 | 94 | 96 | 86 | 124 | 101 | - | 73 |
| Spice | 7 | - | - | - | 1 | 3 | 13 | - | 3 | 6 | 36 | 24 | 5 |
| Stem Vegetable | 7 | 7 | 1 | 6 | 3 | 9 | 7 | 6 | 21 | 18 | 16 | 16 | 7 |
| Stone Vegetable | 59 | 78 | 48 | 47 | 53 | 49 | 66 | 54 | 57 | 76 | 54 | 83 | 51 |
| Tree nut | - | 2 | - | - | 1 | 1 | - | 1 | 2 | 2 | 2 | 2 | - |
| TOT. | 1001 | 714 | 284 | 488 | 502 | 683 | 792 | 601 | 848 | 1134 | 1023 | 951 | 817 |

In particular, analysing the results more in depth it is shown that the food categories with the major number of samples are as follows:

- Pome fruit
(which includes: apples, pears, quince, ...)
- Processed product
(which includes: juice, homogenised, wine, tomato sauce, ...)
- Fruiting vegetable
(which includes: tomato, pepper, aubergine, ...)
- Root and tuber vegetable
(which includes: potato, carrot, cassava, beetroot, ...)
- Citrus fruit
(which includes: grapefruit, orange, lemon, mandarin, ...)
- Leaf vegetable
(which includes: lettuce, scarole, cress, ...)
- Stone fruit
(which includes: apricot, cherry, peach, plum, ...)
- Cereal
(which includes: barley, maize, rice, millet, ...)
- Berry and small fruit
(which includes: grapes, blackberry, strawberry, ...)
- Miscellaneous fruit
(which includes: date, fig, table olive, ...)

In order to give detailed information a percentage of each of the mentioned food classes is mentioned in the Figure 3, where the most relevant food class is constituted by 'POME FRUIT' (15%), followed by 'PROCESSED PRODUCT' (15%) and 'CITRUS FRUIT, ROOT AND TUBER VEGETABLE' (10%).

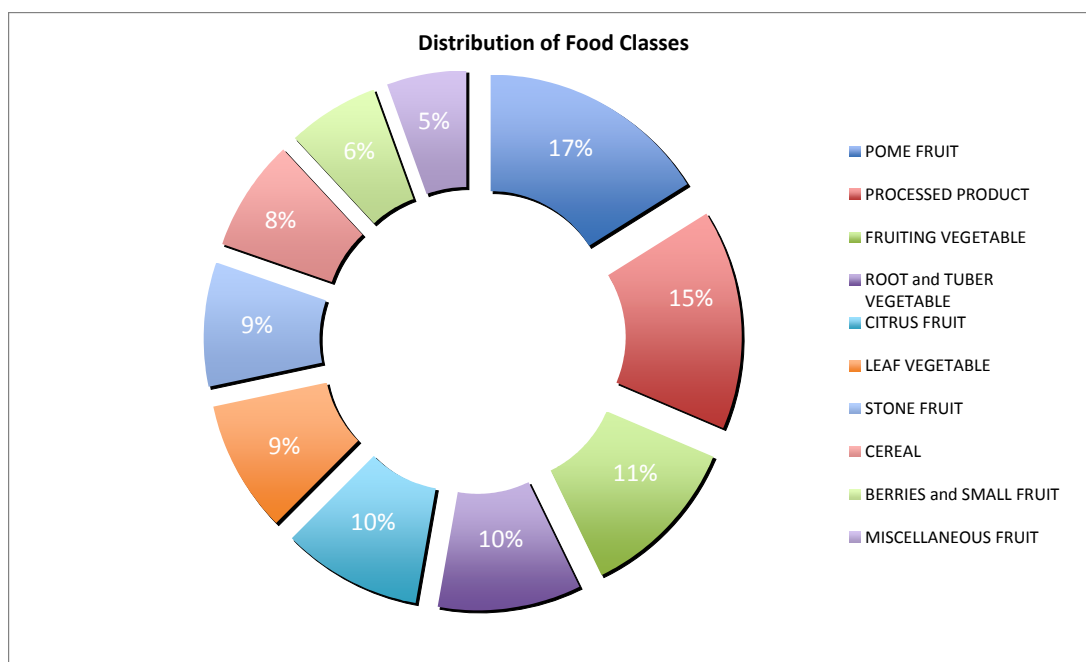


Figure 3: Commodities sampled during the official control plan of the Lombardy Region of pesticide residues in food, the data are disaggregated by food classes.

The analysed samples contained information related to the origin of the foodstuff divided in Lombardy, Italy (excluding Lombardy), European Union (EU), Extra- EU and unknown, as shown in Table 4.

Table 4: Origin of the food matrices, sampled during the official control plan of the Lombardy Region of pesticide residues in food, the results are disaggregated by country of origin and year of sampling.

| | Lombardy | Italy, excluding Lombardy | EU, excluding Italy | Extra EU | Unknown |
|------------|-------------|---------------------------|---------------------|------------|------------|
| 1996 | 510 | 280 | 6 | 1 | 204 |
| 1997 | 210 | 296 | 50 | 42 | 116 |
| 1998 | 107 | 100 | 15 | 5 | 57 |
| 1999 | 185 | 224 | 16 | 22 | 41 |
| 2000 | 147 | 215 | 35 | 48 | 57 |
| 2001 | 158 | 288 | 41 | 72 | 124 |
| 2002 | 234 | 311 | 41 | 104 | 102 |
| 2003 | 163 | 335 | 26 | 41 | 36 |
| 2004 | 306 | 405 | 44 | 57 | 36 |
| 2005 | 471 | 512 | 50 | 66 | 35 |
| 2006 | 302 | 487 | 44 | 170 | 20 |
| 2007 | 325 | 449 | 45 | 125 | 7 |
| 2008 | 381 | 335 | 36 | 50 | 15 |
| TOT | 3499 | 4237 | 449 | 804 | 850 |

The majority of the food matrices analysed are produced within the Italian border (4327) and from the Lombardy Region (3499), this is due to the fact that the sampling plan should reflect the dietary habits and production system of the district where the samples are collected.

Taking as example the district of Pavia, linked to one of the 15 official laboratories in charge of detecting residue of pesticides in food is a well known area for rice production. As a consequence, similar results could be shown for other districts of Lombardy with high production of local and typical food products.

It is also useful to mention that matrices coming from countries outside Europe are analysed by the Inspection Borders and in case a bench of foodstuffs are found with residue of pesticides above the legislative permitted level, they are immediately withdrawn from the country. Therefore, they do not pose an unacceptable risk, per se, for the consumers.

Residue distributions

During the monitoring plan of pesticide residues in food, from 1996 to 2008, a total of 9837 samples were analysed and in 70% of a pesticide residue below the limit of determination was found. In 19% of the total samples only one residue of pesticide was found and in the remaining 11% sample with more than two residues were found, up to nine pesticides residues detected in a sample of pear, analysed in the district of Milan, during the sampling plan of the year 2000.

In addition, it is worth to mention that especially in the fruit, a greater presence of samples with single and multi-residues were found, as compared in vegetables.

This is probably explained by the fact that tree fruit are treated more actively (fruit growing seasons are more long) and they are subject to multiple treatments in their vegetation cycle, both during the flowering, the fruiting and post-harvest.

It is important to mention that the total number of samples where no residues were found is approximately 69% of the total collected samples during the twelve years of monitoring plan taken into consideration; however 30% constituted samples with residue below the MRL and only 1% represented samples with residue concentrations above the maximum permitted level. Details are shown in Table 5.

Table 5: Sample residue distributions according to the detected level of pesticides' residue, the results disaggregated per year of sampling.

| | < LOD | LOD<Residue<MRL | > MRL |
|------|-------|-----------------|-------|
| 1996 | 471 | 516 | 14 |
| 1997 | 411 | 289 | 14 |
| 1998 | 191 | 90 | 3 |
| 1999 | 336 | 147 | 5 |
| 2000 | 354 | 148 | - |
| 2001 | 451 | 217 | 15 |
| 2002 | 578 | 206 | 8 |
| 2003 | 433 | 158 | 10 |
| 2004 | 629 | 210 | 9 |
| 2005 | 866 | 257 | 11 |
| 2006 | 749 | 246 | 28 |
| 2007 | 724 | 215 | 12 |
| 2008 | 682 | 129 | 6 |
| TOT | 6875 | 2828 | 135 |

The top 3 active substances, divided per year, found in food samples are shown below in Figure 4.

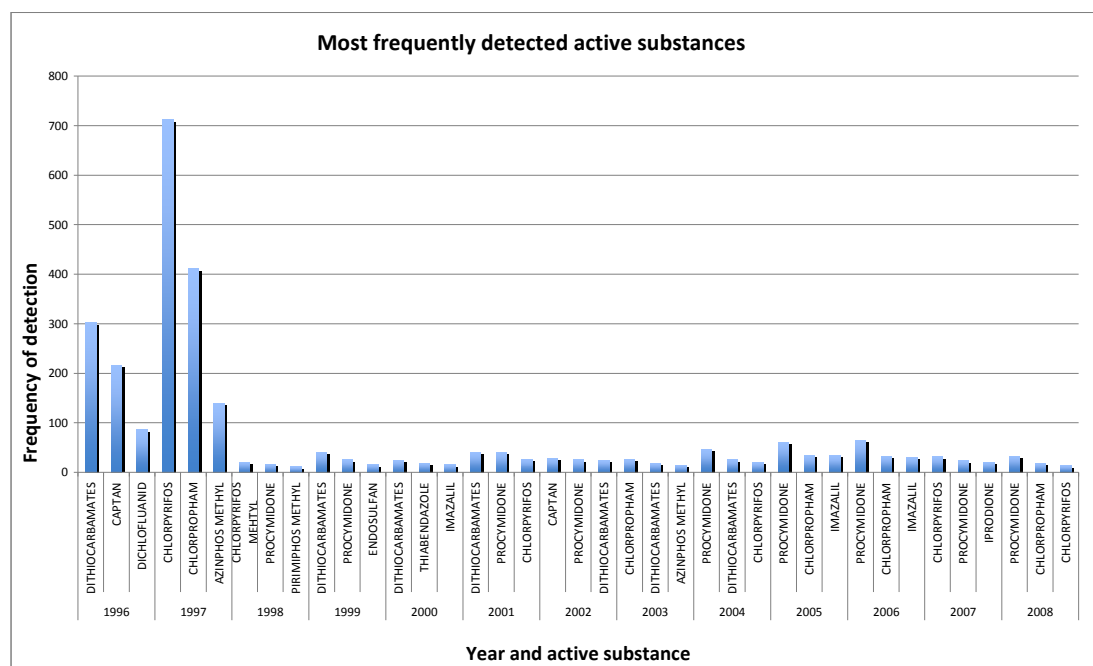


Figure 4: Most frequently detected active substances, divided by year of sampling.

The active substances found more frequent are:

- Chlorpyrifos (insecticide, acaricide)

It is crystalline organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. It is a crystalline

organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests (Ruth, V.A. *et al.* 2006).

- Chlorpropham (plant growth regulator)

It is a plant growth regulator used for pre-emergence control of grass weeds in alfalfa, lima and snap beans, blueberries, cane berries, carrots, cranberries, ladino clover, garlic, seed grass, onions, spinach, sugar beets, tomatoes, safflower, soybeans, gladioli and woody nursery stock. It is also used to inhibit potato sprouting and for sucker control in tobacco (Meister, R.T. 1992).

Chlorpropham is moderately toxic by ingestion. It may cause irritation of the eyes or skin (Occupational Health Services 1992). Symptoms of poisoning in laboratory animals have included listlessness, in co-ordination, nose bleeds, protruding eyes, bloody tears, difficulty in breathing, prostration, inability to urinate, high fevers, and death. Autopsies of animals have shown inflammation of the stomach and intestinal lining, congestion of the brain, lungs and other organs, and degenerative changes in the kidneys and liver (Occupational Health Services 1992).

- Dithiocarbamates (fungicide)

Introduced 40–70 years ago, Dithiocarbamate fungicides still represent an important class widely used in agriculture. They are characterised by a broad spectrum of activity against various plant pathogens, low acute mammal toxicity, and low production costs. In combination with modern systemic fungicides, they are also used to manage resistances and to broaden the spectrum of activity. In Figure 3 they are mentioned as 'Dithiocarbamates' and they comprehend fungicide as Zineb, Maneb, Mancozeb, Propineb and Metiram. In addition, they were some of the most frequently detected pesticides in the European Union, Norway, Iceland, and Liechtenstein, and that this group also had the highest frequency in exceeding maximum residue limits (Crnogorac, G. and Schwack, W. 2009), which refers to total Dithiocarbamates, determined as Carbon Di-sulphite (CS₂) evolved during acid digestion and expressed as mg CS₂/kg.

Active substances not included in Annex 1

According to the Council Regulation 91/414/EEC (91/414/EEC), the active substance authorised on the market are included in the Annex 1 (positive list) of the mentioned Regulation. Taking into consideration exclusively those food samples where the residue of pesticides was found above the legislative limit, it was investigated the possibly wherever some of those substances where not authorised under the Italian legislation. These information were deducted from the Pestidoc database (<http://www.icps.it/pestidoc/>).

In this case, firstly these food matrices could represent an acceptable risk to the health of the consumer, by overcoming the legislative limit and secondly the offence of food fraud, would be reported.

It was then found that in 39 food matrices, with irregular concentration of active substances, the presence of non-authorised pesticides was found. Out of the 39 samples only 6 showed a multi residue concentration with another active substances, included in Annex 1, for the remaining 33 a single residue was detected. The active substances present in most of the samples were Carbon Disulphide, banned in 1991, DDT, banned in 1978 and Dieldrin, banned in 1973.

Food matrices were identified as 16 samples of fruits with major contribution from pome fruits (apple, pear) and 20 samples were constituted by vegetables with high number of fruiting vegetable (Solanaceae), the remaining samples were spices and herbs.

Baby food

During the years 2004-2008, 16 samples of baby food were also analysed; the concentration of pesticide residues were found below the MRL. Samples of baby food covered food and milk for infants milk formulas, soy milk, biscuits for weaning, creams for weaning, nectars and fruit juices for weaning, homogenized, mixed weaning foods, cakes and pastries for weaning, products for children for special diets and other products for weaning foods for early childhood.

Conclusions

The data of the official control plan on pesticide residues in food of plant origin showed for the years 1996-2008, that the number of samples analysed by the official laboratories of the Lombardy Region, is equal to 9387 the overall number of samples is higher than the minimal number set by Ministerial Decree of 23 December, 1992 (23 December 1992).

The number of irregular samples was equal to 135 with a percentage of irregularity equal to 1%.

Samples exceeding maximum residue limits (MRL) are considered irregular as established by Council Regulation 396/2005/EEC (639/2005/EEC) which has harmonised across all EU countries such limits. These limits are set taking into account all categories of consumers including vulnerable groups such as children and vegetarian and include all the European diets. The values of MRLs were established in accordance with an assessment made by ' EFSA risk assessment using models of acute and chronic, and for each active substance were considered toxicological parameters most critical to an assessment more conservative risk for the consumer.

The number of samples without residues was equal to 6882 (69%), the number of samples with residues within the legal limit was 2968 (30%).

Taking into consideration the number of irregular samples through the years in consideration, it remained unchanged (1%) in relationship to a continuing raising number of analysed samples in each year. This success is attributable in part to the activities of regional structures permanently engaged in official control plant protection products in Lombardy and in part to constant revision strictly made by the Italian Ministry of Health and a growing awareness of operators in the use of agricultural pesticides.

Special attention is devoted to investigating samples of fruit and vegetables contain more active, which were 1078, the 11% of the total analysed samples.

It must be emphasised that the occasional overcoming of legal limit (MRL) does not entail any danger to consumer health, when compared with to the appropriate reference value (ADI or ARfD). The trend in irregularity showed a progressive improvement in terms of food safety.

Monitoring of Pesticide Residues in Organic Food of Plant Origin in Lombardy (Italy)

The following chapter of the thesis is represented by a scientific article written in collaboration with co-workers and published in *Journal of Environmental Science and Health, Part B* (Pesticides, Food Contaminants, and Agricultural Wastes) in 2007 (Tasiopoulou, S. *et al.* 2007).

Abstract

Organic agriculture, with its restrictions on the use of synthetic chemical inputs, seems to offer a low-residue alternative to conventional methods. In Europe, the Council Regulation n. 2092/91/EEC regulates the production and trade of organic products and foodstuffs; national and regional legislation in Italy gives specific guidance on the surveillance of organic agriculture. However, monitoring of specific chemical residues in organic foodstuffs is part of the regular controls on food, aiming to safeguard consumer's health.

Monitoring programs are coordinated at the national level by the Ministry of Health and at local level by Regional authorities. In Lombardy, in accordance with the provisions of the General Directorate of Health of the Region and under the supervision of the 15 Local Health Units, a monitoring program of pesticide residues in food of plant origin is undertaken every year. The International Centre for Pesticides and Health Risk Prevention (ICPS), on behalf of the General Directorate of Health of the Region of Lombardy, has been collecting and elaborating the data resulting from the analysis of food samples, carried out by the local laboratories. During the period 2002–2005, a total of 3508 samples food of plant origin were analyzed for pesticide residues, among which were 266 samples of organic farming products. Commodities were classified into groups (citrus fruits, legumes, vegetables, potatoes, processed products, cereals, and fruit other than citrus) and the

outcomes of the analyses were reported by year, origin of the sample and presence/absence of pesticide residues. Results showed that the vast majority of organic farming products were in conformity with the relevant legislation and did not contain detectable pesticide residues. A limited amount of samples had residues at concentration below the Maximum Residue Limit (MRL). Only in one sample the residue level was above the MRL, however it did not pose a concern for public health, as demonstrated by the outcomes of dietary risk assessment. Organic fruits and vegetables can be expected to contain fewer agrochemical residues than conventionally grown alternatives.

There is a widespread belief that organic agriculture products are safer and healthier than conventional foods. It is difficult to come to conclusions, but what should be made clear to the consumer is that “organic” does not automatically equal “safe”. In the absence of adequate comparative data, additional studies in this area of research are required.

Key Words: organic food, pesticide residues, monitoring, fruits and vegetables, consumer risk assessment.

Introduction

Over the last years, increased awareness of food safety issues and environmental concerns has contributed to the growth of organic farming as an ecological production management system that sustains the health of ecosystems from the smallest organisms to human beings and enhances biodiversity and ecological harmony. Organic agriculture, with its restrictions on the use of synthetic chemical inputs such as synthetic pesticides and fertilizers, seems to offer a low-residue alternative to conventional methods and interest of consumers in organic food products is rapidly growing (Sahota, A. 2004 and Richter, T. 2005)

The preference of consumers for organic food has been associated with multiple factors, such as personal health, animal welfare, and environmental protection. However, health-related issues seem to assume greater importance than other concerns (Saba, A. 2003). In a recently published Eurobarometer survey (Eurobarometer 2006) measuring people's

concerns associated with food, 42% of the Europeans consider it likely that the food they eat will damage their health. The presence of pesticides in food is clearly regarded as a potential risk for human health and 70% of EU citizens declare that they are “worried” about pesticide residues in fruit, vegetables or cereals (28% “very worried” and 42% “fairly worried”).

Consumers’ belief that organic agriculture products are safer and healthier than conventional foods was also reflected in an Italian study on perceptions and motivation in purchasing organic products, in which consumers associated organic products with health and healthy eating.

Although these products were regarded as rather expensive, most of the consumers judged them positively (Zanoli, R. 2002). This perception is mainly due to the principles associated with organic food production.

In Europe, the Council Regulation EEC n. 2092/91 (91/2092/EEC), concerning production and trade of organic products and foodstuffs, regulates the organic production of unprocessed and processed agricultural crop and livestock products. According to the principles laid down by this Regulation, pest management should be based on a combination of mechanical and agronomical practices; only in case of immediate threat to the crop, recourse to plant protection products is permitted. The substances allowed as plant protection products are listed in Annex II of the Regulation. The list includes substances of plant or animal origin (e.g. pyrethrins, quassia, lecithin, hydrolyzed proteins), substances for use in traps and/or dispensers (pheromones, metaldehyde, pyrethroids), biocontrol microorganisms (e.g. *Bacillus thuringiensis*), preparations to be surface-spread, and other substances traditionally used in organic farming (copper hydroxide, potassium permanganate, sulphur, paraffin and mineral oils). Moreover, the Regulation describes the principles of the inspection system to be implemented by Member States for controlling all stages in the organic production process.

In Italy, the inspection system is operated by private bodies, with the task to check the consistency of the production methods with the organic farming principles. Private bodies are authorized and supervised at the national level by the Ministry of Agricultural Policy and Forestry under the

Legislative Decree (L.D.) n.220/95 (17 March 1995). Besides inspections regarding the production method, organic foods are also submitted to regular controls, with the purpose of safeguarding consumers’

health. In this respect, a national control program is implemented each year under the coordination of the Ministry of Health–Directorate General for Veterinary Health and Food, with the cooperation of the Regions (23 December 1992 and 30 July 1993). The monitoring programs are coordinated at the local level by the Regional Authorities.

In Lombardy, the General Directorate of Health (Prevention Unit) supervises the regional activities, whereas the operational implementation of controls is handled by the Local Health Units (LHU). The International Centre for Pesticide Safety and Health Risk Prevention (ICPS), under the mandate of the regional General Directorate of Health, collects the pesticide analyses results in order to perform dietary risk assessment for pesticide residues in foods of plant origin.

The objective of this paper is to report the results obtained from the analysis of the pesticide residues in organic food samples in Lombardy during the period 2002–2005. The findings will be analyzed in the light of the general frame of the regional monitoring program of pesticide residues in foodstuff of plant origin. Moreover, it is intended to address the question of safety of organic products for the consumer, by performing a dietary risk assessment when, based on the results, this is considered necessary.

Materials and Methods

In accordance with the provisions of the General Directorate of Health of the Region of Lombardy, a defined number of samples were analyzed within the frame of the monitoring program of pesticide residues in food of plant origin. The local laboratories examined both conventional and organic farming products, and delivered their findings to the 15 Local Health Units of the Region: Bergamo (BG), Brescia (BS), Como (CO), Cremona (CR), Lecco (LC), Lodi (LO), Milan (MI1, MI2, MI3, MI Città), Pavia (PV), Sondrio (SO), Valle Camonica-Sebino (VCS) and Varese (VA). Each year, the laboratories were required to provide the list of the active substances analysed, together with the results of the analyses performed. The findings were reported in an apposite form in which the following information was included for each sample: commodity analyzed, sample origin (Lombardy, rest of Italy, rest of

Europe, non-EU countries), result of the analysis. The samples with pesticide residues above the limit of detection (LOD) were characterised by the name/s of the active substance/s detected and respective concentration in mg/kg; samples with pesticide residues above the national MRL (19 May 200 and 27 August 2004) were identified as irregular.

The International Centre for Pesticide Safety and Health Risk Prevention (ICPS), on behalf of the regional General Directorate of Health, has been collecting the pesticide analyses results provided by the Local Health Units. Since 1996, results have been stored in a Microsoft ACCESS database, which was created for this purpose. The Centre elaborates and subsequently communicates aggregate statistics to the general public through its web site (<http://www.icps.it>).

During the period 2002–2005, a total of 3508 samples were analyzed for pesticide residues, among which were 266 samples of organic farming products. It should be mentioned that the control of pesticide residues in organic food was launched in 2002.

For the purpose of this study, the data on samples of organic foodstuffs were extracted from the Microsoft Access database into an EXCEL spreadsheet in order to carry out a statistical analysis. The raw commodities were classified into groups (citrus fruits, legumes, vegetables, potatoes, processed products, cereals, and fruit other than citrus). The outcomes of the statistical analysis were reported by food group, year, and origin of the sample and presence/absence of pesticide residues.

An exposure assessment was performed in the case where the concentration of the pesticide was above the MRL. The estimation of long-term exposure was performed for two different groups of the Italian population (adults, children and toddlers), based on the average consumption data (Turrini, A. *et al.* 2001) and average body weight values reported previously (66, 51 kg for adults and 41, 61 kg for children/toddlers, respectively). The chronic dietary intake of pesticide residues was calculated as follows:

Residue Intake (mg/kg bw/day) = Residue Concentration (mg/kg) × Food Intake (kg/day)/Body Weight (kg).

An acute exposure assessment was performed taking into account the National Estimated Short-term Intake (NESTI) (WHO 1997) as calculated on the basis of the consumption data for the 97.5th percentile of the Italian

eaters' population (adults and children/toddlers) (WHO 1998). The unit weight used in the calculation was derived from the GEMS/FOOD database (WHO 1998) while a variability factor of 7 was applied.

The calculated dietary intake values were compared to the relevant toxicological endpoints (acceptable daily intake, ADI and acute reference dose, ARfD), established for the pesticide of interest. The estimated dietary intake should be less than the established toxicological value (or less than 100%, when expressed as a percent of the toxicological value).

Results

During the period 2002–2005, within the frame of the pesticides monitoring program implemented by the 15 Local Health Units in the region of Lombardy, 3.508 samples of food of plant origin have been analyzed. Among them, 266 (7.6%) were of organic origin (4.6% in 2002, 9.3% in 2003, 9.2% in 2004 and 7.4% in 2005). A summary of the overall results of the program (total number of samples with residues above the MRL), regarding both conventional and organic food samples, is provided in Table 6.

Table 6: Results of the monitoring program of pesticide residues in Lombardy (2002–2005): total number of samples, samples with detectable pesticide residues [single and multiple residue, residue above the maximum residue limit (MRL)].

| Year | Number of samples | | Samples with pesticide residues | | Residues < LOD | | Residues > MRL | |
|--------------|-------------------|------------|---------------------------------|---------------|----------------|------------|----------------|----------|
| | Conventional | Organic | Conventional | Organic | Conventional | Organic | Conventional | Organic |
| 2002 | 755 | 36 | 210 (62)* | 3 | 545 | 33 | 8 | - |
| 2003 | 666 | 68 | 178 (58)* | 2 (1)* | 488 | 66 | 8 | 1 |
| 2004 | 771 | 78 | 220 (96)* | - | 551 | 78 | 9 | - |
| 2005 | 1050 | 84 | 266 (70)* | 2 (1)* | 784 | 82 | 11 | - |
| Total | 3242 | 266 | 874 (286)* | 7 (2)* | 2368 | 259 | 36 | 1 |

* Multi-residue samples

The number of conventional and organic samples analysed and reported per year in every Local Health Unit by the analytical laboratories involved in the program, is shown in Table 7. It should be remarked that in general, higher numbers of food samples analysed correspond to more densely populated areas.

Table 7: Number of samples analysed and reported during 2002–2005 per Local Health Unit in the region of Lombardy and percentage of the population.

| | 2002 | | 2003 | | 2004 | | 2005 | | % Tot Population |
|--------------|------------------------|------------|------------------------|------------|------------------------|------------|------------------------|-------------|------------------|
| | Conventional (Organic) | Total | Conventional (Organic) | Total | Conventional (Organic) | Total | Conventional (Organic) | Total | |
| BG | 150 (22) | 172 | 111 (26) | 137 | 110 (29) | 139 | 242 (38) | 280 | 10,6 |
| BS | 7 (2) | 9 | 29 | 29 | 113 (1) | 114 | 104 (2) | 106 | 11 |
| CO | 66 | 66 | 61 | 61 | 62 | 62 | 64 | 64 | 5,9 |
| CR | 37 | 37 | 36 (3) | 39 | 37 (2) | 39 | 43 (3) | 46 | 3,7 |
| LC | 21 | 21 | 18 (1) | 19 | 24 | 24 | 24 | 24 | 3,4 |
| LO | 8 | 8 | 19 (1) | 20 | 17 (3) | 20 | 26 (3) | 29 | 2,2 |
| MILANO Città | 140 | 140 | 91 (15) | 106 | 114 (26) | 140 | 108 (22) | 130 | 14,5 |
| MI 1 | 53 | 53 | 49 | 49 | 67 | 67 | 85 | 85 | 10,2 |
| MI 2 | 2 | 2 | 42 (3) | 45 | 33 (1) | 34 | 56 (3) | 59 | 5,7 |
| MI 3 | 9 | 9 | - | - | 29 | 29 | 34 (2) | 36 | 11,1 |
| MN | 77 | 77 | 42 | 42 | - | - | 62 | 62 | 4,1 |
| PV | 86 (10) | 96 | 58 (16) | 74 | 61 (16) | 77 | 69 (11) | 80 | 5,5 |
| SO | 44 | 44 | 42 | 42 | 42 | 42 | 26 | 26 | 2 |
| VA | 36 (1) | 37 | 65 (3) | 68 | 58 | 58 | 98 | 98 | 1,1 |
| VCS | 19 (1) | 20 | 3 | 3 | 4 | 4 | 9 | 9 | 9 |
| TOTAL | 755 (36) | 791 | 666 (68) | 734 | 771 (78) | 849 | 1050 (84) | 1134 | 100 |

The number of active substances analysed by the laboratories during the same period is reported in Table 8. Unfortunately, although all laboratories were requested to supply the list of active substances for which analysis was performed, in some cases this information has not been provided. In total, taking into consideration the overlapping analytical capabilities, the number of different active substances analyzed in Lombardy was 194 in 2002, 191 in 2003, 203 in 2004 and 209 in 2005.

Table 8: Number of active substances analyzed per year in Lombardy laboratories (2002–2005).

| | BG | BS | CO | CR | LC | LO | MILANO Città | MI 1 | MI 2 | MI 3 | MN | PV | SO | VA | VCS |
|-------------|----|----|-----|----|----|-----|--------------|------|------|------|----|----|----|----|-----|
| 2002 | 79 | - | 112 | 74 | 61 | 124 | 125 | 21 | - | - | 54 | 25 | 9 | 39 | - |
| 2003 | 70 | - | 114 | 71 | 78 | 125 | 125 | 30 | 118 | - | - | - | - | 43 | 33 |
| 2004 | 69 | - | 114 | 61 | 32 | 141 | 150 | - | 136 | - | - | 28 | - | - | - |
| 2005 | 68 | - | 112 | 70 | 28 | 142 | 127 | 32 | 142 | - | - | 34 | 68 | 23 | 71 |

The different types of organic commodities sampled were classified into seven groups, as shown in Table 9: citrus fruits (20), legumes (4), vegetables (40), potatoes (27), processed products (90), cereals (36), and fruit other than citrus (49). The most frequently sampled foodstuffs were processed products such as pasta, biscuits, fruit and vegetable juices, cornflakes and flour (34%). Rice samples represented the majority of cereals sampled (32), and 12% of the total samples.

Table 9: Number and percentages of organic food commodities sampled in the period 2002–2005 in Lombardy, divided in seven classes.

| | 2002 | 2003 | 2004 | 2005 | Total | % Total |
|-----------------------------------|-----------|-----------|-----------|-----------|------------|------------|
| Citrus Fruit | 1 | 6 | 6 | 7 | 20 | 7,5 |
| Fruits (other than citrus) | 1 | 14 | 15 | 19 | 49 | 18,4 |
| Legume | - | 3 | 1 | - | 4 | 1,5 |
| Vegetable | 3 | 8 | 16 | 13 | 40 | 15 |
| Potato | 3 | 3 | 11 | 10 | 27 | 10,2 |
| Processed Product | 18 | 24 | 19 | 29 | 90 | 33,9 |
| Cereal | 10 | 10 | 10 | 6 | 36 | 13,5 |
| Total | 36 | 68 | 78 | 84 | 266 | 100 |

Approximately 29% of the collected samples originated from Lombardy, 62% from Italy excluding the area of Lombardy, and only a limited number from EU countries (except Italy) and non-EU countries (2,6% and 3% respectively) (Figure 5).

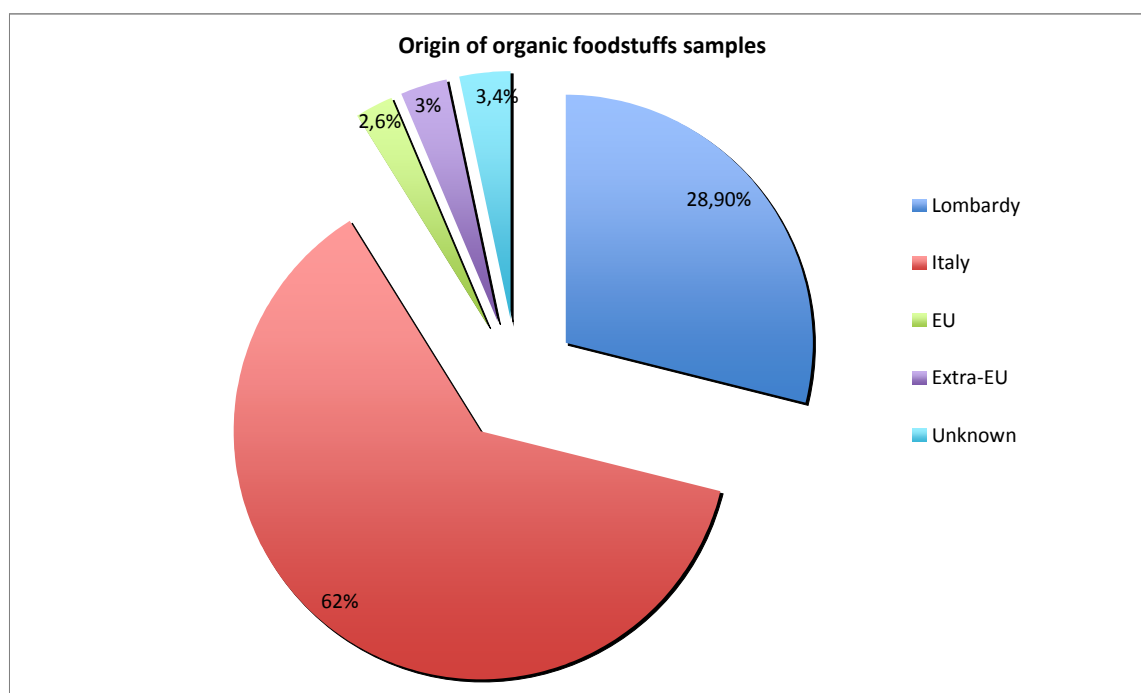


Figure 5: Origin of organic foodstuffs sampled (total number of samples = 266) during the period 2002–2005, in percentage.

Only seven out of 266 organic farming samples contained pesticide residues above the LOD; the active substances detected belong mainly to organophosphorus compounds (Table 10). In two cases multi-residue samples were identified; in one sample of potato four different pesticides were detected (permethrine, tetradifon, dicofol, bromopropylate), whereas one

apple sample was found to contain residues of two active substances (Azinphos-methyl and Carbaryl). In all cases, the concentrations were below the MRL, with the exception of the potato sample where the concentration of one active substance (dicofol) was above the MRL.

Table 10: Lombardy, 2002–2005: samples with detectable pesticide residues, by year, origin, name of active substance detected and chemical class, MRL, residue concentration (expressed in mg/kg of food) and Limit of Detection (LOD).

| | Commodity | Origin | Active Substance | Chemical Class | MRL Italy (mg/kg) | Residue (mg/kg) | LOD (mg/kg) |
|------|-----------|----------|------------------|---------------------|-------------------|-----------------|-------------|
| 2002 | Mandarin | Unknown | Primiphos Methyl | Organophosphorus | 2 | 0,2 | 0,01 |
| | Rice | Italy | Primiphos Methyl | Organophosphorus | 5 | 0,02 | 0,01 |
| | Rice | Italy | Primiphos Methyl | Organophosphorus | 5 | 0,02 | 0,01 |
| 2003 | Kiwi | Extra EU | Permethrin | Synthetic pyetroids | 0,05 | 0,03 | 0,01 |
| | Potato | Italy | Permethrin | Synthetic pyetroids | 0,05 | 0,006 | 0,05 |
| | | | Tetradifon | Organophosphorus | 1,5 | 0,044 | 0,01 |
| | | | Dicofol | Organochlorine | 0,02 | 0,06 | 0,01 |
| | | | Bromopropylate | Benzylate | 0,05 | 0,012 | 0,01 |
| 2005 | Lemon | Italy | Methidathion | Organophosphorus | 2 | 0,22 | 0,01 |
| | Apple | Lombardy | Azinphos Methyl | Organophosphorus | 0,5 | 0,06 | 0,02 |
| | | | Carbaryl | Carbamate | 3 | 0,98 | 0,05 |

The active substances detected in the samples are not included in Annex II of the Council Regulation 91/2092/EEC (91/2092/EEC) and therefore not allowed in organic agriculture. Furthermore, regardless of the fact that their use is only allowed in conventional agriculture, it is reminded that as all plant protection products utilised inside the European Union, these should be authorized under the principles laid down by the Council Directive 91/414/EEC (91/414/EEC). The Directive lays down uniform rules on the evaluation, authorisation, placing on the market and control, within the EU, of plant protection products and the active substances they contain; only products whose active substances are listed in Annex I of the Directive are authorised.

In the case of dicofol, where during the monitoring period 2003, the concentration of residues found in organic potatoes was above the MRL (0.06 mg/kg), a consumer risk assessment was performed. It should be mentioned that the European evaluation for dicofol for inclusion in Annex I of Council Directive 91/414/EEC is still pending (SANCO 2006), and an Acute Reference Dose (ARfD) has not been set. The Acceptable Daily Intake (ADI) was reported at 0.002 mg/kg bw/day (FAO 1992).

Chronic dietary risk assessment (Table 11) performed on the basis of the average consumption data for potatoes for two groups of the Italian population (adults, children and toddlers) (Turrini, A. 2001) shows negligible risk for consumers' health, since dicofol intake is far below the ADI, both for adults (3.5% ADI), and children and toddlers (5%).

Table 11: Chronic dietary exposure of Italian population (adult, children and toddlers) to dicofol found in organic potatoes (monitoring period 2003; residue concentration: 0,06 mg/kg).

| | Body Weight (kg) | Potato Consumption (g/day) | Dicofol Intake ($\mu\text{g}/\text{kg bw}/\text{day}$) | % ADI |
|---------------------------------------|------------------|----------------------------|--|-------|
| Adult (18-64 years old) | 66,51 | 78,7 | 0,071 | 3,5 |
| Children and Toddler (1-17 years old) | 41,61 | 72,1 | 0,104 | 5 |

Regarding acute exposure to dicofol residues, in absence of an established ARfD, a "worst-case scenario" was considered, in which the ADI value was used as the ARfD. Results in Table 12 show that the calculated acute consumption, expressed as a percent of the toxicological endpoint, was below 100%.

Table 12: Acute dietary exposure of Italian population (adult, children and toddlers) to dicofol found in organic potatoes during the monitoring period 2003 (residue concentration 0.06 mg/kg, variability factor 7).

| | Body Weight (kg) | Large Portion (g*) | Unit Weight (g**) | NESTI (mg/kg bw/day) | % ARfD |
|---------------------------------------|------------------|--------------------|-------------------|----------------------|--------|
| Adult (18-64 years old) | 66,51 | 457 | 0,071 | 0,00128 | 63,9 |
| Children and Toddler (1-17 years old) | 41,61 | 394 | 0,104 | 0,00195 | 97,6 |

*Italian population consumption data

** French data

Conclusions

In spite of being properly grown and processed, organic foods are not necessarily free from pesticides and other synthetic chemicals of conventional farming (Magkos, F. *et al.* 2006). Contamination may be due to cultivation on previously contaminated soil, percolation of chemicals through soil, unauthorized use of pesticides, cross-contamination by wind drift, spray drift from neighbouring conventional farms, contaminated groundwater or irrigation water, or even occur during transport, processing and storage. Presence of synthetic chemicals, however, does not necessarily preclude that the food can be described as organic, providing all requirements related to the production process have been fulfilled. Organic fruits and vegetables can be expected to contain fewer agrochemical residues than conventionally grown alternatives.

In our study, the comparison of the monitoring results obtained from conventional and organic food samples showed a 10-fold greater contamination in conventional products (27%) compared to organic food samples (2.6%). Results were similar regarding the presence of multiple residues, present in 0.8% of organic and 8.8% of conventional food samples and in agreement with the findings from other studies (Baker, B.P. *et al.* 2002). In the region of Lombardy, the concentrations of pesticides detected in organic commodities were in their greatest part below the MRL set for conventional products. Only in one sample (organic potatoes), the detected residues were above the MRL; yet the intake of the active substance (dicofol), as calculated for two groups of the Italian population, was far below the ADI (adults 3,5% ADI, children and toddlers 5%). During the same monitoring period, dicofol residues were detected in 20 samples of conventional food products, including potatoes. Dicofol concentrations were below the MRL, with the exception of two samples (pears and strawberries). Therefore, in an attempt to compare organic and conventional foodstuffs in terms of potential risks for human health due to dietary exposure to pesticide residues, conclusions cannot be drawn easily, since in both cases the presence of residues above the set MRL is very low.

The outcomes of the monitoring program of pesticide residues implemented by the Region of Lombardy under the mandate of the Ministry of

Health and with the cooperation of the Local Prevention Units and local laboratories, demonstrate that public health has been safeguarded with success in the recent years. There is a need for more detailed information on analytical methodologies that were used in some of the laboratories . Moreover, given the fact that the complete dataset resulting from the monitoring program is collected and available for elaboration only after the end of each annual monitoring period, improvements in the flow of information are regarded as a prerequisite for checking the completeness of the information provided. It maybe be mentioned that presently actions are being taken in the Region of Lombardy in order to improve the current practices. Furthermore, future efforts will be continued in this direction in order to maintain consumers' trust.

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Deterministic Risk Assessment

In the field of food safety the risk assessment process is established as a means of providing an estimate of the probability and severity of the occurrence of an adverse effect attributable to a particular agent. It should be noted that risks can be prevented and not hazards; the latter being an intrinsic property of an agent. Moreover, the knowledge acquired may be also useful in deciding on the most effective intervention strategies.

Risk assessment is defined as a scientifically based process consisting of the following four steps: i) hazard identification; ii) hazard characterisation; iii) exposure assessment; and iv) risk characterisation.

Hazard identification is the identification of the nature of adverse effects that an agent has as inherent capacity to cause in an organism, system or (sub) population. The step of hazard identification involves a series of in vitro and in vivo studies to define the potential adverse effects of the chemical substance.

The step of hazard characterisation is concerned to define the dose/concentration - response relationship, in order to establish an acceptable intake level, which would be without significant health effect. For dietary intake, hazard characterisation can lead to the definition of reference values: the Acceptable Daily Intake (ADI) for chronic effects and the Acute Reference Dose (ARfD) for acute effects. The ADI expresses the amount of chemical that can be consumed every day for a lifetime without harmful effect, whereas ARfD is the amount of a chemical that can be consumed in a short period of time (one meal or one day) without harmful effects. The critical effect is to be considered in the appropriate study from which the intake dose level that may be consumed without effects from the experiment animals is derived. This level is called the No-Observed Effect Level (NOEL). For humans, the ADI and ARfD values are extrapolated by dividing the NOEL from the appropriate chronic or short-term studies by a safety factor, which accounts for the inter- and intra-species (human variability) differences. Most frequently, a default safety factor of 100 (10 x 10) is applied (Graham, J.D. 1995).

Exposure assessment is the evaluation of the exposure of an organism, system or (sub) population to an agent (and its derivatives). For assessing exposure, it is necessary to specify the amount or concentration of the particular agent that reaches a target organism, system or (sub) population in a specific frequency for a defined duration. In this case, exposure assessment concerns the intake of the substance through diet, which should be defined in terms of concentration of the substance in food and both of food consumption.

Finally, the risk characterisation step involves the determination, including uncertainties, of the probability of occurrence of known and potential adverse effects under defined exposure conditions. Risk characterisation is performed by comparing the estimate of exposure to the safe exposure limit, in this case the ADI or ARfD, for chronic and short term risk respectively.

Introduction

To assess dietary exposure to pesticides it is essential to characterise two main components, food consumption and residue levels of pesticides in food. By combining the two, it is possible to construct intake models that will provide valid exposure estimates. The exposure assessment to pesticide residues through diet significantly differs from the assessment made for other chemical substances such as food additives. This is due to the fact that for pesticides the exposure estimates might be health based and these should be compared to exposure estimates to draw conclusions on risks for health.

Estimating Food Consumption

Estimates of food consumption can be summarised by different parameters, for instance average daily consumption, portion sizes, percentile consumption value related to the total population and “eaters-only” (persons that actually eat the commodity). For predicting long-term exposure, long-term consumption habits and not daily variations must be reflected, thus average daily consumption is commonly used. Examples of food consumption databases used for dietary assessment of chronic pesticide intake are the

World Health Organisation /Global Environment Monitoring System (WHO/GEMS) GEMS/Food Consumption Cluster Diets (WHO, revised August 2006), the German diet database (Banasiak, U. *et al.* 2005 - revised July 2009) and the UK Food consumption databases used within the UK Consumer model (Pesticide Safety Directorate, version 1.1, 2006). Characteristics of these food consumption databases regarding age strata and body weights of the population groups under study are shown in Table 13.

Table 13: Food consumption databases used for dietary assessment of chronic pesticide intake for authorization of pesticides at European level.

| Guideline and Diet | Population group (age and body weight) |
|--|---|
| WHO Guidelines (1997) WHO Cluster Diet (WHO, 2009) | Adult (60 kg bw) |
| WHO Guidelines (1997) German diet (July, 1992) | Children, 2 - 5 years (16. 5 kg bw) |
| UK Consumer Model (PSD, 2006) UK Diet | 10 population groups: Adult, Child (4 classes), Infant Toddler, Elderly (2 classes) |

As regards to the assessment of the acute hazard of pesticide intake, the large amount likely to be consumed in one occasion or in one day (24 hours) is taken into account, based on consumption survey data for individuals for a certain percentile, usually the 97.5th percentile of eaters only. Consumption databases more broadly used for the assessment of acute intake are the GEMS/ Food data sets used by the Joint FAO/WHO Meeting on Pesticide Residue (JMPR) to assess short-term dietary intake of certain

pesticide residues (http://www.who.int/foodsafety/chem/acute_data/en/) and the UK diet (PSD 2006) for adults and toddlers.

National food consumption databases exist in other European countries (Denmark, Finland, France, Germany, Italy, Sweden, the Netherlands, Czech Republic) and world-wide (US, South Africa, New Zealand, Australia) (Boon, E. and van Klaveren J. 2005).

Methods used for data collection and construction of food consumption databases

Sources of food consumption data used for the dietary assessment of contaminants in general and of pesticides more specifically, include food supply data, data from household consumption surveys (household budget method), data collected from individuals and duplicate diets.

- Food supply data, Food Balance Sheets (FBS) is provided at national level and records annual production of food, changes in stocks, imports and exports resulting to an estimate of the average value consumption per capita. FBS give a crude indication where average consumption is slightly over estimated, since waste at the household or individual level is not considered. For example, in the worst cases e.g. for fruits and vegetables it was noted that FBS data are 15% higher than actual consumption compared to detailed national surveys. Moreover, total values for some food groups may be sometimes listed as duplicate entries. International FBS are provided by the FAO (FAOSTAT database), the Organisation for Economic cooperation and Development (OECD) and the Statistical office of the Commission of the European Union-ECE Statistical Division (EUROSTAT). Even though these FBS are compiled in a similar manner, they may be different in food grouping, foodstuffs covered and level of processing of commodities. National FBS compiled by some countries are usually less up-to-date and differ from the international ones.

Using the data from selected FAO Food Balance Sheets, the GEMS/Food Programme (Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme) implemented by the WHO has developed 13 cluster diets (<http://www.who.int/foodsafety/chem/countries.pdf>) comprised of raw and

semi-processed food commodities. These diets are used at international level for assessing long-term dietary intake of contaminants and other agents in food (WHO, 2006). Household budget surveys provide information regarding food availability at the household level, by measuring the purchases of food in terms of expenditure, without further information on the distribution of foods among individual members of the household. Household data may help to refine estimates of the FBS (Kroes, R. *et al.* 2002).

- Individual dietary surveys

Methods that provide information at individual level are more realistic and can be used to increase sensitivity of the crude estimates. Several methods can be used to collect individual dietary data, either by recalling or recording food consumption. Record methods collect information on food intake over one or more days (usually 1-7). Recall methods provide past consumption over the previous day (24 hours recall) or usual food intake (dietary history or food frequency). In food records or food diaries the amounts are recorded by weight after calculating the weights of all ingredients used in the preparation of meals, inedible waste, and cooked weight of the individual portions. In the 24 hours recall method the interviewed subject is asked to recall and describe all food consumed in the previous days, and quantities are usually assessed compared to household measures (e.g. tablespoons etc), food models or photographs. Food frequency questionnaire (FFQ) is a method for assessing food intake over a specified period (daily, weekly, monthly, yearly), consists of a structured questionnaire containing lists of individual foodstuffs or food groups and can be qualitative, semi-quantitative or completely quantitative. Duplicate diet studies, where a duplicate of the foodstuffs consumed is analysed in the laboratory for determining the level of a chemical of interest, have been performed less often. These studies, being more accurate, are used in some cases for the validation of the outcomes of other dietary assessment methods (Kroes, R. *et al.* 2002).

Considerations on food consumption databases

The different methods for estimating dietary intake provide different refinement levels of outputs, spanning from crude per person consumption to detailed individual information. Therefore, they can be used in different circumstances depending on the final aim.

One issue of concern is the fact that existing databases usually considered for dietary risk assessment of pesticide residues are constructed for performing studies on nutritional status of individuals. Thus, they have different aims and consequently their design and points of interest may not be identical. For instance, nutritional studies require data on consumption of total energy, macro and micronutrients, types of lipids etc. whereas other aspects may not be addresses. In contrary, other data would provide useful information for pesticide dietary assessment. This could be for example specific type of fruit or vegetables consumed (e.g. data from apples may differ from peaches etc.), consuming practices (peeled, raw, cooked), up to more detailed data on specific food brands and origin of foods. Moreover, databases developed for risk assessment of pesticide residues require the conversion of food consumed into raw agricultural products. As a result, cooking or other types of food processing are to be taken into account while further translation of meals involves the creation of recipe databases. This last point should be addressed at national level, since the dietary habits may significantly differ between different countries. Also differences in consumption of certain food groups may be due to seasonal fluctuations (e.g. types of fruits and vegetables).

Importance should be given also to specific population groups that are likely to have different consumption patterns but also can be considered more sensitive in respect to the general population as infants and children. For example, it has been noted that the diet of infants and children is composed from a higher percentage of fruits and vegetables compared to adolescents. Food consumption patterns are possible to differ also in some groups of the population due to other reasons (religious practices, location, socio-economic characteristics, beliefs etc.).

Estimating Pesticide Residue Levels

Depending on the purpose of the dietary intake assessment, residue data from supervised trials and surveillance programs may be used. Prior to pesticide authorization, the estimation of intake is based on the results of supervised residue trials performed for this purpose. In the post-marketing assessment of dietary intake monitoring data can be used.

Supervised residue trials

Supervised residue trials are performed in accordance to specific guidelines, in order to assure results to be representative of specific Good Agricultural Practices (GAP). Prior to approval, the potential intake of a pesticide is considered in order to ensure that the exposure levels would not exceed the ADI or ARfD values, and Maximum Residue Limits (MRLs) are proposed accordingly. MRLs represent the maximum concentration of a pesticide residue (expressed as mg/kg) legally permitted if detected in food commodities. MRLs are not proposed according to ADI or ARfD and do not represent toxicological limits but are set on the basis of good agricultural practices that is to say, they represent the safe level when good agricultural practices are followed. Nevertheless, when setting MRLs, justification is required based on estimations of chronic and acute exposure through diet by applying appropriate dietary intake models. Thus, toxicological concerns are also taken into account in order to safeguard consumers' health.

Monitoring data

Monitoring data refers to data gathered from monitoring programs, which are intended to control compliance to the correct use of pesticides and safeguard consumer's health. These programs may have different characteristics and outcomes depending on their final aim. For instance, the U.S. FDA monitoring programme includes regulatory monitoring for surveillance purposes, and incidence/level monitoring to obtain knowledge

about pesticide/commodity combinations of particular interest (U.S. FDA, Centre for Food Safety and Applied Nutrition, Pesticide Program for Residue Monitoring, 1995-2002). Monitoring data are expected to give less conservative and more realistic estimates, since the values recorded are usually lower than the values from supervised field trials.

Several considerations can arise on monitoring data. For instance, when data is available from more than one year there is the question of the data to be selected for assessing dietary exposure e.g. data for how many years should be used, how to treat differences between years or high values due to elevated pressure from disease occurrence, which crops monitoring results to include (e.g. all crops where the pesticide was found or crops where the pesticide was applied) and the sampling scheme followed.

Further considerations on refinement of residue levels estimates

Further refinement while estimating the pesticide residues may be achieved if the following points are considered:

- Residue values after processing

Residues in processed food are usually lower compared to the original raw food commodity due to processing, commercial cooking and preparation. In some cases the processes can result in higher concentrations in specific fractions, for instance when converting fruit to pomace or extracting oil from oil seeds. A number of pesticides are destroyed during heating or boiling, but toxic degradation products may be formed in some cases. Processing factors exist from the JMPR Evaluations of Pesticide Residues and the monographs submitted for registration purposes, nevertheless the latter being under confidentiality. In the coming years this information will be available as foreseen in the recent EC regulation 396/2005.

- Limit of Detection

Another question applying in the case of monitoring data is the evaluation of residue levels under the Limit of Detection. This do not necessary means the absence of pesticide residues but the fact that pesticides may be present at levels which could not be detected or reliably quantified. For the estimates of dietary exposure it can be assumed that all values under the limit of detection are equal to the LOD (worst-case

assumption) rather than zero, which would be the least conservative approach. According to EPA consultations the middle way lies into applying the ½ LOD value, however, a sensitivity analysis is suggested in order to demonstrate the impact of using the different assumptions (EPA 1998).

Where residues are detected above the limit of detection, but below the limit of quantification, a commonly used approach is to assign these results a value of one half of the limit of quantification. If this is done, it is recommended that a sensitivity analysis be performed to evaluate the effect of this assumption.

It should be noted that in monitoring programmes, reporting levels are often established at relatively high concentrations, as historically these programmes have been used to monitor compliance with MRLs. A consequence of dealing with censored data with high reporting levels for several substances in several commodities may be significant uncertainty in the intake estimates (EFSA 2008).

Materials and Methods

Deterministic models used for assessing chronic and acute exposure to pesticides are based on point estimates of the amount of commodities consumed per day and of the level of pesticide residues that can be potentially detected in the commodities. Food consumption data is represented by summary consumption statistics of regional and national databases, while the choice of the residue statistics and the availability of transfer factors determine the degree of refinement of the exposure estimates. The point estimates of exposures are then compared with the toxicological endpoints of interest (ADI or ARfD). In general, if the exposure estimate does not exceed the acceptable intake, the substance is said to be of no concern for consumers.

Chronic intake models

Chronic intake models estimate long-term exposure to pesticide residues through the diet. The most conservative model is the Theoretical Maximum Daily Intake (TMDI), since it is assumed that all the amounts of the commodity eaten have been treated and that residues were found at the MRLs. Intakes are computed for every commodity in the diet treated with the pesticide under evaluation. The sum of those intakes gives the overall estimate of the TMDI (expressed as mg/kg bw/day). If the TMDI does not exceed the acceptable daily intake set for that substance, the chronic intake of that substance in the diet does not pose any concern to human health. On the contrary, when the TMDI exceeds the acceptable toxicological endpoint, a refinement of the chronic dietary exposure estimate is recommended.

The International Estimated Daily Intake (IEDI) and the National Estimated Daily Intake (NEDI) provide a more realistic intake model, being based on the assumptions of average daily food consumption per person and median residues from supervised trials. Hence, the residue level in foodstuffs is represented by a much lower value than the MRL. In addition, the IEDI/NEDI calculation allows for the inclusion of other refinement factors such as the transfer factors due to preparation, cooking or other types of food processing. If the chronic refined estimate still exceeds the acceptable toxicological endpoint the substance is said to pose concern to human health with regard to chronic intake of that substance through the diet (WHO 1997).

Acute intake models

Assessing acute dietary exposure is meaningful for substances that have acute toxicity effects and an ARfD value has been established. The International Estimated Short-term Intake values (IESTI) and the National Estimated Short Term Intake (NESTI) are based on a large portion of a commodity that is consumed on a single occasion or in one day. The large portions are defined as the 97.5th percentile of the consumers of the commodity and are obtained from food consumption survey data for individuals. The acute intake model is intended to reflect the peak exposures

to pesticides through diet in one occurrence or in one day, in the events where high residue levels in a food commodity occurs simultaneously with high-level consumption of the certain food commodity. By definition the acute exposure is computed for every commodity separately. Therefore, there might be an overcoming of the established pertinent toxicological endpoint only for some of the commodities in the diet, while for others the acute exposure is considered acceptable. The algorithm of the model differs depending on the type of the commodity consumed. It should also be noted that, when considering single individual food items such as a single piece of fruit or vegetable, the amounts of a residue might vary between the single units composing a sample. On this basis, default variability factors are included in IESTI/NESTI as a way of reflecting these variations (WHO 1997).

Depending on the data on consumption, the IESTI for each commodity is calculated from the equation defined for each case, as described below. The following definitions apply to all equations:

- LP: highest large portion provided (97.5th percentile of eaters), in kg of food per day
- HR: highest residue in composite sample of edible portion found in data from supervised trials data from which the MRL or STMR was derived, in mg/kg
- HR-P: highest residue in the processed commodity, in mg/kg, calculated by multiplying the HR in the raw commodity by the processing factor
- bw: body weight, in kg, provided by the country for which the large portion, LP, was used
- U: unit weight, in kg, provided by the country in the region where the trials which gave the highest residue were carried out; calculated allowing for the per cent edible portion
- v: variability factor represents the ratio of the 97.5th percentile residue to the mean residue in single units. Default factors for various commodities are listed below
- STMR: supervised trials median residue, in mg/kg
- STMR-P: supervised trials median residue in processed commodity, in mg/kg

Case 1

The concentration of residue in a composite sample (raw or processed) reflects that in the large portion size of the commodity. This is assumed to be the case when the unit weight is < 25 g. This case also applies to meat, liver, kidney, edible offal and eggs.

$$\text{IESTI} = (\text{LP} * (\text{HR or HR-P})) / \text{bw}$$

Case 2

The typical unit, such as a single piece of fruit or vegetable, might have a higher residue than the composite such as when a unit weight of a commodity is > 25 g. The variability factors, v, shown below are applied in the equations. When sufficient data are available on residues in single units to calculate a more realistic variability factor for a commodity, the calculated value should replace the default value of 3 for all commodities. It has to be noted that the 2003 JMPR has proposed to use a variability factor of 3 for all commodities.

When data are available on residues in a single unit and thus allow estimation of the 97.5th percentile residue in a single unit, this value should be used in the first part of the equation for case 2a, with no variability factor, and the HR value derived from data on composite samples should be used in the second part of the equation. For case 2b, the estimated 97.5th percentile residue in a single unit should be used in the equation with no variability factor.

Case 2a

The unit weight of the whole portion is lower than that of the large portion, LP.

$$\text{IESTI} = (\text{U} * (\text{HR or HR-P}) * n + (\text{LP-U}) * (\text{HR or HR-P})) / \text{bw}$$

Case 2b

The unit weight of the whole portion is higher than that of the large portion, LP.

$$\text{IESTI} = (\text{LP} * (\text{HR or HR-P}) * n) / \text{bw}$$

Case 3

When a processed commodity is bulked or blended, the STMR-P value represents the probable highest concentration of residue. This case also applies to milk.

$$\text{IESTI} = (\text{LP} * \text{STMR-P}) / \text{bw}$$

The application of variability factors is recommended when the typical unit, such as a single piece of fruit or vegetable, might have a higher residue than the composite sample (such as when a unit weight of a commodity is > 25g). Based on the Codex sampling design, default variability factors (v) were recommended as listed in the table below (WHO 1997).

However, in 2003 JMPR has proposed to use a variability factor of 3 for all commodities. Moreover, recently, the European Food Safety Authority has published an opinion on the use of variability factors based on studies on supervised trial and market residue data. The analysis of the available data on unit-to-unit variation in pesticide residues estimates the average variability factor as 2.8 for supervised trials and about 3.6 for market surveys. EFSA recommends that consideration should be given to using a default variability factor based on supervised trials for IESTI and NESTI assessments (which use supervised trial data), and a different default variability factor based on market surveys (Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from Commission related to the appropriate variability factor(s) to be used for acute dietary exposure assessment of pesticide residues in fruit and vegetables (EFSA 2005). It is noted that when sufficient data on residues in single units are available for the calculation of a more realistic variability factor for a commodity, the calculated value should replace the default value.

For the calculation of IESTI, the consumption data reported in the latest version of WHO/GEMS database were applied, whereas for NESTI the relative data were retrieved from the UK diet (WHO 2003). The risk assessment for acute dietary intake was conducted by expressing the IESTI/NESTI as a percentage of the established ARfD values.

Results

According to the database, only the samples with a concentration of pesticide residues were taken into consideration, in this section of the thesis. A total of 135 samples, from 1996 to 2008 were therefore found irregular; among those the majority of food commodities (n=111) contained only one detected active substance. However, in addition, 18 samples were found with multiple residues of pesticides, ranging from 2 to 5, (n=18). However, no food samples from organic farming were found with a pesticide concentration above the legislative limit.

Within the food class with the highest number of irregular samples were detected in vegetables, with a considerable amount of in leaf vegetable, including various cultivar of lettuces, cabbages and spinaches. The details of the irregular samples divided by food class are shown in Figure 6.

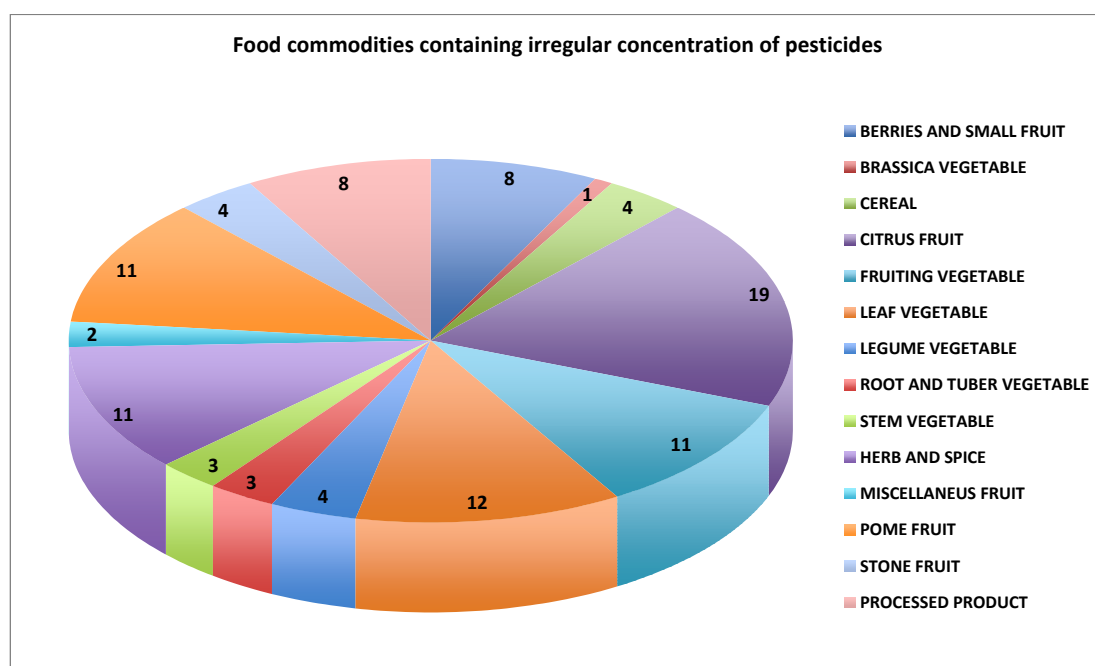


Figure 6: Food commodities with a concentration of pesticides above the legislative limit; the results are expressed as percentage (%) and disaggregated by food classes.

In addition the irregular samples were also analysed according to their origin, where resulted that the majority were from Italy, followed by samples from extra European countries; the complete set of results are shown in Figure 7.

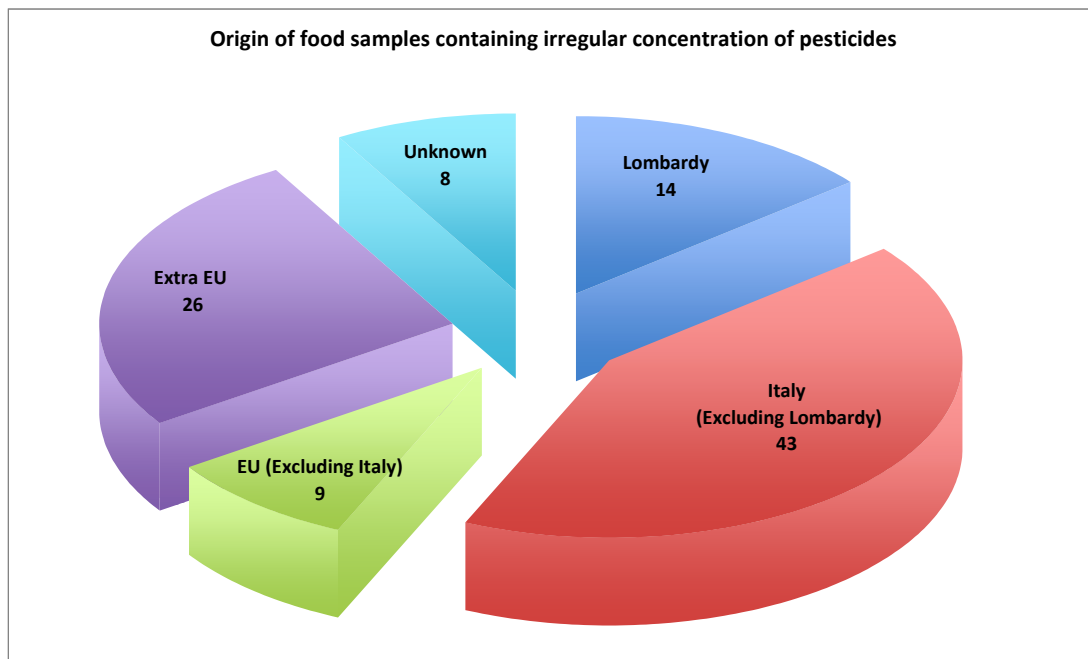


Figure 7: Origin of irregular samples, the results are expressed as percentage (%).

Among these set of 'irregular' samples the most detected active principles belonged to the functional classes of insecticides and fungicide, in particular they were Dimethoate and Procymidone, respectively.

It is also relevant to mention that some of the irregular samples contained active substances not included in Annex 1 of the EU Regulation 91/414, at the time of the sampling. For example, the fungicide Dichlofluanid were found in 4 samples, from 2005 to 2008, whereas the decision of non-inclusion in Annex 1 was taken in 2003. Therefore in these cases, in addition to the possible risk for the health of the consumers, an offence of fraud could be notified. A similar approach could be done for food samples containing concentration of Bromopropylate and Parathion Methyl, found in 2003 and 2004. However the decision of non-inclusion, for those active substances was taken in 2002 and 2003, respectively; therefore a certain amount of time is given to the users to finish their stock of pesticides, before going out of trade.

A revised version of the model for calculating the acute and chronic consumer exposure (revision 2), which includes additional features for refined intake calculations, was used. The revised model, named PRIMO (Pesticide Risk assessment MOdel), merging information on 17 European Diets. It can be downloaded at the EFSA website in the section related to MRL settings (EFSA 2007) (<http://www.efsa.europa.eu/en/mrls/mrlteam.htm>).

In order to assess the consumers exposure (chronic and acute) assessment of residue of pesticides in food matrices all the irregular samples

taken from the monitoring programme of the Lombard region were used. The toxicological endpoints and the correspondent MRL coupling food matrix and pesticide were collected from the EU Pesticide Database at:

http://ec.europa.eu/sanco_pesticides/public/index.cfm

The EFSA model can be used in two modes:

The first is to use it as a first "screening tool" based on conservative assumptions. In this case the calculations for chronic and acute risk assessment will be based on the MRL values.

The second mode is to perform "refined calculations" where the MRLs are replaced by other values in the chronic and/or acute calculation spreadsheet (e.g. STMRs, HR-values).

Therefore, a first series of run were performed using the current EU MRL, coupling active principle and food commodities, to see if there is a possible risk for the consumer, see Table 14, below.

Table 14: Exposure assessment of food commodities, using the EFSA PRIMo model and current MRLs (food commodity/active substances) as highest residue, expressed as mg/kg. Comparison with the toxicological endpoints for acute and chronic exposure (ADI of ARfD) is indicated.

| Matrix | Origin | Active Substance | MRL | ADI (mg/kg bw/day) | Source | ARfD (mg/kg bw) | Source | %ADI | Diet | %ARfD |
|--------------------|----------|--------------------|------|--------------------|-----------|-----------------|-----------|-------|-----------------|--------|
| Lettuce | Lombardy | CHLOROTHALONIL | 0,01 | 0,03 | JMPR 1994 | 0,6 | COM 2006 | 0 | IT Adult | 0 |
| | | DITHIOCARBAMATE | 5 | 0,006 | COM 2004 | 0,08 | COM 2004 | 31,4 | IT Adult | 168,2 |
| Flowering Brassica | Lombardy | CHLOROTHALONIL | 3 | 0,03 | JMPR 1994 | 0,6 | COM 2006 | 0,7 | IT Adult | 33 |
| | | DITHIOCARBAMATE | 1 | 0,006 | COM 2004 | 0,08 | COM 2004 | 1,2 | IT Adult | 82,6 |
| Lettuce | Lombardy | CHLOROTHALONIL | 0,01 | 0,03 | JMPR 1994 | 0,6 | COM 2006 | 0 | IT Adult | 0 |
| | | DITHIOCARBAMATE | 5 | 0,006 | COM 2004 | 0,08 | COM 2004 | 31,4 | IT Adult | 168,2 |
| Pear | Lombardy | DITHIOCARBAMATE | 5 | 0,006 | COM 2004 | 0,08 | COM 2004 | 28,8 | IT Kids/Toddler | 569,2 |
| Courgette | Italy | DIENDRIN | 0,02 | 0,0001 | JMPR 1994 | - | - | 3,6 | IT Kids/Toddler | - |
| Wheat | Lombardy | DITHIOCARBAMATE | 1 | 0,006 | COM 2004 | 0,08 | COM 2004 | 110,8 | IT Kids/Toddler | 18,1 |
| Lettuce | Italy | DITHIOCARBAMATE | 5 | 0,006 | COM 2004 | 0,08 | COM 2004 | 31,4 | IT Adult | 168,2 |
| Grape | Italy | ENDOSULFAN | 0,5 | 0,06 | JMPR 2006 | 0,02 | JMPR 2006 | 0,1 | IT Adult | 163,5 |
| Pear | Italy | DITHIOCARBAMATE | 5 | 0,006 | COM 2004 | 0,08 | COM 2004 | 28,8 | IT Kids/Toddler | 569,2 |
| Lemon | Extra-EU | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 0,6 | IT Adult | 344,5 |
| Orange | Extra-EU | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 9,6 | IT Kids/Toddler | 1326,2 |
| | | THIABENDAZOLE | 5 | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 2,4 | IT Kids/Toddler | 221 |
| Orange | Italy | THIABENDAZOLE | 5 | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 2,4 | IT Kids/Toddler | 221 |
| Lemon | Unknown | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 0,6 | IT Adult | 344,5 |
| Potato | Italy | CHLORPROPHAM | 10 | 0,05 | JMPR 2005 | 0,5 | COM 2003 | 17,9 | IT Kids/Toddler | 307,5 |
| Potato | EU | CHLORPROPHAM | 10 | 0,05 | JMPR 2005 | 0,5 | COM 2003 | 17,9 | IT Kids/Toddler | 307,5 |
| Mandarin | EU | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 4,2 | IT Adult | 556 |
| Lemon | EU | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 0,6 | IT Adult | 344,5 |
| Lemon | EU | THIABENDAZOLE | 5 | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 0,2 | IT Kids/Toddler | 57,4 |
| Apple | Extra-EU | THIABENDAZOLE | 5 | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 4,4 | IT Kids/Toddler | 163,3 |
| Pear | Italy | CHLORPYRIFOS EHTYL | 0,5 | 0,01 | JMPR 2001 | 0,1 | COM 2005 | 0,2 | IT Kids/Toddler | 455,4 |
| Lemon | Unknown | IMAZALIL | 5 | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 0,6 | IT Adult | 344,5 |
| Apple | Italy | BIFENTHRIN | 0,3 | 0,015 | EFSA 2008 | 0,03 | EFSA 2008 | 1,8 | IT Kids/Toddler | 98 |
| | | CYPERMETHRIN | 1 | 0,02 | JMPR 2006 | 0,04 | COM 2004 | 4 | IT Adult | 244,9 |
| | | CHLORPYRIFOS | 0,5 | 0,01 | JMPR 2004 | 0,1 | COM 2005 | 4,3 | IT Adult | 49 |
| | | DIPHENYLAMINE | 5 | 0,075 | EFSA 2008 | - | EFSA 2008 | 5,9 | IT Kids/Toddler | - |
| | | PROPYZAMIDE | 0,02 | 0,02 | DIR 03/39 | NOT APPL | DIR 03/39 | 0,1 | IT Adult | - |
| | | Mandarin | EU | CHLORPYRIFOS EHTYL | 2 | 0,01 | JMPR 2001 | 0,1 | COM 2005 | 4,2 |
| Potato | Italy | CHLORPROPHAM | 10 | 0,05 | JMPR 2005 | 0,5 | COM 2003 | 17,9 | IT Kids/Toddler | 307,5 |
| Potato | EU | CHLORPROPHAM | 10 | 0,05 | JMPR 2005 | 0,5 | COM 2003 | 17,9 | IT Kids/Toddler | 307,5 |

This table shows the correlation between European MRLs and the toxicological endpoints for chronic (ADI) and acute (ARfD) exposure; the net results are expressed as percentage of the mentioned endpoints. In this case only results that may cause a risk for the consumers are highlighted (>100%). Therefore, it is important to highlight that even using the current MRLs, which should constitute a safe level for the consumer; a total of 34 cases may pose an unacceptable risk for the population. In most of the cases the risk is linked to the acute exposure of pesticides, only one case shows an unacceptable risk for chronic exposure.

However, two are the main parameters of this assessment: the first one is, as mentioned before, the MRL, the second important parameter is represented by the consumption of each commodity, according to the various available European diets. Therefore, for a more accurate assessment, the Italian diet should be used with the monitoring data of pesticides residues in food, from Lombardy region. Unfortunately the Italian diet, especially for acute exposure, is not available for all the assessed commodities; therefore the diet correspondent to the worst-case scenario was used. This provides a certain grade of uncertainties for whole assessment.

The quality of the food consumption data is relevant for exposure assessments in the same way as it is for nutrient assessments, and will be influenced by measurement errors, including under-reporting. Measurement errors in dietary surveys include errors in reporting of food intake, estimation of portion size, food coding and data entry. It is then conceivable that food chemical exposure estimates based on the food consumption data will be underestimated (Lambe, J. 2002).

In addition, for the calculation related to the exposure assessment of pesticide residues, the highest value (HR) found during the monitoring programme was used. Therefore the model was primarily used for “refined calculations”.

As described in the previous chapter of the thesis, 0,1% of the samples were found with pesticide residues higher than the legislative limit. The number of active substances was equal to the highest residue was then compared with the related toxicological endpoints (ADI and ARfD) for calculating the related consumer exposure. In order to have a more conservative assumption, for chronic exposure the highest calculated TMDI was taken into consideration even though if it was related to a diet different than the Italian one, the results were then expressed as percentage of ADI. For the acute exposure, for each commodity, the calculation is

based on the highest reported consumption expressed as kg bw. If no data on the unit weight was available from diet used, an average European unit weight was used for the IESTI calculation.

In case of harm for the consumer; when the comparison with the related toxicological endpoint (ADI of ARfD) the highest value was reported and, when available, the data correspondent to the Italian diet; the details are shown in Table 15.

Table 15: Exposure assessment of food commodities, using the EFSA PRIMo model and highest detected residue of active substance, expressed as mg/kg. Comparison with the toxicological endpoints for acute and chronic exposure (ADI of ARfD) is indicated.

| Matrix | Food Class | Active Substance | HR | Annex 1 | ADI (mg/kg bw) | Source | ARfD (mg/kg bw/day) | Source | % ADI |
|----------|-----------------------|------------------|--------|---------|----------------|--------------|---------------------|--------------|--------|
| Wheat | Cereal | DIAZINON | 1,4 | NO 2007 | 0,0002 | EFSA 2006 | 0,025 | EFSA 2006 | 4652,5 |
| Wheat | Cereal | DITHIOCARBAMATE | 17 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 1883,2 |
| Mint | Spice | DIMETHOATE | 232,25 | YES | 0,002 | JMPR 2003 | 0,01 | EFSA 2006 | 386,1 |
| Mint | Spice | DIMETHOATE | 140 | YES | 0,002 | JMPR 2003 | 0,01 | EFSA 2006 | 232,7 |
| Lettuce | Leaf Vegetable | DITHIOCARBAMATE | 20 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 125,8 |
| Mint | Spice | DIMETHOATE | 56,12 | YES | 0,002 | JMPR 2003 | 0,01 | EFSA 2006 | 93,3 |
| Mint | Spice | DIMETHOATE | 55,54 | YES | 0,002 | JMPR 2003 | 0,01 | EFSA 2006 | 92,3 |
| Lettuce | Leaf Vegetable | DITHIOCARBAMATE | 12,5 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 78,6 |
| Lettuce | Leaf Vegetable | DITHIOCARBAMATE | 9,2 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 57,1 |
| Mint | Spice | DIMETHOATE | 28,72 | YES | 0,002 | JMPR 2003 | 0,01 | EFSA 2006 | 47,7 |
| Pear | Pome Fruit | DITHIOCARBAMATE | 4,8 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 27,7 |
| Orange | Citrus Fruit | IMAZALIL | 10 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 19,2 |
| Cabbage | Brassica Vegetable | DITHIOCARBAMATE | 15,5 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 18,4 |
| Pear | Pome Fruit | DITHIOCARBAMATE | 3 | NO 1991 | 0,006 | COM 2004 | 0,08 | COM 2004 | 17,3 |
| Lemon | Citrus Fruit | IMAZALIL | 9 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 17,3 |
| Orange | Citrus Fruit | THIABENDAZOLE | 22 | YES | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 10,6 |
| Orange | Citrus Fruit | PARATHION METHYL | 0,65 | NO 2003 | 0,003 | JMPR 2003 | 0,03 | JMPR 2003 | 10,4 |
| Orange | Citrus Fruit | IMAZALIL | 5 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 9,6 |
| Apple | Pome Fruit | THIABENDAZOLE | 11,5 | YES | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 9,1 |
| Grape | Berry and Small Fruit | ENDOSULFAN | 1,3 | NO 2006 | 0,06 | JMPR 2006 | 0,02 | JMPR 2006 | 8,9 |
| Apricot | Stone Fruit | OMETHOATE | 0,19 | NO 2002 | 0,0003 | EFSA 2006 | 0,002 | EFSA 2006 | 6,9 |
| Lemon | Citrus Fruit | IMAZALIL | 11,9 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 5,9 |
| Mandarin | Citrus Fruit | IMAZALIL | 7,06 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 5,9 |
| Grape | Berry and Small Fruit | FENITROTHION | 1,5 | NO 2007 | 0,005 | EFSA 2006 | 0,013 | EFSA 2006 | 3,9 |
| Grape | Berry and Small Fruit | FENITROTHION | 0,85 | NO 2007 | 0,005 | EFSA 2006 | 0,013 | EFSA 2006 | 2,2 |
| Mandarin | Citrus Fruit | THIABENDAZOLE | 8,96 | YES | 0,1 | JMPR 2006 | 0,3 | JMPR 2006 | 1,9 |
| Lemon | Citrus Fruit | IMAZALIL | 8,05 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 1,1 |
| Lemon | Citrus Fruit | CARBARYL | 2 | NO 2007 | 0,0075 | EFSA 2006 | 0,01 | EFSA 2006 | 0,9 |
| Peach | Stone Fruit | METHAMIDOPHOS | 0,1 | NO 2008 | 0,004 | JMPR 2004 | 0,003 | COM 2007 | 0,9 |
| Lemon | Citrus Fruit | IMAZALIL | 5,3 | YES | 0,025 | EFSA 2010 | 0,05 | EFSA 2010 | 0,7 |
| Grape | Berry and Small Fruit | PROCYMIDONE | 1,1 | NO 2008 | 0,028 | DAR/COM 2007 | 0,012 | DAR/COM 2007 | 0,5 |

HAPERITIF Indicator

To provide a harmonised European approach for pesticide risk indicators, the Sixth EU Framework Programme recently financed the HAIR (HARmonised environmental Indicators for pesticide Risk) project. This paper illustrates the methodology underlying a new indicator-HAPERITIF (HARmonised PESTicide Risk Trend Indicator for Food), developed in HAIR, for tracking acute and chronic pesticide risk trends for consumers (Calliera, M. *et al.* 2006).

HAPERITIF can be applied to provide information on acute and chronic risk of consumers (HAPERITIF_{ac} and HAPERITIF_{chr} respectively). The acute indicator, HAPERITIF_{ac}, is based on the ratio between an Estimated Short Term Intake (ESTI) and the Acute Reference Dose (ARfD) while the chronic indicator HAPERITIF_{chr} is based on the ratio between the Estimated Daily Intake (EDI) and the Acceptable Daily Intake (ADI). Both ARfD and ADI, for a specific pesticide, are established at international level (WHO 1997).

HAPERITIF, follows a stepwise approach as reported in Figure 16.

Step 1: quantification of pesticide residues on crops;

Step 2: prediction of pesticide residues on foods;

Step 3: exposure estimate;

Step 4: calculation of the indicator.

The different steps are characterised by a decision tree procedure depending on the availability of input data.

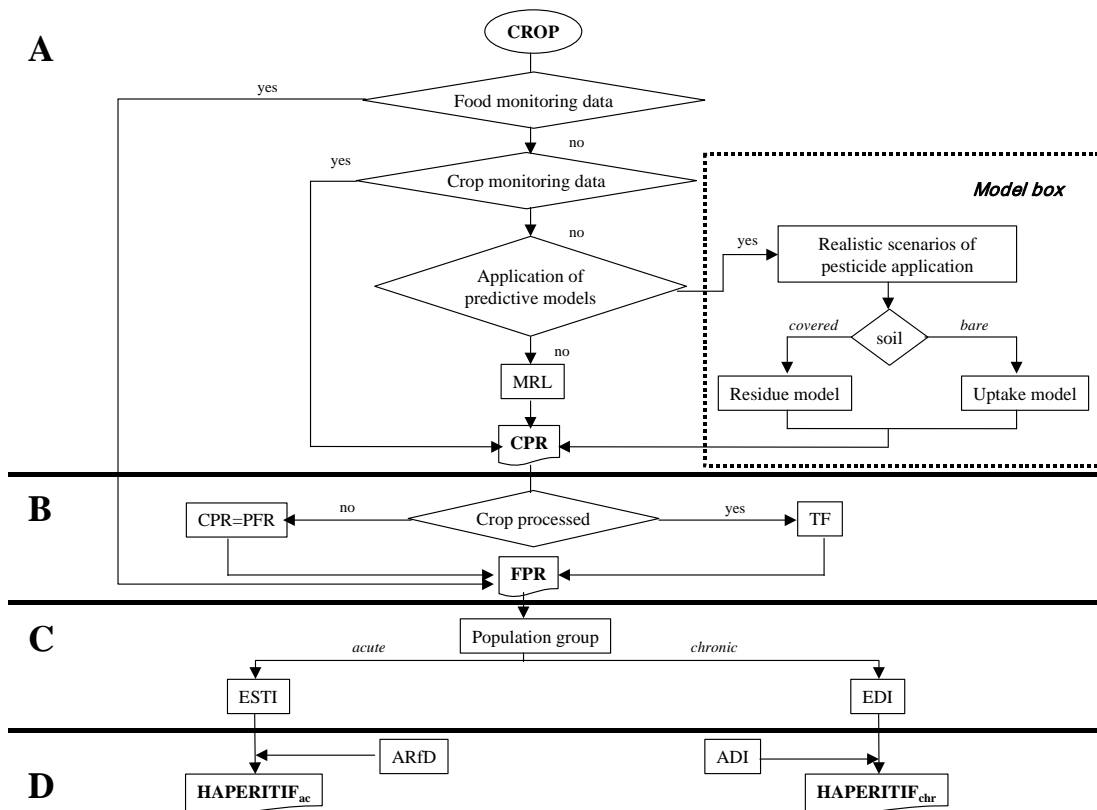


Figure 8: Overall scheme of the HAPERITIF indicator (MRL=Maximum Residue Level, CPR=Crop Pesticide Residue, FPR=Food Pesticide Residue, ESTI=Estimated Short Term Intake, EDI= Estimated Daily Intake, ADI= Acceptable Daily Intake, ARfD= Acute Reference Dose)

Acute exposure

Considering the acute exposure, HAPERITIF_{ac} takes into account the unit to unit and the potential variability within a commodity as suggested by WHO for the definition of IESTI the International Estimated Short-term Intake (WHO 2003). The approach proposed by WHO was followed for the evaluation of acute exposure; however, in HAPERITIF_{ac} the reference acute exposure has been named ESTI (Estimated Short Term Intake), to avoid the distinction, accepted at international level, between national (NESTI) and international (IESTI) estimated short-term intake.

According to WHO, three different exposure scenarios, depending on the consumption data, are necessary to evaluate consumer acute exposure, and all those cases have been taken into account for HAPERITIF_{ac} as described in the previous chapter of the thesis.

Chronic exposure

For the chronic exposure, HAPERITIF_{chr} is based on the Estimated Daily Intake (EDI), which provides a realistic estimate of long-term intakes of pesticide residues (WHO 1997). The mean residue or MR(FPR) is the most likely level that would result from the use of the pesticide at the maximum approved doses and timing under Good Agricultural Practice.

EDI is expressed as follows:

$$EDI = \frac{D_v \times MR(FPR)}{bw}$$

EDI: Estimated Daily Intake (mg/kg bw/day)

MR(FPR): median residue detected (mg/kg) level in the edible portion when available or median residue from models.

D_v: mean dietary intake (mg/person/day)

Aggregation

The indicator can be applied both to evaluate the acute (HAPERITIF_{ac}) or the chronic (HAPERITIF_{chr}) pesticide risk for consumer associated to the consumption of one commodity (crop) or to a particular typology of diet. The proposed methodology can also be applied at different levels of aggregation. The different level of aggregation are briefly described below:

1) One a.i. residue in a single commodity. This is the simplest level of aggregation. It calculates the Exposure/Toxicological Ratio between ESTI and ARfD for the acute indicator (HAPERITIF_{ac}) or EDI and ADI for the chronic indicator (HAPERITIF_{chr}) for each active ingredient applied on a particular crop or commodity. This approach can lead to compare the risk of different a.i. residues that are present in a particular commodity and then to identify the most hazardous substances to the consumer health.

2) Several a.i. residues in a single commodity. This level of application take into account the possibility of multi residues exposure of consumers as consequence of the simultaneous presence of more pesticide in a single commodity. In this case HAPERITIF can be calculated considering both the acute and chronic exposure, according to the following equation:

$$HAPERITIF_{(ac\ or\ chr.)} = 95^{th}\ percentile \frac{EXP_{a.i.}}{TOX_{a.i.}}$$

where:

EXP_{a.i.}: Estimated Intake Chronic or Acute, calculated for each a.i. in a single commodity

TOX_{a.i.}: ADI or ArfD, depending on acute or chronic indicator, of the different a.i. considered

As a conservative approach, the 95th percentile of all the exposure/toxicity ratios is taken into account. At this level of application the indicator can be used to monitor the time trend risk associated to the food consumption of a particular commodity (crop). In fact, the evaluation could be repeated for several years, using the first one as benchmark against which the success of new strategies can be evaluated. The indicator can be calculated for a particular country, region, or territory, or at EU level.

3) One a.i. residue in several commodities. The third level of aggregation considers the case where one a.i. is utilized on several crops. This case should cause only higher levels of chronic exposure for consumers, due to compound residues in more commodities. The chronic indicator will be calculated considering the overall exposure deriving from consumers' diets according to the following equation:

$$HAPERITIF_{chr} = \sum_{crop=1}^n \frac{EDI_{crop,a.i.}}{ADI_{a.i.}}$$

where:

EDI_{crop,a.i.}: Estimated Daily Intake calculated for all crops with residues of a single a.i.

ADI_{a.i.}: ADI of the specific a.i.

4) Several a.i. residues in several commodities. This level of aggregation can be used as a risk trend analysis system for different categories of consumers who use a particular diet. In this case too, the 95th percentile of the aggregation of the exposure/toxicity ratio is considered, and 1 is the threshold value identified.

$$HAPERITIF_{chr} = 95^{th} \text{ percentile} \sum_{crop=1}^n \frac{EXP_{crop,a.i.}}{TOX_{a.i.}}$$

All the levels of aggregation described previously can be computed for a particular region, country, or at EU level

HAPERITIF application in a post harvest treatment

The active ingredient Chlorprofam is used as an example of the application of the indicator for post harvest treatments in potatoes.

The values of HR and MP for the application comes from 12 years monitoring data (1996-2008).

The indicator can be applied both to evaluate the acute (HAPERITIF_{ac}) or the chronic (HAPERITIF_{chr}) pesticide risk for consumer associated to the consumption of potatoes comes from the areas on which the monitoring data are made. This is an example of the simplest level of aggregation. The Exposure/Toxicological Ratio between ESTI and ARfD for the acute indicator (HAPERITIF_{ac}) or EDI and ADI for the chronic indicator (HAPERITIF_{chr}) are calculated. The results have been presented in the Figure 9.

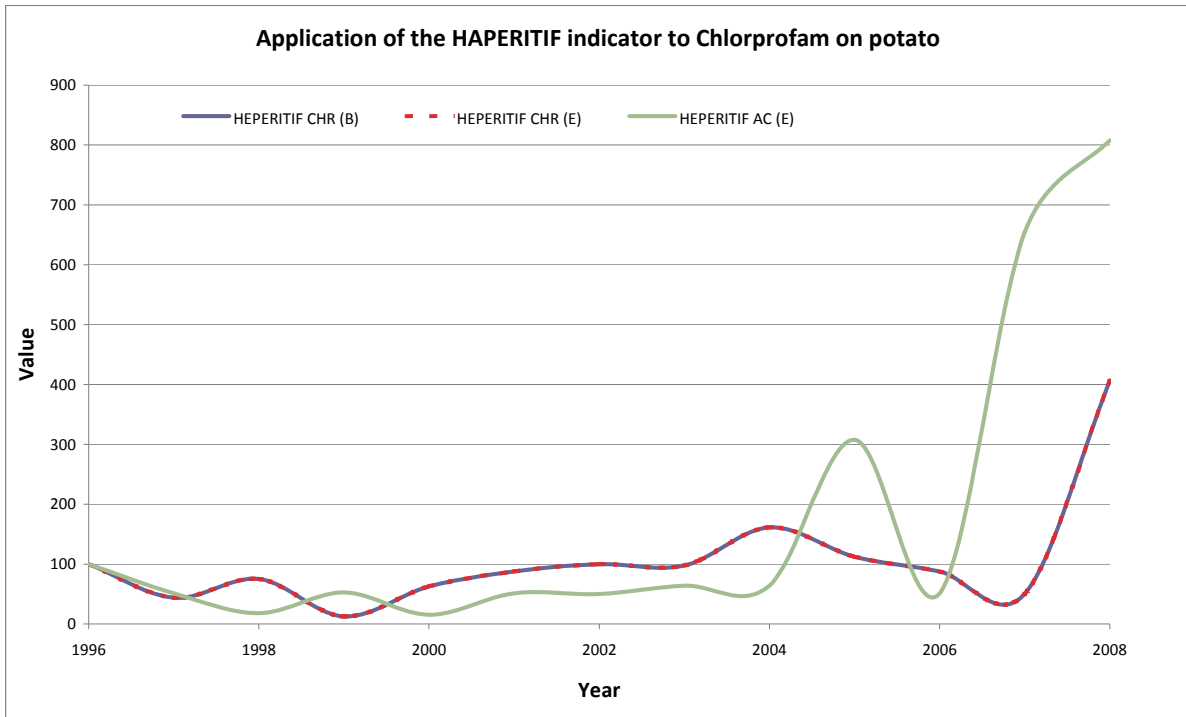


Figure 9: Example of the application of HAPERITIF acute and chronic for Chlorprofam used in post harvest treatments. On X axis calendar years are reported, on Y axis the values of HAPERITIF indicator for chronic (blue and red line) and acute (green line) are reported.

In the figure above, the same level of aggregation for the HAPERITIF indicator is shown taking into consideration the following:

- 1) $HEPERITIF_{CHR} (B)$: long term exposure for pesticide residues, using the WHO Cluster Diet B, as model of intake and the median residue detected in the edible (EDI)
- 2) $HEPERITIF_{CHR} (E)$: long term exposure for pesticide residues, using the WHO Cluster Diet E, as model of intake and the median residue detected in the edible (EDI)
- 3) $HEPERITIF_{AC} (E)$: short term exposure for pesticide residues, using the WHO Diet, as model of intake and the ESTI calculation in case the unit weight of the whole portion is lower than that of the large portion, F, as indicated in the equations 2a, described in the previous chapter.

The first two indicators differed from the potato's intake across the population. In fact, a higher consumption is linked to the cluster diet E rather than B. However, the difference in consumption was not significantly relevant, therefore the two lines (red and blue) resulted overlapped.

Conclusions

The most commonly detected pesticides in irregular samples are Dimethoate (n=16), Procymidone (n=15), Ethion and Chlorotalonyl (n=10), Dithiocarbamate (n=9); which mainly belongs to the functional classes of fungicide and insecticide.

Using the EFSA model for exposure assessment, it has to be noted that in case of overcoming of the acute toxicological endpoint, expressed in percentage of ARfD, the diet associated with the results are mainly from Northern Europe (Germany and UK), in case of absence of the Italian data. Therefore these results could be taken, with a high degree of uncertainties, when associated with residues of pesticides found in Lombardy coupled with the Italian diet. On the other hand, the results shown for chronic exposure, expressed as percentage of ADI, are more accurate, being able to retrieve the Italian consumption data for the selected commodity.

However, it has to be noted that for the irregular samples coming from extra European countries; they were immediately withdrawn from the Italy once arrived at the inspections borders. Even though these commodities had entered the Italian market, once analysed by the inspection bodies, they are withdrawn from the market, according to the RASFF.

In addition, some of the actives substances found in irregular samples were already withdrawn at the time of sampling (e.g. DDT, Esachlorobenzene). Therefore, more over the health of the consumers is noticed a convict of fraud.

In relation to the use of the HEPERITIF indicator, for acute and chronic exposure, the two times trend risk was compared and it was possible to note the constant trend for the chronic risk while for the acute one, despite a general increase during the overall period, show peak over the unit ($ESTI/ARfD > 1$) than the chronic exposure in consideration. These trends are calculated using only monitoring data that represent a realistic exposure scenario. In this example is clear that for consumer eating potatoes comes from the area of monitoring data, the acute exposure should be considered relevant for health implication.

One of the aspects that the deterministic approach, used in this section of the thesis, could not solve is the cumulative exposure for multi-residue samples. The principal reasons for this are that the level of protection provided by the deterministic approach is uncertain and that some details of the probabilistic methodology require further work (EFSA 2009).

Probabilistic risk assessment

The probabilistic approach has been applied quite recently in the field of pesticide exposure assessment for estimating the acute intake of several pesticides in the diet. This technique allows the simulation of real live occurrence of actual food consumption together with the whole range of observed levels of pesticide residues in food. On the contrary, in point estimate analysis only a single number can be used to characterize each input and, therefore, many possible values of that input are ignored. One other advantage of the probabilistic approach versus the deterministic model is that it is possible to evaluate the acute exposure to pesticides in more than one commodity at the same time. Moreover, the output shows a full range of exposures instead of a single value, giving the possibility to explore the coverage of the population exposed on the basis of the established toxicological endpoint.

It should also be pointed out the necessity for transparency to be maintained, by indicating the sources and software used, as well as the way results are presented. Last but not least, it should be noted that while the point estimates cover the 97.5% of the population of eaters only, the probabilistic approach provides a whole range of percentiles of exposures as well as the percentages of exposures that may lay over the ARfD.

Introduction

As regards the dietary assessment of pesticides, the probabilistic model includes two main sets of input data linked to each other by the software code: a) food intake dataset of the consumers, from which an individual is selected and all eating occasions are searched for items that may contain the target chemical, b) a dataset providing information of the probability of the target chemical to be present in the food items.

Currently, simulations of possible combinations of dietary intakes and residue values can be carried out by several computer software packages developed

for this purpose. Most broadly known are the @Risk software (Palisade Corporation), the Crystal Ball® software (Decisioneering, Inc.), and the specifically developed for dietary exposure assessment programs Monte Carlo Risk Analysis (MCRA, Stochastic modelling of chemical intake from food, developed in collaboration between RIKILT and Biometris in the context of the Dutch Programme for the Quality of Agricultural Products-KAP), and the Creme software, for probabilistic modelling. However it has to be mentioned that the first three models were developed not specifically for pesticide risk exposure, while Creme Food is a tool, which provides accurate and reliable information on the population's exposure to chemicals from many different sources. Creme Food utilises published and peer reviewed science to calculate these values by combining input data sets from product usage habits, amount of product used per occasion, chemical monitoring laboratories, demographic data sets, market information, industry and other data. Therefore this latter software was the most appropriate within the remit of the thesis.

Estimating intake from one commodity for one person on one day requires multiplying the amount of commodity they consume by the concentration of pesticide it contains, and then dividing by body weight. However, risk managers need to know how often intakes exceed the ARfD, when considered for multiple persons and multiple days. The probabilistic approach estimates this by taking consumption and body weights for multiple persons and multiple days and combining it with different concentrations, selected at random. Consumption and body weight data are derived from national dietary surveys, and the concentrations are derived from monitoring or field trial data. This process is illustrated below:

1. Select one “person-day” record from the dietary survey, comprising consumption and body weight.
2. Sample a single concentration at random from a distribution estimated from the residue data.
3. Calculate the modelled intake for this person-day by multiplying consumption with concentration and dividing by body weight.
4. Repeat steps 1-3 for a large number of person-days, calculating a modelled intake for each.
5. Determine the percentage of modelled intakes for all the person-days that are below the ARfD for the pesticide.

The frequency distribution of the exposure estimates is examined in regards to the percentage of exposures being above the toxicological endpoint. The algorithm used for computing the exposure estimates may also include the modelling of the unit-to-unit variability following different options, depending on the actual data available (Boon, E. *et al.* 2003).

The results in Sections 3-5 were generated by one probabilistic model developed in Ireland (Creme). This model modelled variability in consumption using dietary surveys, from various countries in Europe (McNamara, C. *et al.* 2003); however since the monitoring data used for the assessment are from the Lombardy region the Italian consumption data was used (Turrini, A. *et al.* 1996).

Food consumption data

Since the input data on food consumption is required to be in the form a range of individual consumptions, it is expected that the methods used should allow for collecting information on every individual's dietary habits. In addition, these data should be as more accurate as possible, therefore quantitative data is needed. In consequence, the most appropriate methods for data collection would be the 24-hours recall data or dietary records. Quantitative Food frequency questionnaires may also be of use.

Models estimating the intake of pesticides use data referring to raw agricultural commodities therefore it is necessary to translate the actual food eaten to agricultural products. This is can be possible by the use of recipe databases that are sometimes available at national level. This type of database provides quantitative information on the ingredients of the composite foods consumed, (for instance the amount of flour used for making a pizza). Other points of interest could be information on specific brand marks, market share, special population features (for instance infants) etc. Relevant information would be the proportion and the parts of a raw commodity being actually consumed, since the original product may include a non-edible part, for instance peels etc. This may have a considerable effect on the total level of residues present on the commodity. Information from food consumption surveys about every day consumption practices could be useful in this respect.

For probabilistic assessment of acute dietary exposure to pesticides, it is important to see the correlation of food items that can be included in the model.

Unless only one commodity is included in the model, the use of actual individual consumption data is preferred, since the whole dataset allows for correlations to be investigated.

Residue datasets

As discussed previously, the estimation of the residue values can be derived either from supervised field trials or from monitoring programs. When applying probabilistic models it is possible to enter as input data all the raw data available, instead of using only the median and highest Residue values. The residue values datasets should address the following considerations: a) what is the range of values of residue concentrations (levels of residue concentration) and b) what is the probability that these residues will be present in the food (frequency).

Supervised field trial data might be regarded as of better quality since trials are performed under experimental conditions. Nevertheless, it must not be forgotten the fact that their final aim is to be utilised for registration purposes and thus their outcomes often overestimate the real values. Moreover, given the fact that only a small number of trials is requested, the number of resulting residue values is limited. On the other hand, data being of better quality is not necessary more realistic, whereas monitoring data can be more representative of the reality.

On this basis, the U.S. Environmental Protection Agency suggested performing "bridging" (or "reduced use") studies, seeking to compare or "bridge" the residue data resulting from the maximum application rates used to determine tolerances and the more typical ranges actually applied. These studies can be used to establish a relationship among residues from field trials conducted at the maximum application scenario (maximum application rate, highest application frequency and shortest PHI) and residues expected at the range of more typical ranges. In this way a broader and more complete range of values will be generated.

In general, bridging studies consist of one or more field trials using several different application rates. The applications should occur at the same location and at the same time because of the potential impact of environmental conditions and variability in study conducts on the result. Therefore only controlled field trials specifically designed can be used and data are then used to establish the relationship between application rate and resulting residue level. One application

rate in each field trial should be at the maximum rate and at least two other lower rates should be selected so that the relation between application rate and residue level can be calculated. In some cases it might be preferable to use exaggerated rates, particularly if residues under the limit of quantification are expected, so quantifiable residues will result. Once a determination is made that it is appropriate to adjust residue levels from maximum rate/minimum PHI field trials with information obtained from reduced-rate field trials, it becomes necessary to incorporate these data into a Monte Carlo analysis. The first step of this incorporation is to adjust the field trial data that would have been developed earlier for tolerance setting purposes to residues that would have been found had lower application rates been used (EPA 2000).

As mentioned above, monitoring data can be more representative of the reality, depending on the sampling methods. As an example, when data is collected for the U.S. Market Basket surveys the sampling is made on a single serving basis (i.e. single apple etc.) from commercial retail establishments (supermarkets etc.) applying a rigorous statistical design, often according to OPP (Office of Pesticides Program) reviewed protocols. In contrast, monitoring data collected by the FDA is intended for tolerance enforcement therefore sampling is not intended to be statistically representative. On the other hand, United States Department of Agriculture/ Pesticide Data Program (USDA/PDP) sampling which is performed as closely as possible to the point of consumption is statistically designed for use in dietary risk assessment in order to be representative of residue concentration in US, and samples are prepared as for consumption.

When probabilistic risk assessment models are intended to be used for comparison between country statuses, harmonisation of data collection should be previously performed. This applies particularly to systemisation of data collection, sampling and analytical methods in use. As an example, where analysis of foodstuffs is performed by accredited laboratories, the results can be comparable since methods used are standardised and validated.

For instance, in the Europe Union the competent authorities of the Member States are asked to perform regular inspections of foodstuffs at national level and to report the results from national monitoring programs to the Commission. These results may vary significantly between countries also due to several factors e.g. sampling strategies, methods of analysis used, and differences in national MRLs.

Under this light, EU has recommended since 1996 the participation of MS in a coordinated monitoring program entitled "Monitoring of pesticide Residues in Products of Plant Origin in the European Union" which also includes information from Norway, Iceland and Liechtenstein (European Commission, Health & Consumer Protection Directorate-General, 1996 - 2004). This program was designed as a rolling program in a series of 3-year cycles and covers major pesticide-commodity combinations as selected from the WHO/GEMS European Diet. The sampling design is based on a statistical method proposed by Codex Alimentarius and the minimum numbers of samples of each commodity are fixed at a different level for each country, according to the population and consumer numbers.

Sources of variability

Separating variability and uncertainty is useful for identifying parameters for which additional data are needed. It is reminded that uncertainty is linked to lack of information, whereas variability to observed differences attributable the true diversity in a population or exposure parameter and results from natural random processes.

Unit variability

Unit variability of food samples needs to be considered when the typical consumption portion, for example one fruit, is different from the sample taken for analysis, for example a sample of ten fruits. For the determination of pesticide residues, measurements are usually performed in composite samples where the distribution of residues among individual items may be different, with some items containing more pesticide than others. In a batch of food items previously treated with a pesticide, the residue of the pesticide remaining on/in single food items at the time of consumption differs, due to a variety of factors.

It is important to take account of this variation when assessing acute dietary exposure to pesticides in medium and large-sized food items (for instance apples or melons).

According to the FAO guidelines, variability should be considered for food commodities, which have, unit weight more than 25 g, whereas for weights under 25 g it is assumed the residue data reflect the residue levels of the commodity consumed. When residue data on individual items is available, this can be used directly.

Information on variability is relatively limited. Moreover, existing variability studies from analysis of retail samples are not standardized and are performed on batches taken from various sampling locations. Besides, in order to obtain satisfactory conclusions, findings should reflect more closely real life conditions, as happens for instance with variability studies performed with samples from locations at the end of the distribution flow. Variability studies may also be generated from field trials. In this case the within batch variability is expected to be lower and the measured values more uniform (Earl, M. *et al.* 2000). In fact, after examination the range of variability factors from existing studies where residues were measured separately in individual food items, it was concluded that on average, variability factors estimated from samples collected in the marketplace were higher than those from samples obtained in supervised trials (EFSA 2005).

In the deterministic model, variability is addressed through the “variability factors” which are based on the 97.5th percentile of the distribution of residues; i.e. the level that is exceeded by 2.5% of residues in food items. Variability factors are defined as the ratio between the 97.5th percentile value and the mean composite sample value. In the probabilistic approach however, one single value for variability is not appropriate for applying in single simulations of a probabilistic exposure analysis. On the contrary, variation in residue levels between units of one composite sample can also be described as following a distribution. Currently there is a lack of guidelines on how to apply variability within the probabilistic approach, though diverse ways have been proposed, for instance the use of different distributions (Boon, E. 2004)

The U.S. EPA, in order to address the problem of residue data on single units, has developed a technique known as “the decomposition method”, resulting in a new set of data of residues on individual items (EPA 1999). This methodology consists of extrapolating from data on pesticide residues in composite samples of fruits and vegetables to residue levels in single units of fruits and vegetables. Given the composite sample mean, the composite sample variance, and the number of

units in each composite sample, it is possible to estimate the mean and variance of the pesticide residues present on single units of fruits and vegetables. These parameters can then be applied to generate information on the level of residue in fruits and vegetables. This information can then be incorporated into a probabilistic exposure estimation model, such as the Creme software, in order to estimate exposure to pesticide residues in foods and the risk attendant to that exposure. This methodology has a higher degree of accuracy when more than 30 composite samples have detectable residues.

Other organizations have developed similar methodologies for extrapolating from residue levels in composite samples to residue levels in single units, however and the results are similar to those of OPP. This is expected since the methods developed originate from the same fundamental assumption that residues on individual serving sizes of fruits and vegetables follow a lognormal distribution, as established from earlier goodness-of-fit studies (EPA 1999).

Processing

As discussed, processing raw commodities results usually into lower levels of residues compared to the processed foods due to different ways of preparation, cooking etc. The effects of processing depend on the pesticide characteristics and the product processed. However, in order to have an estimate of the changes due to processing, processing factors are calculated. These factors usually result as a part of the pesticide registration studies and in spite of the fact that conditions of their calculation vary from every day life habits they can provide some information.

In the point estimate approach only one food (group) - processing type combination can be addressed at a time, which can result in worst-case estimations of exposure (no effect of processing). Sometimes, in food consumption surveys, there may be information available on the percentage of people consuming a specific food item peeled or not peeled, for instance apples. When no information on processing is available from the food consumption survey, general assumptions on processing habits may be derived from other sources (e.g. literature).

When information on processing practices is incorporated in the analyses using the probabilistic approach, a more realistic estimation of exposure is possible compared to the worst-case assumption that nobody peels their apple or the too

optimistic situation that everybody does. For example, in a study on the Dutch general population the exposure decreased with more than 20% compared to the worst-case assumption. Moreover with the probabilistic approach different types of processing per food (peeling, not peeling, juicing) can be addressed in one analysis. When doing this each food (group) - processing type combination should be linked to the correct variability factor (e.g. apples eaten whole are subjected to variability, while those mixed in juices are not) (Boon, E. 2004).

Other sources of variability

Other causes of variability within the exposure model may be present; nevertheless they are not usually taken into account. For instance, residue values reported may vary also due to lab-to-lab variations owing to analytical methods or sampling errors. Variations of body weight values within a population, as well as food portions consumed may also be another source of variability.

Sources of uncertainty

In the case of monitoring data, lack of information on residues values under the LOD presents a significant source of uncertainty. This obstacle is usually addressed by assigning a fixed value, most often the value equal to the LOD or $\frac{1}{2}$ of LOD. Useful information regarding the probability to encounter the substance under examination in the treated commodity could be derived from the percent of crop treated (PCT) and the percentage of imported versus domestic crop, when this are available. When the percent of crop treated is known, it is possible to assign a probability that a residue level at LOD could be near to zero rather than near the LOD value, resulting to more accurate estimations. PCT adjustments should only be applied to distributional residues (e.g., in acute probabilistic acute analysis), but not to single residues values (e.g. in deterministic point estimate analysis). Information on the PCT in the U.S. could be obtained from USDA (Biological and Economical Analysis Division, (BEAD) and National Agricultural Statistical Service/ Agricultural Chemical Usage Reports) and from the DPR Pesticide Use Report Data (DPR MT-3, 2004).

Residue levels reported may be affected also as a result of the decline and degradation. Decline effects are due to the Pre Harvest Intervals maintained between pesticide applications, whereas storage, transportation, shelf-life periods account for the degradation of residues. However, these factors are difficult to be assessed and thus they are not considered.

As sources of uncertainty may also be considered the ones that result from the lack of information regarding food consumption, such as differences between categories of consumers, use patterns of food and underreporting.

Sensitivity analysis

Sensitivity analysis may be used to rank the model's input assumptions with respect to their contribution to the outcome variability and uncertainty. In this way we can be aware of the manner in which alternative selections will affect the final conclusions. Models may be expanded to include additional components that may be of importance by using sensitivity analysis to get closer to true value exposures. Sensitivity analysis can be done for a limited set of inputs. If more inputs are investigated in a sensitivity analysis in an experimental design, statistical methods like analysis of variance can be used to quantify the main effects and possible interactions when simultaneously changing more than one input. By assessing the results of sensitivity analysis it is possible to evaluate whether the sensitivity of the model might be a matter of concern or of relatively small importance.

Validation of probabilistic models

In general, validation of the probabilistic models regarding dietary exposure to toxic substances it is intended to demonstrate that the model applied does not overestimate the "true" exposure and at the same time that it provides a more realistic picture compared to the conservative calculation methods. Validating a model presupposes the examination of the following: the validity of distributions used to represent the input values, the adjustments made for potential correlations, the number of iterations applied, the methodology used to sample from input values, the number of observations needed to obtain reliable estimates of consumption and residue distributions, the representativeness of data and the percentiles considered.

For toxic substances as pesticides, where the main focus is the possibility of exceeding a certain toxicological limit, validations should be concentrated to the upper percentiles of exposure distributions, for instance 99 or 99.9 percentiles, whereas comparing mean and median exposures are beyond interest. The validation can be statistically strengthened by calculating confidence intervals to quantify the uncertainty of the Monte Carlo estimate (Lopez, A. *et al.* 2003).

Several validation studies have been performed for assessing the "fitness of purpose" of probabilistic models. As "true" intake values are considered the values originating from the analysis of duplicate diets, although these data also include a level of uncertainty, for instance sample values under the limit of detection.

A validation study of the Monte Carlo Risk Assessment (MCRA) model was carried out by Lopez *et al.* taking as a reference population 282 infants aged 8-12 months in the Basque Country (Lopez, A. *et al.* 2003). The residue data were based on the Pesticide Monitoring Programs carried out by the Autonomous Communities in Spain, and when these not available, the Spanish MRLs were applied. Food consumption inputs were derived from 1-day food diaries and recipe study carried out as a part of the Monte Carlo project. Three approaches were used: a) a visual comparison of the graphs representing the cumulative probabilistic distributions of the modelled, conservative and duplicate diet studies, b) a statistical test of a high percentile and c) the comparison for each infant of the duplicate diet, conservative and model intake values, analysing 19 pesticides and validated for 6. It was found that the probabilistic model reduces the bias of conservative methods and does not underestimated intakes.

Boon *et al.* conducted a similar validation exercise of the MCRA model in a population of Dutch infants using a duplicate diet study, addressing six pesticides in total (1). Food consumption data was derived from a food diary where participants were asked to record the food consumed on the same day and translated to raw agricultural commodities using the conversion model Primary Agricultural Products (CPAP) developed by the RIKILT Institute (Institute of Food Safety, Wageningen, the Netherlands) and pesticide residue measurements from the Dutch monitoring programmes in 2000 and 2001 were used. The model was considered validated when the outcome was higher than the "true" intake and at the same time lower than the point estimate. It was shown that the intake exposures estimated by the model were closer to the real ones, compared to the point estimates.

Materials and Methods

The aim of the current analysis is to provide an example of assessment of acute dietary exposure to pesticide residues using probabilistic approach with the help of Creme software.

Chlorpropham was selected as a reference substance for this example, since it is already included in the Annex I of the Council Directive 91/414 and there were adequate monitoring data available as well as a final evaluation document of the substance prepared by Joint FAO/WHO Meeting for Pesticide Residues (JMPR).

Chlorpropham is mainly used as a post harvest treatment on potatoes for anti-sprouting purposes. In E.U. and U.S. the accepted applications for this substance on edible commodities regard potatoes only, while it can be further used for weed control as a pre- or post emergence herbicide for flower bulbs and ornamental plants. The authorized treatment on potatoes is spraying by means of hot fogging equipment directly on the stores, whereas no PHI was judged to be necessary as concluded in the review report of the Standing Committee on the Food Chain and Animal Health. In the U.S., Chlorpropham is registered for post-harvest treatment on potato as an emulsifiable concentrate used by direct spraying of a 1% aqueous emulsion on potato tubers moving along a conveyor line or as an aerosol fog at a standard application rate of 0.015 kg a.i./t whereas no withholding period is identified.

The Maximum Residue Limit set for Chlorpropham by the JMPR Commission is 30 mg/kg for ware potatoes. However in the EU legislation, the MRLs for this substance are currently under review, since the existing MRLs were set on 1982 by the Directive 82/528/EEC (Official Journal L 234 of 09.08.1982) for trading purposes (0,05 mg/kg).

In terms of toxicological endpoints the ADI and ARfD in the final European review report is set to 0,05 mg/kg bw/day and 0,5 mg/kg bw/day respectively by the JMPR (FAO Pesticide Management, JMPR Evaluations for Pesticide Residues, 2001).

Modelling exposure using the Creme software programme

Creme Software Ltd (Creme) was formed in 2005 following five years of research resulting in the development of the most advanced methods of food safety exposure assessment. This research was conducted at Trinity College Dublin Ireland in collaboration with European partners. The Creme model was originally developed and scientifically validated in this EU FP5 framework project (Monte Carlo), the principal investigator was Prof. Mike Gibney (University College Dublin).

Today Creme Food is on release version 3 of its software, which provides the most user-friendly, accurate and detailed dietary exposure assessment solution in the market. The scientifically advanced models allow users to understand the impact of a range of items including food ingredients, chemicals, contaminants, additives, flavourings, pesticides, veterinary residues, nutrients, nanotechnology and functional food ingredients on consumers in different market sectors across Europe and the rest of the world.

The Creme Food statistical models combine population's food consumption patterns with data on chemical concentrations in foods, ingredients or raw agricultural commodities to determine dietary exposure to the chemicals of interest. Exposure results are expressed in statistics across the population, for example the mean exposure for the population and the 97.5th percentile representing the high-end exposure results. All of this data is pre-installed and ready to go, providing the options of running both deterministic and probabilistic scenarios. Any new or additional data required by the user can be installed in Creme directly by the user.

Food consumption data

The food consumption data were collected through seven days dietary recalls for each respondent and include 314114 individuals of all ages (Turrini, A. *et al.* 2001). Furthermore, population is divided in 2 different age strata (adult and child) of the Italian population. The average daily intake rates were divided by each individual's reported body weight to generate the intake rates (g of potatoes consumed /kg of bw per day). The data extracted in this exercise, regarding potato consumption, considered the consumption of potatoes (including baked, boiled,

chips, fried and other various types of potato dishes in one group) for the total population (consumers and non consumers), as well as data for consumers-only.

Subjects data

The Subjects table records each survey participant's unique Subject ID, along with various demographic and characteristic information, such as:

1. Day Count: the number of days that the subject completed the survey.
2. Gender: the subject's gender.
3. Bodyweight

Other fields may also be available, depending on the survey, such as: Region, Socio-Economic Group, Socio-Class Group, Age in months, Household composition, Taking Vitamins or Minerals, Area Code, Vegetarian/Vegan information, Institutionalised.

The default fields provided when a new Subjects table is created are: Subject Code, Day Count, Bodyweight (kg), Height (m).

As with any table type, the user may add extra fields and data as required. This extra information may be used to filter for different subsets of the population when performing exposure assessments.

Residue data

Monitoring data were derived from the pesticide residues database of the Lombardy Region, available on the Internet site of ICPS at www.icps.it. The monitoring data used were derived from the database resulting from the monitoring programme undertaken in the region of Lombardy (North Italy), from 1996 until 2008. Analyses were performed in six different laboratories. The monitoring residue dataset includes in total 233 residue samples, from which 212 were above the Limit of Determination and 21 below. The range of monitored values includes residues from 0.01 mg/kg (LOD) to 6.30 mg/kg (HR). The majority of the samples were produced in Italy (145), 38 were from European Countries, 27 were from Lombardy (excluding Italy) and for 23 the country of origin was not mentioned.

Iterations

When any uncertainty is present in an assessment, be it due to distributions or variability models, then it is not sufficient to gather a single set of statistics for daily average intake of a product or chemical.

There are two approaches to this issue, and the choice depends on the exact form of the uncertainty or variability. For example, it may be the case that the concentration is known to be variable from one food commodity to another with a known standard deviation. On the other hand, it may be the case that the concentration is the same across all food commodities of the same type, but with unknown concentration.

This was the case chosen for monitoring data of Chlorprofam, where most of the residue concentrations were expressed and only few samples were below the LOD. Therefore it was run an assessment with one uncertainty iteration and the Creme Food software analysed every individual in subset of the survey you are interested in, and then calculate the statistics using subject weights.

For the case study on Chlorprofam, 1 uncertainty iteration was used, with 10 variability iterations equivalent to simulating 19780 subjects for the total population, 3220 for toddlers (age ≤ 18) and 16560 for adults (age > 18).

Results and Conclusions

All the relevant information mentioned in the previous chapters of the thesis were plotted in the Creme Food Software. Three different data set were prepared for each year of sampling (1996-2008), the main differences were related to the age of the population present in the survey, considering total population, adult (age ≥ 18) and child (age ≤ 18). Therefore, for each year of sampling three assessments were run, taking into consideration the highest possible percentile (P99.9) allowed by the software and the acute exposure. This is because the aim of the study was to cover the majority of the population with particular attention to the most sensitive classes (child). In addition, the deterministic approach computation that was, developed in

the previous chapter of the thesis highlighted that most of the risks to consumers were derived by acute exposure. Therefore, only the above-mentioned type of exposure was considered in the probabilistic approach.

For general population (Figure 10), the acute exposure calculated as 99,9th percentile of the intake of Chlorprofam in association with the consumption of potato, which is relatively low compared with the toxicological endpoint for acute exposure (ARfD=0,5).

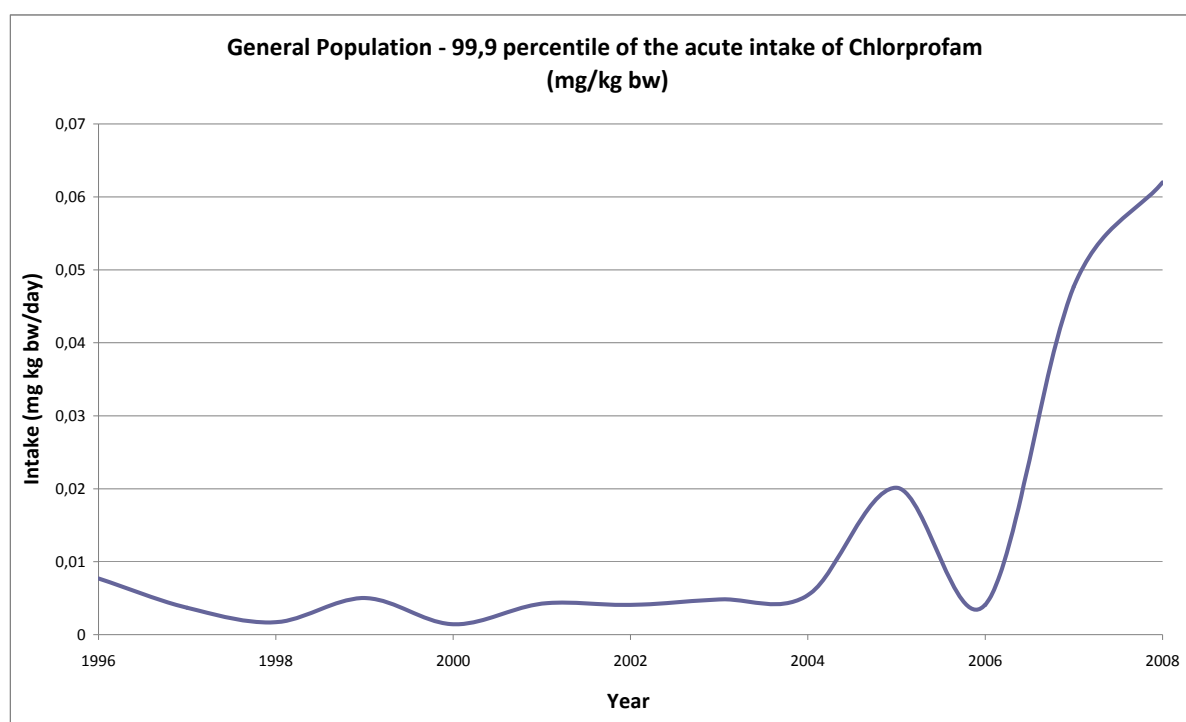


Figure 10: Acute probabilistic exposure comparing consumption of potato with residue data of Chlorprofam through the years from 1996 to 2008, for general population. X axis: Calendar years and Y axis: 99,9th percentile of the intake on Chlorprofam (mg/kg bw)

It has to be noted that the slope of the curve is relatively stable through all years of sampling. However the high peaks found in 2005, 2007 and 2008 could depend from the high number of samples detected and the relative high concentration of Chlorprofam found in some samples.

A similar shape could be found comparing the probabilistic exposure assessment of Chlorpropham in child and adult (Figure 11). It is highlighted that the exposure for children is higher than the adult exposure during all years; this was especially more evident in 2005, 2007 and 2008. However, it has to be considered that, even the exposure of child and adults is high in some years. This did not represent an unacceptable risk for both categories of consumers because the value of the toxicological endpoint, for acute exposure (ARfD) was not overtaken.

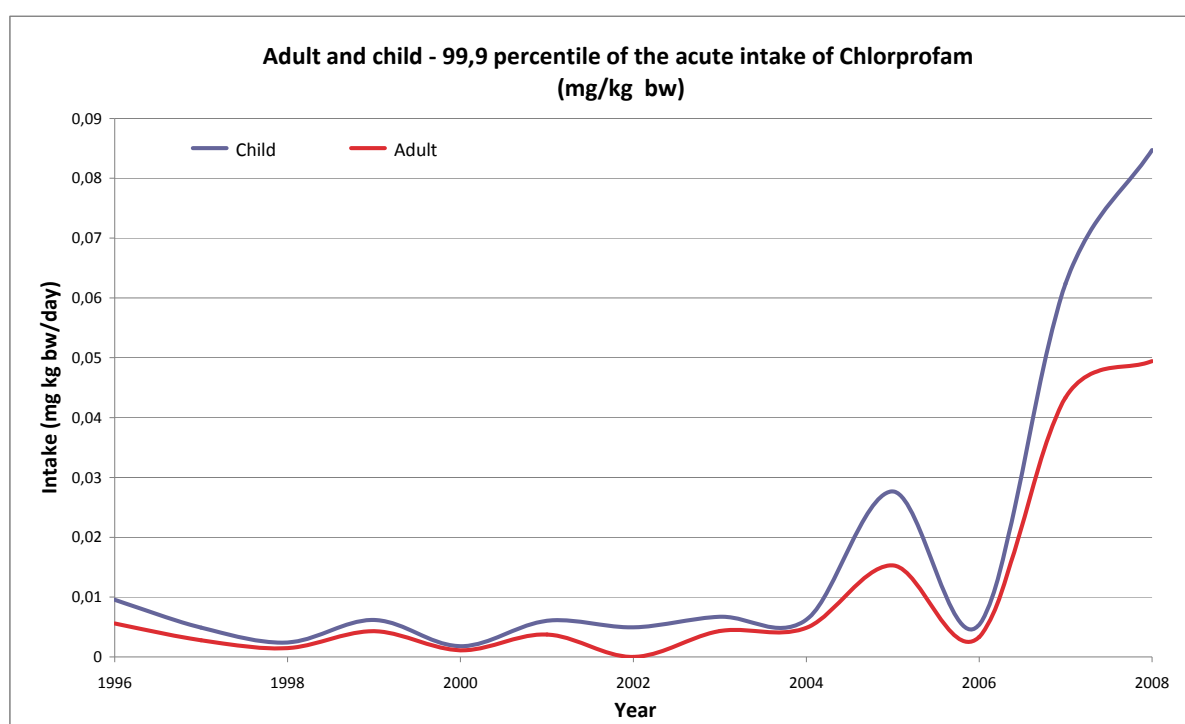


Figure 11: Acute probabilistic exposure (adult and children) comparing consumption of potato with residue data of Chlorpropham through the years from 1996 to 2008. X axis: Calendar years and Y axis: 99.9th percentile of the intake of Chlorprofam (mg/kg bw)

In addition, analysing the data in Figure 12, taking into consideration the 95% confidence interval of the 99,9th percentile, median, minimum and maximum of the exposure to Chlorpropham, for the general population; it has to be noted the shape of the curve followed more the fluctuation of the detected residue of pesticide rather than the consumption potato pattern across all population.

It is clearly visible that especially in the years 2007 and 2008, the error boxes showed a huge variation in the data. This could be explained by the large

amount of food sampled and analysed in those years and by the variation in the detected concentrations.

In addition, it has to be mentioned that a similar tendency was already discovered and discussed in the previous chapter on the thesis, in the section related to the use the Haperitif Indicator; that highlighted an increase in residue concentration during the last years monitoring (2007 and 2008) and a relative high peak in 2005.

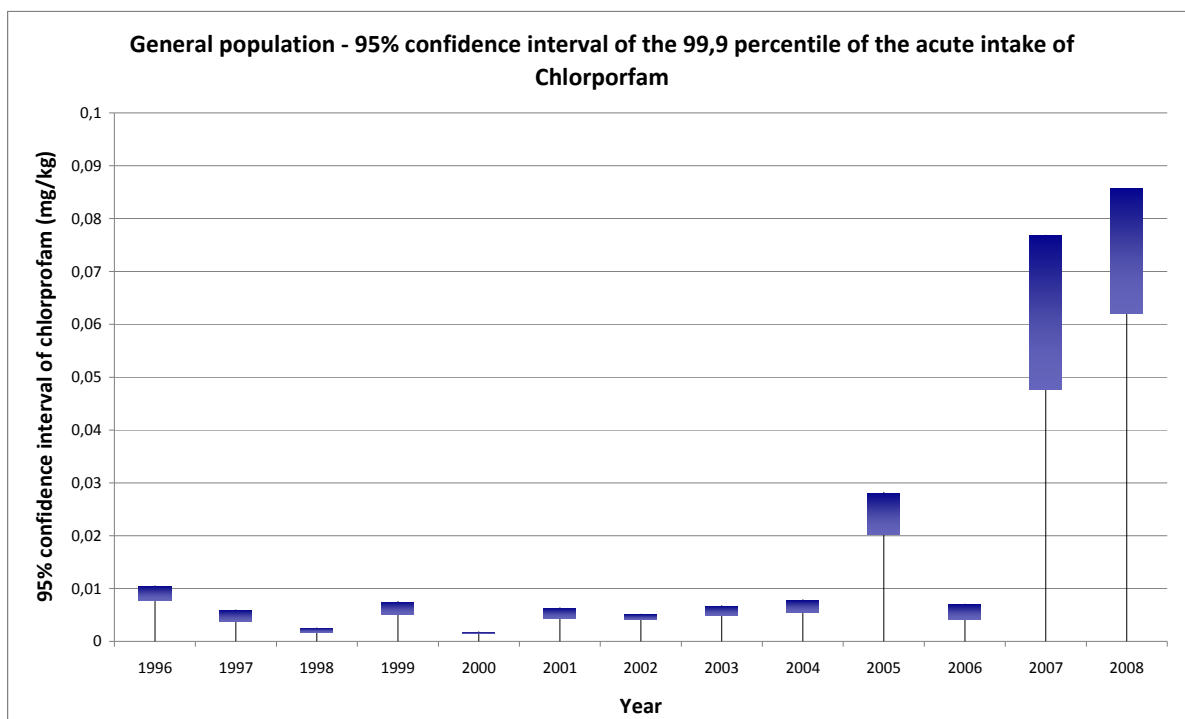


Figure 12: Bar chart showing the fluctuation of Chlorpropham acute exposure during 1996 to 2008 in general population. X axis: Calendar years and Y axis: 95% confidence interval of the 99,9th percentile, maximum and minimum of Chlorpropham (mg/kg)

A second level of application of the probabilistic approach would consist to assess the impact on the health of the consumer on a pesticide over all the commodities where it was detected. For this work example the insecticide Chlorpyrifos was used.

Chlorpyrifos is a crystalline organophosphate insecticide that inhibits acetylcholinesterase and is used to control insect pests. It is used on a variety of food and feed crops, golf courses, as a non-structural wood treatment, and as an

adult mosquitocide. According to the evaluation of JMPR 2004, the toxicological endpoints for acute and chronic exposure are 0,1 and 0,01 mg/kg.

Most of the parameters used in the previous assessment of Chlorprofam were also used in the present work example; therefore the Italian food consumption data were collected through dietary recalls and included 314114 individuals of all ages. Furthermore, population is divided in 2 different age strata (adult and child), the residue data were from the monitoring assessment of pesticide in Lombardy (1996-2008). In addition, in order to have comparable results, the number of iterations remained unchanged.

Chlorpyrifos was detected in 206 food commodities; most of them belonged to the category of pome fruit (n=102) and citrus fruit (n=45), from which apples represented the highest contribution to the exposure of Chlorpyrifos.

As described in the previous example of Chlorprofam, the acute exposure was exclusively taken into consideration; the results are expressed in details in Figures 13 and 14. The acute exposure was calculated considering the 99,9th percentile for the total population divided into two sub-groups (adult and children) filtered by age. Comparing the exposure, it was noted, that there was no overtaking of the correspondent toxicological endpoint (ARfD= 0,1 mg/kg), that is an order of degree higher than the highest detected residue. However it was noted a high exposure during the year 2007 of monitoring (see Figures 13 and 14, red lines); in this case the thorough analysis of the original dataset was required. It was then noted that in the mentioned year, a food sample of herb contained a concentration of Chlorpyrifos above the legislative permitted level, was found. Therefore it could be assumed that it was withdrawn by the national market, as soon as the official control of pesticide residues was performed. Therefore, for this example, it was then excluded from a refined assessment. In order to give a complete set of information it has to be noted that the red line, in both figures, represented the first assessment, with the complete set of data, including the illegal sample.

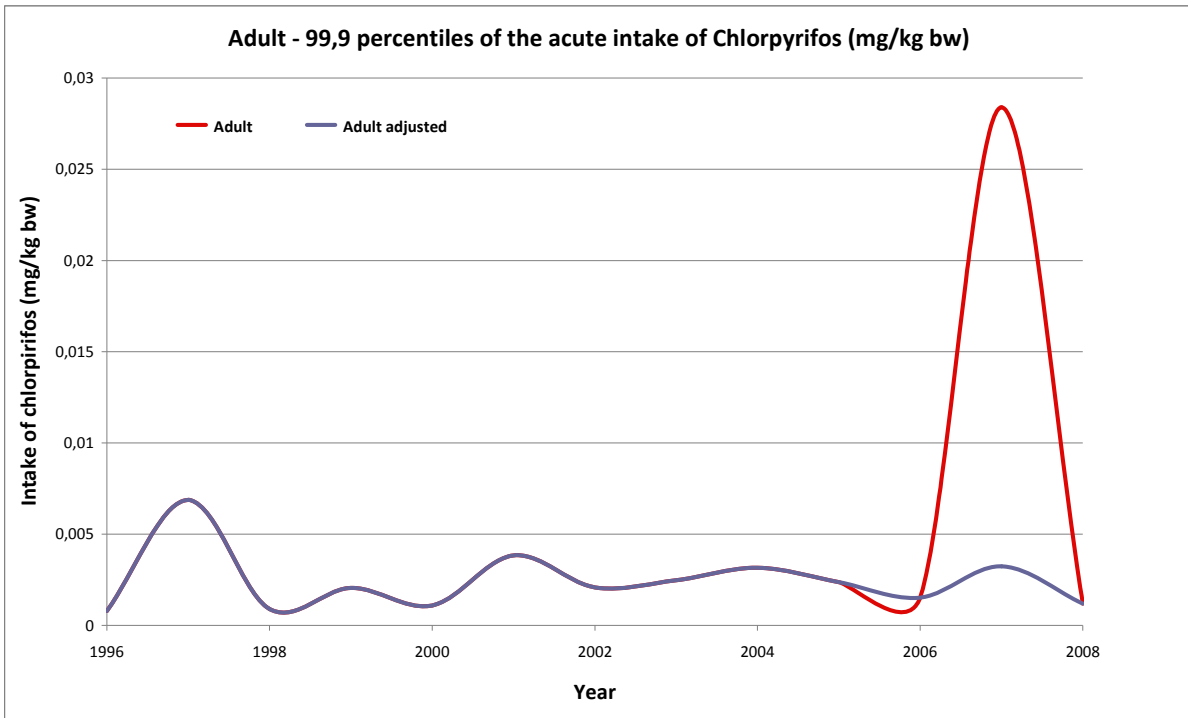


Figure 13: Acute probabilistic exposure, for adult, comparing residue of Chlorpyrifos through the years from 1996 to 2008, in all food commodities. X axis: Calendar years and Y axis: 99,9th percentile of the intake of Chlorpyrifos (mg/kg bw)

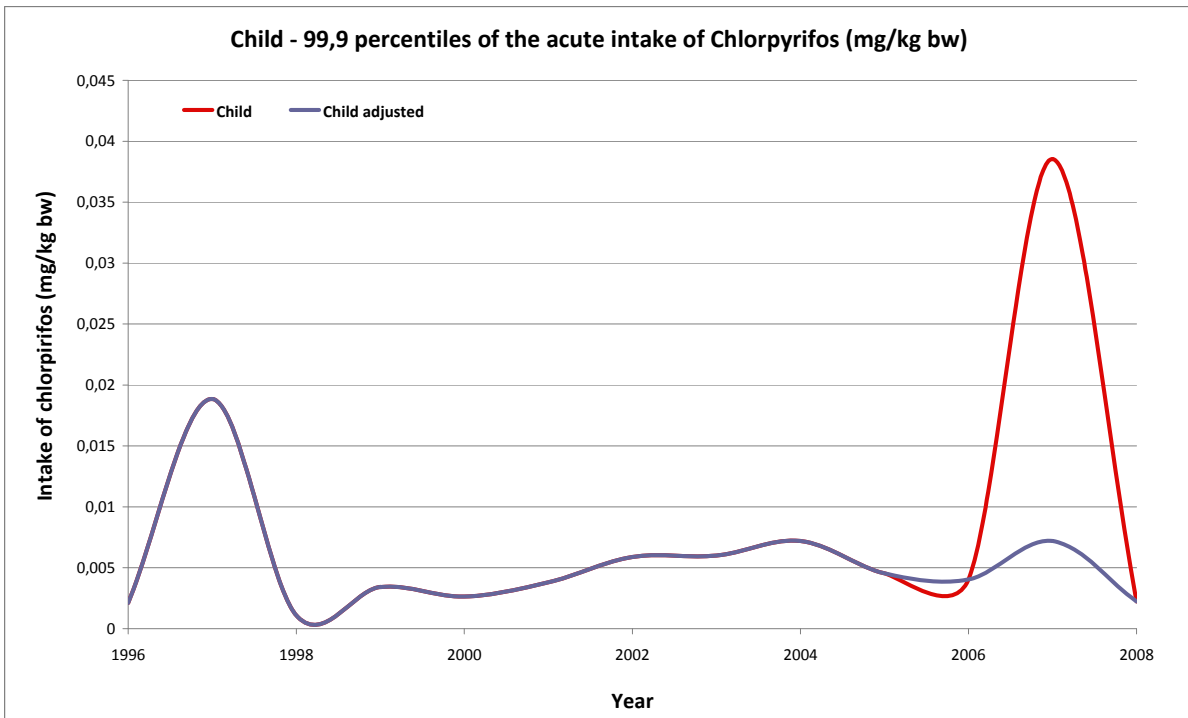


Figure 14: Acute probabilistic exposure, for child, comparing residue of Chlorpyrifos through the years from 1996 to 2008, in all food commodities. X axis: Calendar years and Y axis: 99,9th percentile of the intake of Chlorpyrifos (mg/kg bw)

According to the Regulation EC No. 396/2005 from the European Parliament and the Council has required that cumulative and synergistic effects of pesticides be considered when Maximum Residue Levels (MRLs) would be adopted. Therefore, a third level of aggregation was assessed, in the thesis, considering cumulative effects of pesticides with the same mechanism of actions. For this all the organophosphates (OP) detected in the monitoring year 2006, were considered.

OPs were identified as belonging to a Common Assessment Group (CAG) because they inhibit Acetylcholin esterase (AChE) and based on their toxicokinetic and toxicodynamic characteristics acute Cumulative Risk Assessment (CRA) was performed.

Table 16: Organophosphates (OP) compounds used in the cumulative risk assessment with the relative toxicological endpoint for acute exposure (ARfD and ARfD refined).

| Active Substance | ARfD (mg/kg bw/day) | Source | ARfD refined (mg/kg bw/day) | Source |
|---------------------|---------------------|-----------|-----------------------------|---------------|
| AZINPHOS-METHYL | 0,01 | SCoFAH 06 | 0,1 | JMPR 2007 |
| CHLORPYRIFOS | 0,1 | Dir 05/72 | 0,1 | JMPR 2004 |
| CHLORPYRIFOS-METHYL | NA | - | 0,1 | SANCO/3061/99 |
| DIAZINON | 0,025 | EFSA 06 | 0,025 | EFSA 06 |
| DIMETHOATE | 0,01 | Dir 07/25 | 0,02 | JMPR 2003 |
| FENITROTHION | 0,013 | EFSA 06 | 0,013 | EFSA 06 |
| FENTHION | 0,01 | JMPR 2000 | 0,01 | JMPR 1997 |
| PHOSALONE | 0,1 | EFSA 06 | 0,3 | JMPR 2001 |
| MALATHION | 0,3 | EFSA 06 | - | - |
| PARATHION-METHYL | 0,03 | JMPR 2003 | 0,03 | JMPR 1995 |
| PIRIMIPHOS-ETHYL | - | - | - | - |

11 organophosphates were detected in the monitoring year 2006 and as first step of the assessment the Acute Reference Dose (ARfD) was reported (Table 16). In addition, since some of the ARfD were calculated taking into consideration toxicological studies where the inhibition of the AChE was not considered a refined calculation was necessary to compare each toxicological endpoints according to the same common toxic effect.

A first deterministic CRA was performed using the Hazard Index (HI) approach on the basis of the common toxicological end-point (inhibition of AChE). By definition the HI is the sum of the ratios between the exposure and the reference value (ADI or ARfD) for each component (hazard quotient, HQ). A ratio less than 1 means that the combined risk is considered acceptable (EFSA 2009). Chlorpyrifos was then selected as Index Compound (IC) and the potencies of all other chemicals were normalised to the IC, calculating a Relative Potency Factors (RPF); which is

the ratio between the ARfD of the index compound and the ARfD on the selected pesticide. For a complete set of information the ARfD and the correspondent RPF are expressed in Table 17.

Table 17: Acute Reference Dose and correspondent Relative Potency Factors (PRF) for the Organophosphate compounds. The Index Compound (IC) is indicated.

| Active Substance | ARfD (mg/kg bw/day) | Relative Potency Factor |
|--------------------------|---------------------|-------------------------|
| PHOSALONE | 0,3 | 0,33 |
| AZINPHOS-METHYL | 0,1 | 1,00 |
| CHLORPYRIFOS (IC) | 0,1 | 1,00 |
| CHLORPYRIFOS-METHYL | 0,1 | 1,00 |
| PARATHION-METHYL | 0,03 | 3,33 |
| DIAZINON | 0,025 | 4,00 |
| DIMETHOATE | 0,02 | 5,00 |
| FENITROTHION | 0,013 | 7,69 |
| CARBARYL | 0,01 | 10,00 |
| FENTHION | 0,01 | 10,00 |
| PIRIMIPHOS-ETHYL | 0,01 | 10,00 |

A first deterministic CRA was performed using the Hazard Index (HI) approach. For each one of the compounds the National Estimate of Short Term Intake (NESTI) was calculated according to the FAO/WHO acute dietary intake assessment using the Italian consumption diet. The NESTI values were then reported and the results were disaggregated by food commodity and correlated to the hazard index of the index compound. A complete list of the acute exposure per commodity is show in Figures 15 and 16.

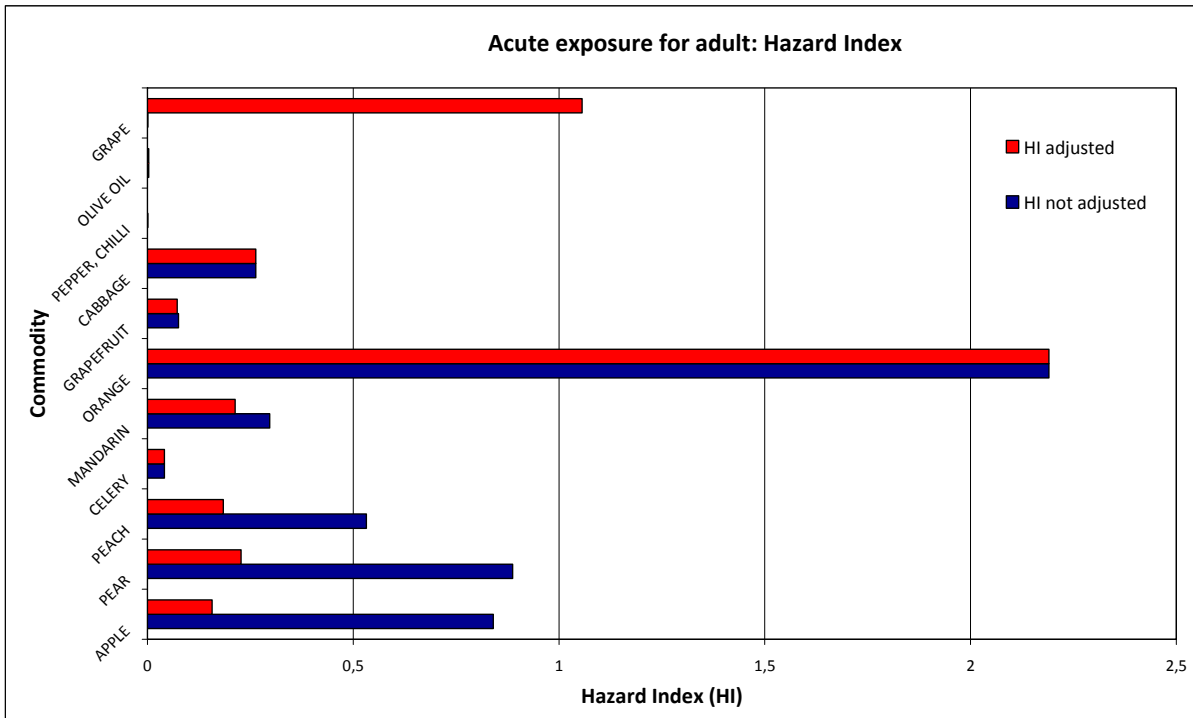


Figure 15: Acute exposure, for adult, using the hazard index approach. The results are disaggregated by food commodities.

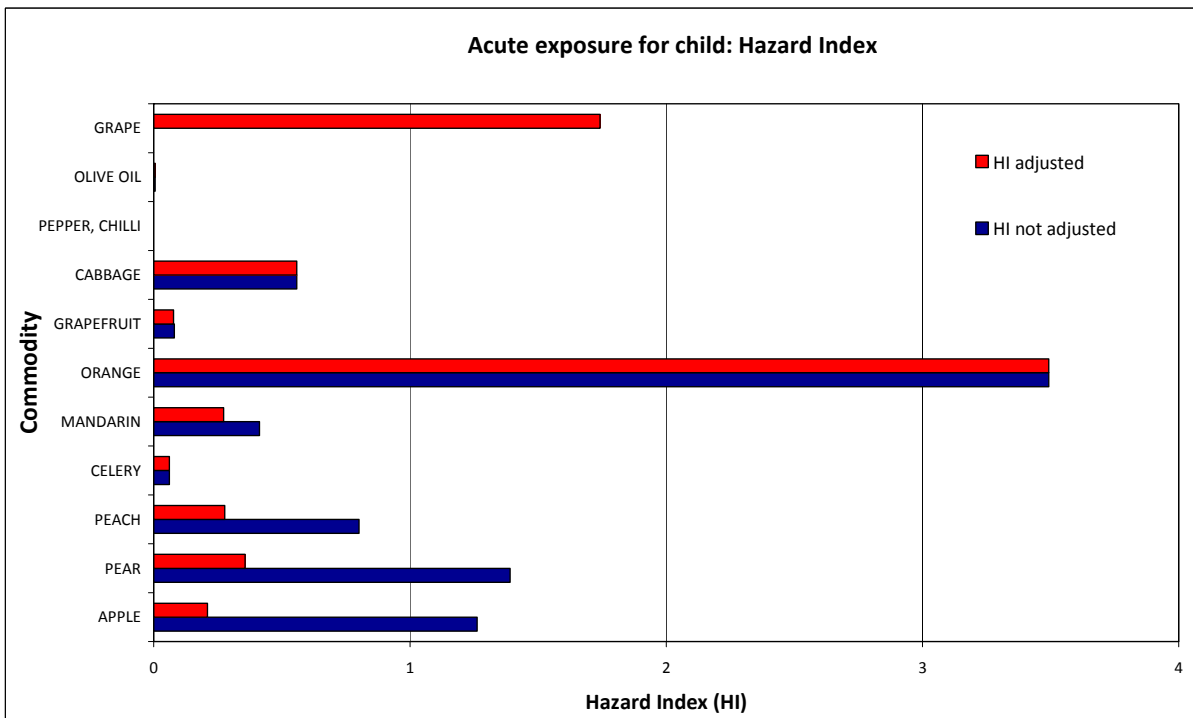


Figure 16: Acute exposure, for children, using the hazard index approach. The results are disaggregated by food commodities.

In Figures 15 and 16 it is highlighted the acute cumulative HI was > 1 for mint, apple, pear and orange and after adjustment, for mint, orange and grape. In addition, it has to be mentioned that all HI were calculated without taking into account processing factors and due to the high concentration of pesticide found in mint samples the was promptly withdrawn from the Italian market.

In addition, a probabilistic acute CRA based on RPFs was performed. The monitoring data for OPs (total of 1024 samples) were imported in the software Creme Food Software, the Italian consumption data stored in Creme were analysed to obtain averages and large portion estimates, for adult and child; which are shown, in details, in Figures 17 and 18.

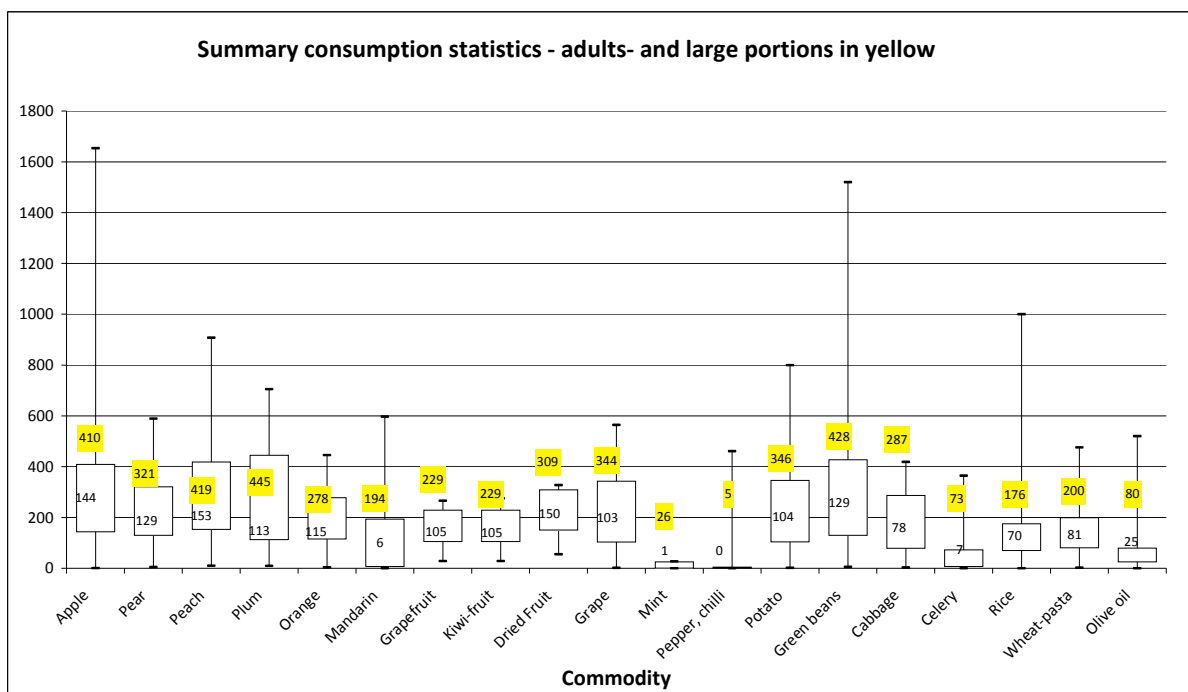


Figure 17: Summary consumption statistics, for adult population, indicates minimum, maximum and median consumption. The large portion is highlighted in yellow.

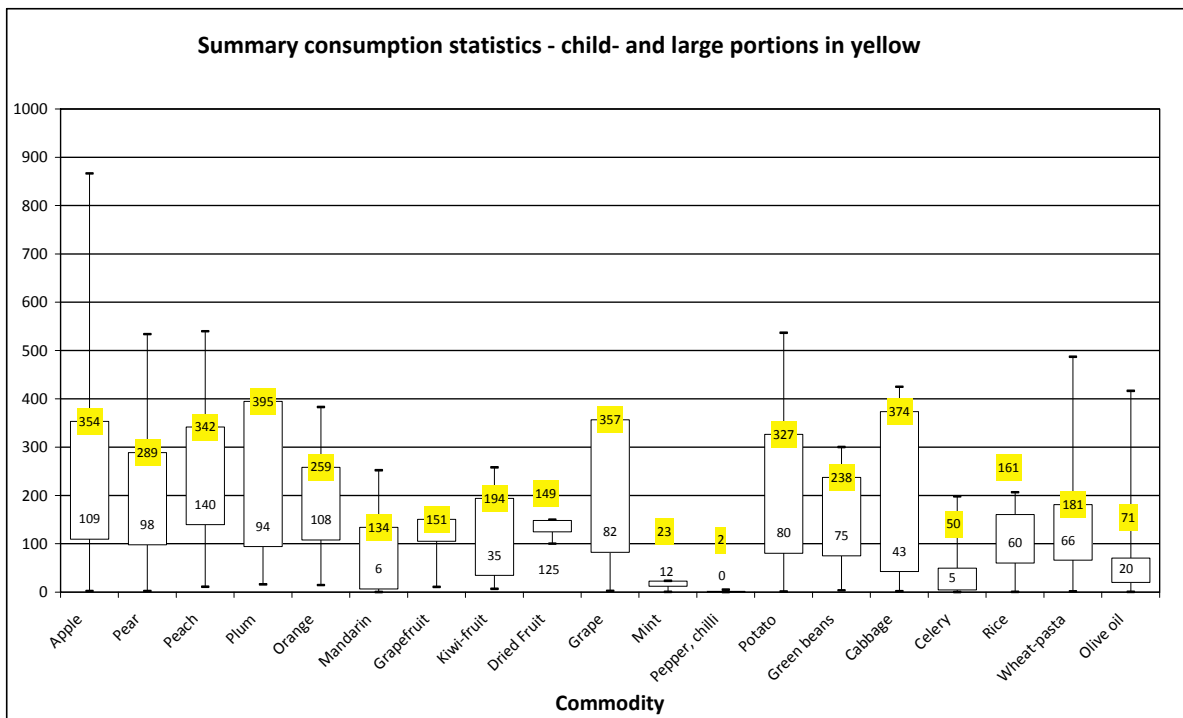


Figure 18: Summary consumption statistics, for child, indicating minimum, maximum and median consumption. The large portion is highlighted in yellow.

The data in the above two figures showed that a high contribution of consumption is not always linked to a high exposure to a chemicals. For example, apples and grape represented a high degree of consumption, according to the Italian diet. However they did not significantly contributed to the total acute exposure of OPs. Whereas mint, that showed a low consumption, was relevant for calculating acute exposure, due mainly to the high concentration of chemicals detected in these types of food samples. However, due to the high concentration of Chlorpyrifos in mint samples the correspondent data were excluded from the cumulative assessment. Therefore, Figure 17 and 18 do not contain this information.

Residue levels in Creme were adjusted according to the RPF and a probabilistic risk assessment was obtained, using the aforementioned Italian consumption diet and the data residue of pesticides from the monitoring year 2006. Probabilistic acute cumulative assessment indicates that the intake of the 99.99th percentile of adults and toddlers was below the set ARfD of the Index Compound. The results are show in details in Figures 19 and 20.

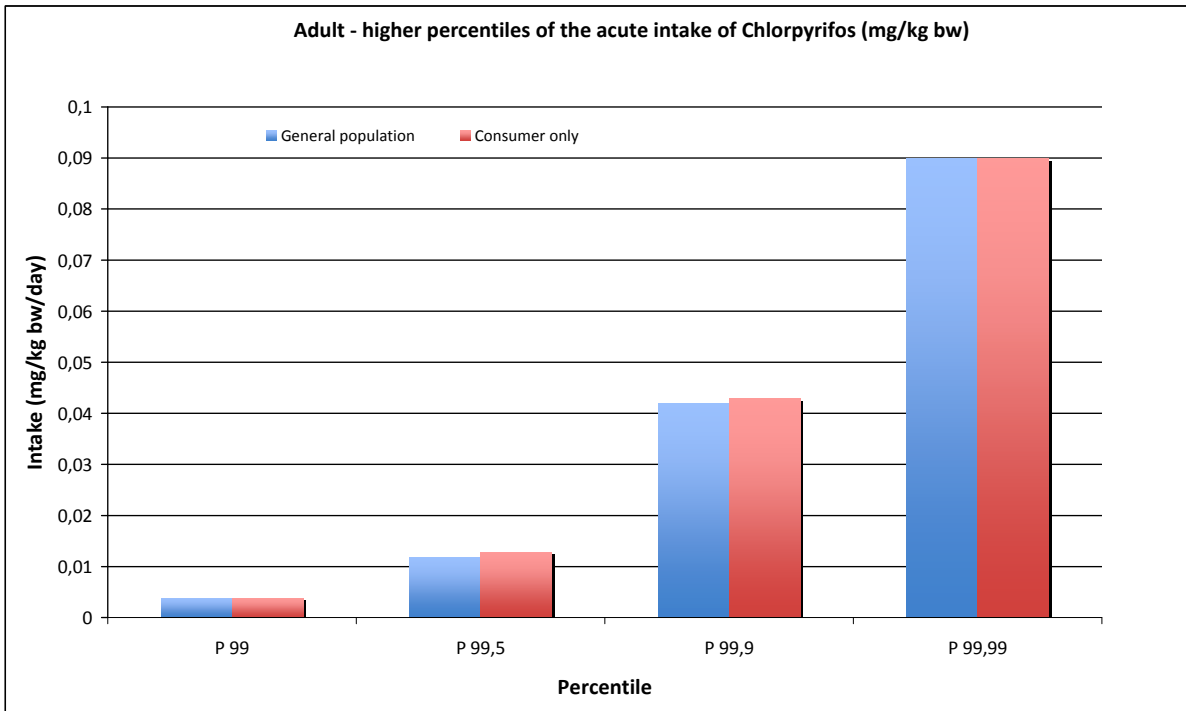


Figure 19: High percentile of the acute intake, for adult, of Chlorpyrifos.

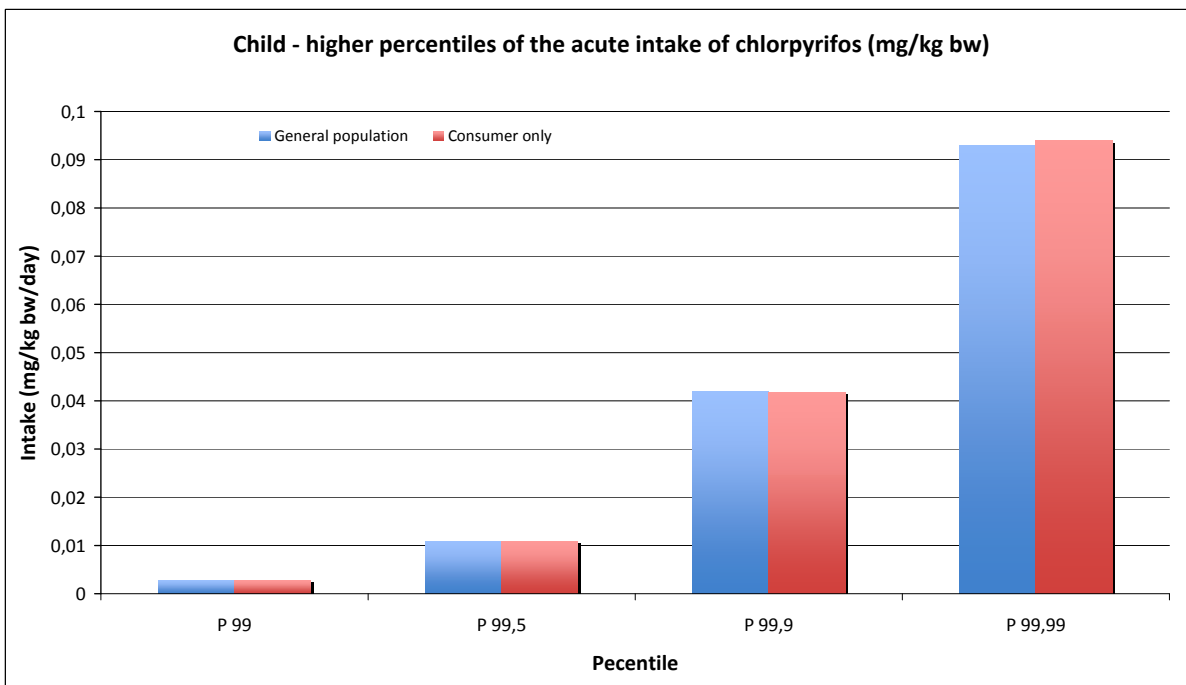


Figure 20: High percentile of the acute intake, for toddler, of Chlorpyrifos.

Overall Conclusions

The first aim of the thesis was to describe and analyse the results of the pesticide monitoring programme in Lombardy Region, from 1996 to 2008. The data of the official control plan on pesticide residues in food of plant origin showed that the number of samples analysed by the official laboratories of the Lombardy Region was equal to 9387 indicating that the overall number of samples was higher than the minimal number set by Ministerial Decree of 23 December, 1992. It is also relevant to mention that the number of irregular samples was equal to 135 with a percentage of irregularity equal to 1%.

Samples exceeding Maximum Residue Limits (MRL) are considered irregular as established by Council Regulation 396/2005/EEC (396/2005/EEC) which has harmonised across all EU countries such limits. These limits were set taking into account all categories of consumers including vulnerable groups such as children and vegetarian and include all the European diets. The values of MRLs were established in accordance with an assessment made by ' EFSA risk assessment using models of acute and chronic, and for each active substance were considered toxicological parameters most critical to an assessment more conservative risk for the consumer.

The number of samples without residues was equal to 6882 (69%); the number of samples with residues within the legal limit was 2968 (30%).

Taking into consideration the number of irregular samples through the years in consideration, it remained substantially unchanged (1%) despite the annual increase of the number of analysed samples. This is attributable in part to the activities of regional structures permanently engaged in official control plant protection products in Lombardy and in part to the constant revision strictly made by the Italian Ministry of Health and a growing awareness of operators in the use of agricultural pesticides.

Special attention was devoted in investigating samples of fruit and vegetables contain more than one active substance, which were 11% of the total analysed samples.

It must be emphasised that the MRL is not a toxicological limit and a violation is not necessarily a cause of concern for public or animal health. For pesticides authorized for agricultural use, the MRLs are set at the maximum safe level that one would expect if the pesticide is used according to the rules and restrictions specified in the authorisation.

A section of the thesis was especially dedicated to the analysis of pesticide residues in foods from organic markets. It was then found that in spite of being properly grown and processed, organic foods are not necessarily free from pesticides used in conventional farming. Contamination may be due to cultivation on previously contaminated soil, percolation of chemicals through soil, unauthorized use of pesticides, cross-contamination with wind drift, spray drift from neighbouring conventional farms, contaminated groundwater or irrigation water, or even occurred during transport, processing and storage. Presence of synthetic chemicals, however, does not necessarily preclude that the food can be described as organic, provided that all the requirements related to the production process have been fulfilled. Organic fruits and vegetables can be expected to contain fewer agrochemical residues than conventionally grown alternatives.

In our study, the comparison of the monitoring results obtained from conventional and organic food samples showed a 10-fold greater contamination in conventional products (27%) compared to organic food samples (2.6%). Results were similar regarding the presence of multiple residues, present in 0.8% of organic and 8.8% of conventional food samples and in agreement with the findings from other studies (Baker, B.P: *et al.* 2002). In the region of Lombardy, the concentrations of pesticides detected in organic commodities were in their greatest part below the MRL set for conventional products. Only in one sample (organic potatoes), the residues found were above the MRL; yet the intake of the active substance (Dicofol), as calculated for two groups of the Italian population, was far below the ADI (adults 3,5% ADI, children and toddlers 5%). During the same monitoring period, Dicofol residues were detected in 20 samples of conventional food products, including potatoes. Dicofol concentrations were below the MRL, with the exception of two samples (pears and strawberries). Therefore, in an attempt to compare organic and conventional foodstuffs in terms of potential risks for human health due to dietary exposure to pesticide residues, conclusions cannot be drawn easily, since in both cases the presence of residues above the set MRL is very low.

The outcomes of the monitoring program of pesticide residues implemented by the Region of Lombardy under the mandate of the Ministry of Health and with the cooperation of the Local Prevention Units and local laboratories, demonstrated that public health has been safeguarded with success in the last years. Moreover, given the fact that the complete dataset resulting from the monitoring program is collected and available after the end of each annual monitoring period; improvements in the flow of information are regarded as a prerequisite for checking the completeness of the information provided. It should be mentioned that presently the Region of Lombardy is taking action in order to improve the current practices and future efforts would continue in this direction in order to maintain consumers' trust.

A further step to understand the exposure of consumers to residue of pesticides in food was obtained by using the deterministic approach developed by EFSA in the recent past (PRIMo Model). It was found that among the detected 135 irregular samples, only 31 might cause harm to the health of the consumer. The most commonly found pesticides in irregular samples were Dimethoate, Procymidone, Ethion and Chlorotalonyl and Dithiocarbamate; which mainly belong to the functional classes of fungicide and insecticide.

Using the EFSA model for exposure assessment, it has to be noted that in case of overcoming of the acute toxicological endpoint, expressed in percentage of ARfD, the diet associated with the results are mainly from Northern Europe (Germany and UK), in case of absence of the Italian data. Therefore these results could be taken, with a high degree of uncertainties, as associated with residues of pesticides found in Lombardy coupled with the Italian diet. On the other hand, the results for chronic exposure, expressed as percentage of ADI, were found to be more accurate, being able to retrieve the Italian consumption data for the selected commodity.

However, it has to be noted that for the irregular samples coming from extra European countries; they were immediately withdrawn from Italy on arrival at the inspections borders. Even though these commodities had entered the Italian market, once analysed by the inspection bodies, they are withdrawn from the market, according to the RASFF.

One of the aspects that the deterministic approach, used in this section of the thesis, that could not be solved was the cumulative exposure for multi-residue samples.

In addition, some of the active substances found in irregular samples resulted already withdrawn at the time of sampling (e.g. DDT, Esachlorobenzene). Therefore, the health of the consumers was noticed a convict of fraud, in case the active substance was withdrawn from the market more than 3 years before the date of sampling.

An additional step was constituted by the use of the probabilistic method (Creme Software) to calculate the cumulative exposure of pesticides for the consumers. In this case three levels of aggregation were tested taking into account residues of Chlorprofam on one crop (potato), residues of the insecticide Chlorpyrifos in all food commodities and residues of the chemical group of Organophosphate. All three sets were plotted in the software along with the Italian consumption data (Turrini, A. *et al.* 2001), where the probabilistic acute cumulative assessment indicated that the intake of the 99.9th percentile of adults and children was below the set toxicological reference value for acute exposure.

Therefore, it maybe concluded that, even though, in this thesis only a relative small amount of data was treated, the actual European legislation and its implementation by the Region of Lombardy under the mandate of the Ministry of Health were highlighted wherein, of the safety to consumer health was assured to some degree. However, further implementation could be envisaged: for example, it was stated that each year the number of collected samples was higher than the minimal number set by Italian Ministry of Health, whereas, the number of irregular samples remained substantially unchanged through all years. This could represent a tool for better implementation of monitoring control, to reduce the number of analytical determination by focusing more on particular food commodities that are more prone to chemical contamination or focusing on certain classes of pesticides with high potential risk to the consumers.

It has to be noted that the purpose of the monitoring programme of pesticides at National level, from 2010 onwards, is not only to identify samples above the limit of quantification but also to assess the consumer exposure. For this it could be relevant to establish a database on the authorised GAPs and pesticide uses at national level. In addition, it could be envisaged the implementation of the new format, developed by EFSA, for reporting the pesticides monitoring results, to put efforts in recording and reporting the production method (conventional vs. organic) of the analysed samples, to report possible reasons for the MRL overrun and to clearly

indicate if, as a consequences of a sample exceeding the MRLs, the lot was not put on the market and therefore was not available for consumption.

It has to be acknowledged that, during the recent years, a lot of progress was made to calculate and reduce the risk of consumers, derived by pesticides' exposure. However, as part of the Risk Assessment Paradigm, the communication of risk plays an important role. Reading newspapers, we are often in contact with reported food scares, which might distort the risk perception of the general consumers. Therefore, there might be the need to reinforce the long-term investment, at European and National level, in promotion to inform consumers and educational campaigns on food-borne risks; this would help to build an individual awareness towards the risks from pesticide residues in food commodities.

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