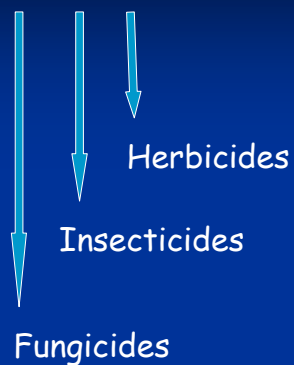


Health effects due to pesticide exposure: regulatory aspects and new challenges

Emanuela Testai
Istituto Superiore di Sanità- Roma (Italy)
testai@iss.it



Pesticides = Plant Protection Products



To be effective on pests :
reactive compounds



Possible health effects



Different mode of action

Different targets

Exposure to Pesticides

* Professional exposure:

processes of production and packaging;
transport; storage; agricultural operators.

* General population exposure :

presence of residues in food and drinking
water; pesticide use in private and public
building e presence of residues on furniture
and surfaces

Regulatory aspects

In Europe pesticides are regulated by EU Dir. 91/414/CEE. Those which can be commercialized are included in a positive list known as Annex 1. The EU Directive deals with the harmonization of pesticide registration procedure in all the MS.

The inclusion depends on a **risk assessment evaluation** (Monograph) carried out by a Rapporteur Member State, commented and discussed by the other MS in EPCO Meeting and in the EFSA PPP-Panel.

In each MS the competent authority is responsible for a provisional registration of those pesticides which are not yet included in Annex 1. This procedure foresees the evaluation by the National experts of a registration dossier prepared by the Company .

Regulatory aspects

Studies included within the Registration Dossier must be carried out according to specific **OECD** Test Guidelines in research centers which are certified for **GLP** (Good Laboratory Practice)

GLP compliance of research centers and **CRO** is certified in Europe by National Monitoring Authority inspecting them and auditing their studies every other year.

OECD TG \rightleftharpoons GLP

Quality

MAD (Mutual Acceptance of Data)

No duplication of regulatory testing
Commercial exchanges easier

TOXICOLOGICAL RISK ASSESSMENT: A 4 STEP PROCESS

1. HAZARD IDENTIFICATION

Which is the difference between **hazard** and **risk**?

The **hazard** is an intrinsic feature of the chemical

The **risk** is the probability to experience an adverse effect following exposure to the chemical



A lion represents without any doubt a 'hazard' for humans

A man without any defense in front of a lion has a high 'risk' to be attacked and damaged. In these conditions only two factors could reduce the risk, without affecting the hazard: 1. the lion has just eaten. 2. the man works in a cirque .

If the same lion is closed in a cage, its hazard is again the same, but the risk to be attacked for the man outside the cage tends to zero (it will not be =0 since there is always the possibility that the cage could be damaged and /or open)

1. The hazard identification represents a qualitative aspect of RA and give answers to the questions:

what does the chemical do?

Which are the produced effects ?

Are they reversible or persistent?

Which are the target organs/tissues?

Which are the mechanisms

Which is the general behavior within the organism?

2. DOSE-RESPONSE RELATIONSHIP

Quantitative Aspects

'Omnia venenum sunt: nec sine veneno quicquam existit. Dosis sola facit ut venenum non sit' (Paracelso)

At what concentration does the effect occur ?

The threshold for the effect can be achieved with :

A single exposure (acute and generally at high doses)
accidents, poisonings

Repeated exposure (low doses for prolonged times;
the low doses are generally non toxic if taken
singularly cumulative effects)

Which is the critical effect?

The effect is considered critical if:

- It is the one present at the lowest dose.
- It is the most relevant effects from a toxicological point of view (e.g. Hair loss *vs.* early marker of hepatic damage)

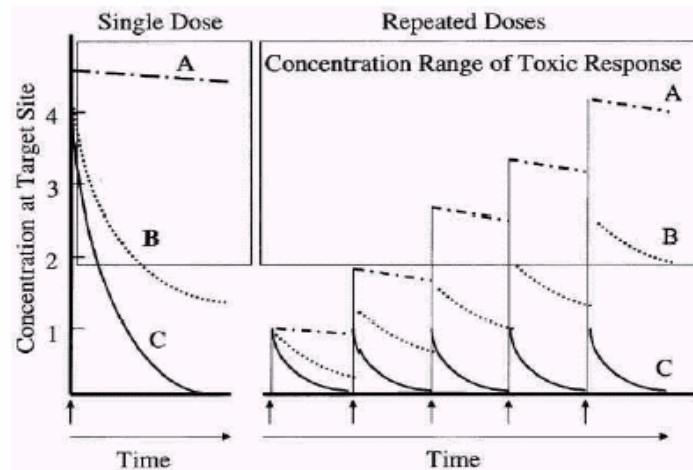
Preventing the critical effect represents a total prevention for human health

Exposure Duration & Frequency

Given a certain dose, its toxicity is higher on increasing duration and frequency of exposure. It may be due to:

- a. Bioaccumulation of the chemical within the organism \Rightarrow frequency of administration is higher than rate of elimination \Rightarrow the xenobiotic concentration becomes higher than the toxicity threshold (importance of toxicokinetics)

Relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate




2. Cumulative damages \Rightarrow administration rate is higher than damage repair process within the organisms

It is possible to have cumulative damages without any bioaccumulation of the chemical \Rightarrow recovery time is usually higher than elimination time.

Es: exposure to ethanol results in steatosis (lipid accumulation in the liver); ethanol is eliminated in a very short time, but lipids are removed from the hepatic tissue in a much longer time \Rightarrow outcome of chronic effects (cirrhosis) in heavy drinkers.

3. EXPOSURE EVALUATION


Which is the level of exposure?

- external  At which concentration the chemical is present in different matrices or environment (diet/air /water/working place/consumer products)?

Which is the preferred route(s) of exposure?
(inhalatory, percutaneous, oral..)

Which the pattern of exposure ? Working hours,
environmentally, dietary,...

Which is the toxicologically relevant specie? (e.g.,
pesticides degradation products)

- internal  At what concentration the chemical is present in different organ/tissue and in the target site?

Which the fate of the chemical within the body?

Which is the concentration of the chemical
species toxicologically relevant?

ADME

Biomarkers of exposure

Biomonitoring studies and
'body-burden' measurments



4. RISK CHARACTERIZATION

Which is the probability to have an effect and at what extent in the exposed population?



Dose-response relationship data are compared with information of the extent of exposure to estimate the probability to observe the toxic effect within the population.

Variability factors:

1. **Exposure** (duration, dose, route): which are the groups with higher levels of exposure?
2. **Susceptibility** (age, patho/physiological status, genetic and/or acquired factors): which are the more susceptible groups, at the same levels of exposure?

When the more vulnerable groups are protected, all the population is protected

Uncertainty factors:

Quality of the available studies and of the experimental results

Adequacy of the experimental model (relevant animal specie, study duration)

Extrapolation of animal data → human and high experimental doses → low actual exposure dose

Introduction of uncertainty factors (UF) to take into account variability and uncertainties in the achievement of reference values.

The Registration Dossier provides information on the active substance (a.s.) and on different formulations which will be commercialized.

Data on the a.s.

- * Identity and physico-chemical data (eg: boiling point, vapour pressure, Henry's constant, density, solubility, logPow, explosivity, inflammability)
- * Uses and efficacy
- * Analytical methods e related l.o.d. (in different matrices: soil, fruit, vegetables...) (HPLC, GC, GC/MS, HPTLC, scintillation,..... multiresidue methods)

- * Toxicological studies for the evaluation of health effects on humans and animals :

Toxicokinetics studies :ADME
(AbsorptionDistributionMetabolismExcretion-
including a dermal absorption study)

- Administration of the a.s. (radiolabeled or not) via oral route and i.v.
- Determination of plasma concentration $f(t)$ to calculate AUC , $t_{1/2}$ e Cl_i
- Determination of $f(t)$ of urinary and fecal excretion and content in expired air of parental a.s. and its metabolites
- Identification of metabolites
- Determination of bioaccumulation potential after repeated exposure and residues in different organs $f(t)$

- * Acute toxicity studies on rat (oral, dermal and inhalatory LD_{50})

- * Skin and Eye irritation in rabbit

- * Skin Sensitization on guinea pig

Important for C&L (classification and Labelling)

- * Short term repeated toxicity

28 and 90 days with rodents (rat and mice);
28, 90 days and 1 year with dog - oral, dermal
and inhalatory route.

Identification of a NOAEL (No Observed Adverse Effect Level) and a LOAEL (Low Observed Adverse Effect Level) Propedeutical to long term repeated toxicity studies and relevant for ARfD and AOEL definition

* **Genotoxicity studies**

in vitro mutagenicity test on bacteria and mammalian cells,
in vitro test for chromosomal aberration, in vivo
micronuclei test

* **Chronic toxicity and cancerogenicity studies**

2 years (rat) and 18 months (mice) oral route (diet)

Identification of a NOAEL and a LOAEL and major
effects and target organ

* **Reproductive toxicity and developmental toxicity
studies**

Two generation study (rat) and teratogenesis with rat and
rabbit

Identification of a NOAEL and a LOAEL and major effects

**ADI = Acceptable Daily Intake = the dose which
can be taken every day in one life without any
health effect**

Calculated on the basis of the lowest NOAEL from toxicity
studies (usually chronic toxicity or 1 year dog), to which an
uncertainty factor (UF) is applied, varying depending on the
nature of the effects and on the quality of the studies

No genotoxic and reprotox effect present and NOEL derived
from a chronic study, UF = 100 applied (10 for interspecies
and 10 for intra-species variability).

Above mentioned effects present or chronic toxicity data not
available, higher UF used (200-500-1000).

Lowest NOAEL = $1.4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ coming from the chronic
toxicity study in mice $\rightarrow \text{ADI} = 0.014 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$

ARfD = Acute Reference Dose

It defines the acute risks for group of individuals which are exposed to particularly high levels of the a.s. for limited period of time.

Calculated only if the a.s. has an elevated acute toxicity and/or specific and relevant toxic effects (i.e. neurotoxicity not present in chronic toxicity studies or developmental effects), by applying an UF to the value of the lowest NOAEL coming from a short term repeated toxicity study, using the more appropriate route of exposure.

AOEL= Acceptable Operator Exposure Level

Calculated on the basis of the lowest NOAEL from a short term repeated toxicity or reproductive toxicity studies, using the more appropriate route of exposure of workers, corrected for oral or dermal absorption factors to which an UF (usually ≥ 100) is applied . Two AOEL values can be defined : a systemic and a percutaneous one.

The more appropriate exposure scenario are defined and operator exposure modeled to identify the AOEL and the IPD (individual protection devices) which should be used and mentioned in the label.

* Identification of relevant residues to calculate MRL (Maximum Residues Levels)

A.s. and/or relevant metabolite residues testing in agricultural specimen and livestock.

Data on residues and n° of agricultural products for which pesticide use is foreseen MRL, considering the ADI value.

The sum of MRL present in all the products on which the pesticide use is authorized defined as TMDI (Theoretical Maximum Daily Intake), should be a small % of the ADI (since other route of exposure may contribute to total exposure) and never above the ADI.

The unrealistic TMDI value is often replaced by the EDI (Estimated Daily Intake), which is an estimated consumption based on typical diets.

MRL = 0.05 mg/kg on different crops, based on the WHO model a TMDI = 0.0016 mg/kg bw is obtained accounting for 11% of the ADI ($0.014 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$).

* Environmental Fate (water, air, soil)

Identification of relevant metabolites

Application of environmental modeling

* Effects on non target organisms (ecotoxicology)

Studies on terrestrial vertebrates, aquatic species, arthropodes, soil macro- and micro-organisms

Risk characterization and overall risk assessment

Identification of R and S Phrases (label)

Data on the formulation

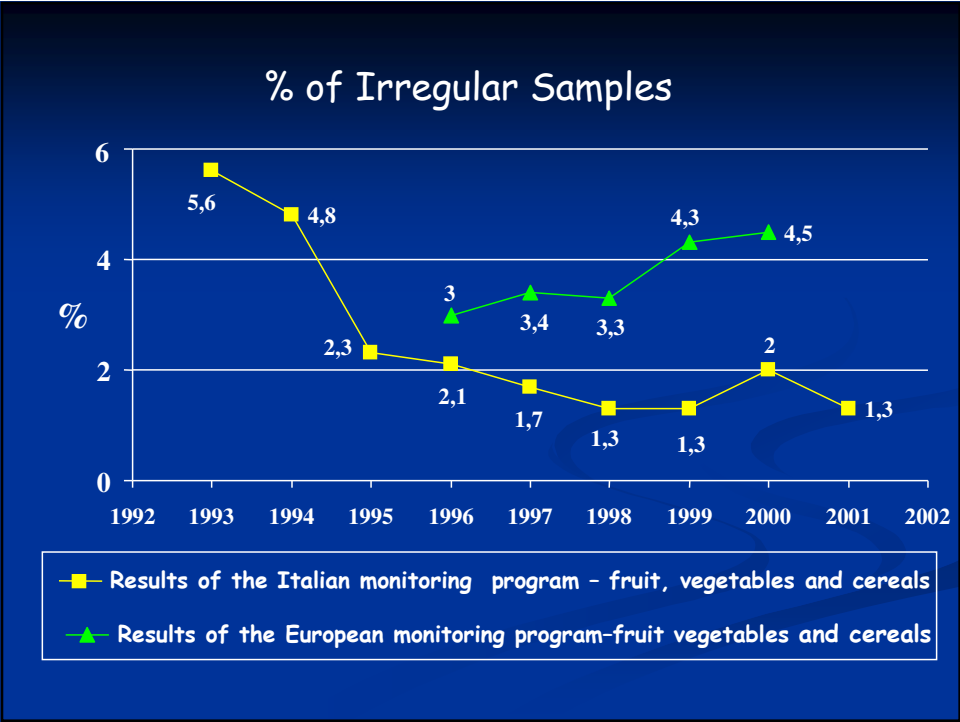
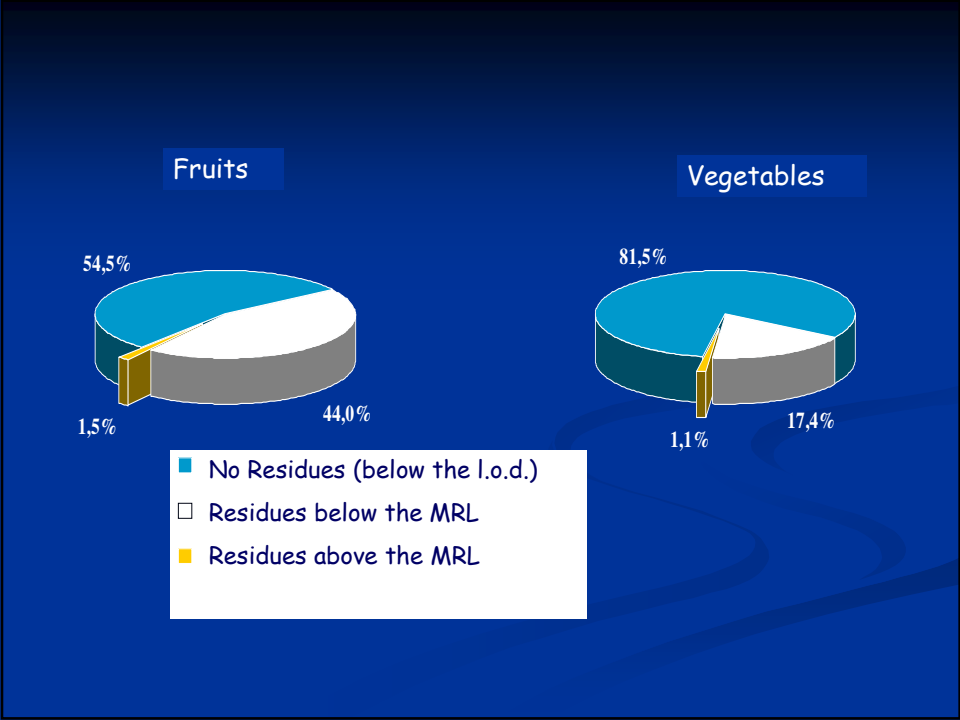
- * Composition and physico-chemical data (% of the a.s., identification & quantification of other components: surfactants, solvents, etc.)
- * Uses and efficacy
- * Analytical methods and related l.o.d.
- * Oral, dermal and if relevant inhalation toxicity studies. Skin and Eye irritation, skin sensitization
- * Environmental fate and ecotox

Each MS has a National Plan to control residues in agricultural products (EU Dir. 90/642/CEE), whose results should be compiled in a report to be sent at EU level.

Limit values have been established also to control drinking water quality both for the a.s. (0.1 µg/l-admissible level) and the relevant metabolites. Limits not driven by specific toxicological evaluations, but conservative enough. Monitoring plans should be in place in each MS.

The situation in Italy

About 9000/year analyzed samples : 86% Fruits and 122% Vegetables more than requested at EU levels.



About 1-1.5% samples with residues above the MRL

Decreasing Trend in the number of irregular samples with time (5.6% in 1993)

21%-25% of samples with more than 1 residues

A.s. more frequently found in fruit and vegetables :
Procimidon, Chlorotalonil, Chlorpyrifos, Endosulfan, Imazalyl,
Azinfos-methyl, Metidathion, Diphenylamine and Malathion
(old generation pesticides)

A.s. more frequently found in water: triazines and their
metabolites , bentazone and molinate

New pesticides are absolutely better or
monitoring plans should be updated?

OPEN ISSUES

1

Risk assessment is based on data on single chemicals. Possible interactions between different pesticides or of pesticides with other chemicals for human use (e.g. drugs, diet, smoke) are ignored.

2

More susceptible individuals (due to age, genetic or acquired reasons) are poorly considered and it is not clear if the UFs include these difference in vulnerability.

1 Possible consequences due to combined exposure to different pesticides

Combined exposure to non genotoxic pesticides, with different targets, different mode of action, no TK interactions, at doses \leq NOAEL: total independence

Combined exposure to pesticides each at doses \leq NOAEL with the same target and mechanisms : dose additivity.

Combined exposure to pesticides each at doses \geq NOAEL independently on their mechanisms : additivity (synergism / antagonism cannot be ruled out).

In developmental toxicity, beside the mechanism of action, the window of exposure (corresponding to different organogenetic phases) is crucial.

1

Multiple exposure to different pesticides



Same food containing multiple residues

Different food, each containing 1 residue

EDI values calculated for an average Italian diet are generally 2-5% ADI

Considering the possibility of dose additivity and summing up % EDI values, 20-50 different pesticides showing the same target and the same mode of action should be ingested to reach the ADI value.

Children and susceptibility to pesticides

1993: USA National Research Council Document on *'Pesticides in the diets of infants and children'* indicated the lack of knowledge on *age-related susceptibility*

1996: *Food Quality Protection Act* indicated the possibility to use additional UF of 10 to protect children. Special focus on OPT pesticides and their neurotoxic effects (request to conduct a cumulative risk assessment for this class of pesticides, taking into account they have the same target and mechanisms)

Too much? Or the contrary?