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# Exposure to pesticides residues from consumption of Italian blood oranges

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## Abstract

The paper reports the results of a 5-year study aimed to evaluate pesticide levels, deriving from orchards activities, on the Italian most common orange cultivar (*Citrus Sinensis, L. Osbeck, cv. Tarocco*). The study, using a Bayesian approach, allowed both the qualitative (the number) and quantitative distributions (the amount) of pesticides to be determined with its own probability value. Multi-residue analyses of 460 samples highlighted the presence of: ethyl and methyl chlorpyrifos, dicofol, etofenprox, fenazaquin, fenitrotion, imazalil, malathion and metalaxil-m. 30.5% of samples contained just one pesticide, 2.16% two pesticides and 0.65% of samples had three pesticides at the same time. The most common residue was ethyl chlorpyrifos followed by methyl chlorpyrifos.. The Estimated Daily Intake (EDI) values for ethyl and methyl chlorpyrifos, as well as the distance from safety level (NOAEL), were calculated. The risk has been differentiated in order: 1) to take account also the period of real citrus consuming (180 days); 2) to differentiate the risk derived from eating oranges that contain a certain level of chlorpyrifos from those unspecified. The most likely EDI values for ethyl chlorpyrifos, derived from Italian blood orange consumption are: 0.01 and 0.006 mg/day, calculated on 180 and 365 days, respectively. Considering the probability of occurrence of ethyl chlorpyrifos, the EDI values are reduced up to: $2.6*10^{-3}$ and $1.3*10^{-3}$ mg/day, respectively. Analogously for methyl chlorpyrifos, the EDI most likely values are: 0.09 and 0.04 mg/day, respectively. Considering its probability of occurrence the EDI values are: $6.7*10^{-3}$ and $3.4*10^{-3}$ mg/day, respectively. The results confirmed that levels of pesticides in Italian Tarocco oranges deriving from a controlled chain are safe.


**Keywords:** EDI, NOAEL, Tarocco oranges, risk assessment, safety, @ risk, Monte Carlo.

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## Introduction

The increase in recorded incidence of food-borne illness have driven the more industrialised countries to develop more effective food safety control systems, based both on process (design) and on performance-based (product) systems (Cho and Hooker, 2009). All food operators are requested that they are managing food safety aspects correctly, developing appropriate control procedures, analyses and monitoring activities. A risk-based approach is strongly recommended. One of the most important aspect concerns the presence of phytosanitary products.

The most comprehensive report of pesticide residues in Italy is prepared annually by an important environmental association (Lega Ambiente, 2008). They reported the results of the analysis of 10,048 vegetables and fruits carried out in 2007 by Italian Public Authorities involved in food control (Chiappara and Fallico, 2008), and highlight that the number of irregular samples of oranges declined from 1.3% to 1.0% but that 47.4% of samples contained at least one pesticide. This improvement is significant also for Sicilian production, where of 80 samples of analysed citrus (lemons and oranges): 57 (71.2%) were pesticides free, 16 (20%) contained one pesticide, and 6 samples (7.6%) had more than one pesticide residue. The number of irregular samples in the citrus chain, according to results of national monitoring plan for pesticides residues on food products (P.N.R.A.), co-ordinated by The General Directorate for food safety and nutrition of Italian Ministry of Health, has decreased significantly in the last decade. In fact, it declined from 5.6% in 1993 to 1% in 2006.

The total Italian production of citrus fruit (medium value 2004-2006) is about 3.4 million tons, with an invested area of 170.000 hectares. Sicily supplies 52.2% of orange production

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and 50% of Italian orange production comprises blood oranges; this percentage increases up to 70% in Sicily where the cultivar *Tarocco* is predominant. (Tribulato, Maccarrone, La Rosa, 2007). In blood oranges there are many healthy substances, with antioxidant activity (Maccarrone, 2004). In fact their juice contains anthocyanins, up to about 100 mg/l in freshly-squeezed *Tarocco* juices (Arena et al. 2001), ascorbic acid up to about 800 mg/l (Maccarrone, 2004), hydroxycinnamic acids derivates like coumaric acid (Rapisarda et al., 1998).

The most widely used class of phytosanitary products in farming are the organophosphorus compounds. They represent about the 40% or registered products. They can be very dangerous as inhibit the normal action of acetylcholinesterase (AChE). The most common one is chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate]. Alternatively in post-harvest treatment thiabendazole and imazalil are currently applied to control mould decay in citrus fruit (Schirra et al., 1996).

Many studies have been carried out in order to evaluate the decay kinetics of some pesticides currently used on citrus fruit. Montemurro et al. (2002) found that degradation kinetics of chlorpyrifos depends on commercial formulation as well as for fenitrothion (Montemurro et al., 2005). Schirra et al. (2005) evaluated the residue levels, as well as persistence and storage performance of citrus fruits treated with fludioxonil.

The importance of a risk-based approach to guarantee food safety/quality is strongly recommended both by Regulatory Agencies (Reg. CE 852/04; FAO, 2006) and other bodies (ISO 22000:05; GLOBAL/GAP). Many methods have been proposed to perform risk assessment. The simplest ones, qualitative or semi-quantitative, take in account just the probability and the consequences (impact) of the negative event(s). Exhaustive methods to

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perform risk assessment, from general applications to food safety/quality, are available in literature (Tennant, 1997; Vose, 2000; FAO, 2006). Risk assessment is based both on *deterministic approach* when the calculated risk is based on a point estimate, usually the worst-case value for each input variable; instead in a *probabilistic risk assessment model* both variability and uncertainty in input variables can be taken into consideration, by using probability distributions to represent the input variables instead of point estimates (Vose, 2000; Spanjersberg et al. 2007). Vose (1998) proposed a QRA, based on Monte Carlo simulation method, to evaluate microbial food safety. The probabilistic approach has been used by Slob and Pieters (1998) to estimate human acceptable exposure limits (RfD, ADI, TDI) using distributions for extrapolation factors instead of single uncertainty factors, more conservative; beside Slob (2006) published a study on the probabilistic dietary exposure assessment taking into account variability in both amount and frequency of consumption. The probabilistic model has been also used (Spanjesberg et al., 2007) for allergen risk assessment, resulting in a more exhaustive risk assessment respect to traditional deterministic risk assessment. Beside probabilistic modelling has been used for exposure assessment of operator and residential non-dietary exposure for pestidide products (Lunchick, 2001), for cumulative risk assessment of the exposure to organophosphorus and carbamate insecticides in the Ducht diet (Boon et al., 2008) and also to estimate children's short-term residential exposure to lead (Canales, Leckie, 2007).

The aims of this paper were: a multi-annual evaluation, both qualitative and quantitative, of pesticide residues on blood oranges (*Citrus Sinensis L. Osbeck, Cl Tarocco*); to estimate the daily intake of residues by consumption of blood oranges; the probability of consumers' overexposing to exposure levels above the TDI.

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## Materials and methods

This study has been carried out in collaboration with the Italian leading company of blood oranges (*Oranfrizer, Scordia (CT)*). It produces both for the internal market and, overall, for the international one (UK, Germany, Japan, etc.). The company in the last decade has adopted many quality schemes, as well as: farm inspections and chemical controls of product.

*Sampling and Multiresidual analysis*

During the 5-years of the study (2003-2007), 460 samples were collected as indicated in Table 1. Within 48 hours from picking, in triple, a multi-residue analysis was carried out. According to ISTISAN 97/23 protocol. A purée was obtained from1 kg of oranges. Puree (10 g) was mixed with diatomaceous earth (13 g), transferred into a glass column (20 x 200 mm) and eluted with four aliquots (25 ml each) of a mixture dichloromethane-ethylacetate (50-50). The eluted sample was dried under reduced pressure, re-dissolved with methanol (1 ml) and an aliquot (0.1 µL) injected to GC-MS in splitless conditions.

The GC-MS system was a SHIMADZU GC 17A-MD 5800, equipped with a column SLB™-5ms (L × I.D. 30 m × 0.25 mm, $d_f$ 0.25 µm), with a linear flow of 1.0 ml/min., a linear velocity of 36.3 cm/sec. The sampling time was of 5.0 min., the injector temperature was of 270.0 °C. The oven temperature program was initially set at 50.0 °C with a hold of 1 min, after was ramped to 120.0 °C at 25 °C/min, with a hold of 1 min and finally was ramped to 300.0 °C at 5 °C/min, with a hold of 13.20 min. The total oven program was of 53.0 min. The interface temperature of detector was of 280.0 °C and the range mass was from 54.00 *m/z* to 580.0 *m/z,* working in Selected Ion Monitoring *(SIM)* target qualifier ions were used, both for the identification and quantification of each compound. Recovery (%) and reproducibility ($(RSD)_R$ %), for each of the detected compounds, were studied at four fortification levels



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(0.001 – 1.00 mg/kg), for a total of 8 replicates. All but one, fenazaquin (80%), the recovery was always higher than 90%. RSD values are reported in table 3.

Statistics and risk analysis were carried out with the software Excel 2003 (Microsoft Inc.) and @Risk (ver. 4.5, Palisade Inc.), respectively. All distribution are analysed between the 5° and 95° percentiles.

*Qualitative Analysis*

For each of the studied years and for the total of 5-years, the number and kind of phytosanitary products on each orange sample was evaluated. To this purpose, all analytical results where reported in an excel spreadsheet, assigning: the value *one* when the chemical compound was present, *zero* in all others cells. According to Vose (1998), data on presence/absence (successes or failures) have been used as inputs to determine the probability of an event occurs. The Beta distribution describes the true probability (p) of an event occurring, given (n) and (s) obtained from spread sheets, respectively (Vose, 1998). In order to estimate the number of phytochemicals present at the same time on *Tarocco* oranges (up to 6), and each associated probability, a Binomial distribution was used. The distributions are :

p = BETA (n+1;n-s+1); s= Binomial (n;p)

This qualitative analysis was carried out in order to evaluate the probability of finding: ethyl chlorpyrifos, methyl chlorpyrifos, or both of them.

*Quantitative Analysis*

The aim of this part of the study was, for each and the five years, to evaluate the level (mg/kg) of each phytochemical present in *Tarocco* oranges. Moreover for each phytochemical the associated best-fitted distribution was obtained. For each sample and for each phytochemical in Table 3 are reported: the RSD, the number and the percentage of positive

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samples, the MRL, the average value, the standard deviation, the minimum and maximum value, the best fitted distribution.

*Risk Assessment*

For the most important residues found in blood oranges the *Estimated Daily Intake (EDI) and the Potential risk (EDI /NOAEL)* for consumers were calculated for the period 2003-2007. In both cases a probabilistic approach was used (@Risk, ver. 4.5, Palisade Inc.). The EDI (Food chemical concentration x food consumption) was estimated according to Douglass and Tennant (1997) multiplying the distribution of the phytochemical concentration (mg/kg) in *Tarocco* oranges with the distribution of orange *pro capita* consumption in Italy (ISMEA, 2008) in the same period. The simulations were run, as single, for ethyl chlorpyrifos and methyl chlorpyrifos, as multiple intakes for the sum of ethyl and methyl chlorpyrifos.

In order to characterise the risk (FAO, 2006), the NOAEL (Non Observed Adverse Effect Level), the highest tested dose on animals without adverse effect, was obtained from literature (INCHEM, 1999). Analogously to other food risk assessment studies (Spanjersberg et al., 2007; Zhang et al., 2008), the potential risk for consumers, the relationship between the chemical intake distribution and the safety factors distribution for both ethyl and methyl chlorpyrifos as single, and as multiple intakes for the sum of them, was estimated dividing the two distributions (*in practice how far are the pesticides concentrations on oranges from safety concentrations*).

In order to use the same units of EDI, mg/day, safety factor NOAEL was multiplied for 60 Kg (the average weight of a person) (WHO and FAO, 2004).

In all risk analyses the following assumptions were done:

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1. Pesticides analyses on oranges, according to official methods (ISTISAN, 97/23), must be carried out on the entire fruit (peel and edible portion). In *Tarocco* oranges the ratio between *flavedo* + *albedo*= peel and edible portions (pulp) is around: 10/90; while the most part of pesticides remains on *flavedo*, only a small amount, usually 3-5%, transfers into the *albedo* (edible portion). As the strong difference in pesticides concentrations between the peel and the edible portion is largely compensated by the ratio between the two parts: the data concerning pesticides have not been modified.
2. Usually the consumption *pro capite* of a food commodity is calculated per year (365 days), as the fresh oranges consumption is concentrated between December and May, a 6 months (180 day) *pro capite* consumption, and relatives simulations, have been also arbitrary calculated.
3. Lastly, in order to differentiate the risks by pesticides exposure of the generic population by the consumer that really eat Italian *Tarocco* oranges, one more input has been added. In fact, the distribution of pesticide(s) concentration has been multiplied for the distribution of probability of the presence of the pesticide(s) (Output of qualitative analysis).

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## Results and discussion

Multi-residue analysis of 460 samples of Italian Tarocco oranges, for the years 2003-2007, highlighted the presence of the following pesticides: ethyl and methyl chlorpyrifos, dicofol, etofenprox, fenazaquin, fenitrotion, imazalil, malathion, metalaxil-m.

*Qualitative Analysis*

Orange samples, for each year, were analysed in order to know the probability to have, at the same time, one, two or three pesticides residues. Results of the binomial distribution, are reported in Table 2.

Of the analysed samples, over the five studied years, 140 (30.5%) contained just one pesticide; 9 samples two pesticides (2.16%); two samples 3 pesticides (0.65%). None of the samples had four, or a higher number, of pesticide residues. Moreover, the probability distributions (Figure 1a) highlighted that: although 140 is the most probable number, it must be expected that *Tarocco* oranges with just one pesticide will fall in the range between: 124-157 (26.95-34.13%); and between 5 and 15 (1.08-3.26%) samples with two pesticides (Figure 1b), and 1 to 6 (0.22-1.30%) with three (Figure 1c). In 2003, 11 samples (16.7%) contained just one pesticide, with an acceptable range between 7-17 (Table 2). In this case the most common pesticide was ethyl chlorpyrifos, followed by methyl chlorpyrifos and three samples containing: dicofol, etofenprox and malathion, respectively. The two samples with two and three pesticides contained ethyl and methyl chlorpyrifos; ethyl, methyl chlorpyrifos plus fenanzaquin, respectively.

Of the 92 samples analysed in 2004, 31 contained one pesticide: ethyl chlorpyrifos. Just one sample contained two pesticides: ethyl chlorpyrifos and dicofol at the same time. The

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probability distribution, concerning the presence of one pesticide, has shown acceptable range between 24 and 39 samples; and up to 4 samples for the presence of two pesticides (Table 2). In 2005, 43.7% of samples contained just one pesticide, distributed as follows: 47 samples ethyl chlorpyrifos, 19 methyl chlorpyrifos, 2 metalaxil-m, 1 with fenitrotion. The 3.75% showed at the same time two pesticides, distributed as follows: 2 samples with ethyl and methyl chlorpyrifos, one sample each: methyl chlorpyrifos and metalaxil-m; ethyl chlorpyrifos and etofenprox; etofenprox and fenazaquin.

In 2006 the 18.6% of samples contained just one pesticide, distributed as follows: 14 samples ethyl chlorpyrifos, two samples fenitrotion, one with methyl chlorpyrifos and etofenprox, respectively. Two samples contained ethyl and methyl chlorpyrifos. One sample at the same time contained methyl chlorpyrifos, dicofol and fenitrotion.

In 2007 the 28.6% of samples contained one pesticide residue of which 6 samples contained ethyl chlorpyrifos and 5 samples contained imazalil.

All pesticides found in *Tarocco* oranges, in the five years which were studied conformed with the legislation (Ministerial Decree of 27/08/2004 ).

As chlorpyrifos, both as ethyl and methyl, was the most commonly occurring residue, a specific qualitative analysis was carried out in order to evaluate the probability as single or in co-presence. Results are in figures 2a, 2b and 2c.

In particular, the 23.16% of samples have shown only ethyl chlorpyrifos residues, with a probability of about 4.5%, with an acceptable range between 20% and 26.5% (figure 2a);

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instead the 7.8% of samples have shown only methyl chlorpyrifos residues, with a probability of about 7%; this probability distribution, reported in figure 2b, has shown acceptable ranges between 27 and 46 samples, corresponding to 5.87% and 10% respectively. The most probable number of samples with ethyl and methyl chlorpyrifos, in the five studied years, is equal to 6, representing the 1.5% of samples (figure 2c); the acceptable range is between 0.65% and 2.6%.

*Quantitative Analysis*

Table 3 reports the results of quantitative analyses, concerning: ethyl and methyl chlorpyrifos, dicofol, etofenprox, fenazaquin, fenitrotion, imazalil, malathion and metalaxil-m, calculated in positive orange samples. Moreover the table reports, for each molecule, the admitted Maximum Residue Level in oranges and, when the pesticide was present at least in five samples, the best fitted distribution.

As described above, ethyl and methyl chlorpyrifos are the most commonly coccuring pesticides, deriving from farming activities, in blood oranges followed by etofenprox and fenithrotion. The presence of imazalil (Table 3) must be discussed separately. In fact, it is present in a very low number of samples compared to literature data (Fernandez et al., 2001). This is normal if we consider that it is not used during farming. But, is the most used post-harvest fungicide in citrus fruit, given with the waxing of product. The presence of this molecule at this stage of the chain may indicate that a few lots are not supplied directly by farmers but from other citrus business operators.

The average level of ethyl chlorpyrifos was: 0.07 mg/kg of oranges, with the lowest value, in positive samples, of 0.003 mg/kg and the highest value of 0.29 mg/kg. The concentration was

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always lower than the MRL and was normal distributed. The average level of methyl chlorpyrifos was 0.56 mg/kg, with the highest value, measured in 2005, of 2.05 mg/kg. The last value was four fold higher the MRL (Table 3). Also methyl chlorpyrifos was normally distributed. Dicofol was present in three samples over the five studied years with an average value of 6.69 mg/kg; but, in 2003, one sample showed, with 20 mg/kg, the highest measured concentration of pesticide; this value was ten fold higher than the MRL value; instead, in 2004, one sample showed, with 0.07 mg/kg, a concentration twenty-eight times lower than MRL, and in 2006 seventy-four times lower. All but one of the remaining pesticides have shown very low concentrations and far from MRL. The only exception was imazalil with an average value of 3.3 mg/kg and the highest measured value, 4.8 mg/kg, next to the MRL (Table 3).

Data shown above are qualitatively similar to pesticides residues found in Spanish oranges (Fernandez et al., 2001), but quantitatively shown some differences. In fact, without considering differences of imazalil, explained above, it is possible to see that: ethyl chlorpyrifos is largely used in Italian samples than in Spanish ones, but at lower levels, 0.07 mg/kg and 0.16 mg/kg, respectively; Dicofol was present in 18.7% of the Spanish with an average value of 0.28 mg/kg, while in Italian *Tarocco* oranges was present in the 0.65% of samples with an average value, excluding the sample with 20 mg/kg, of: 0.04 mg/kg. Fernandez et al. (2001) report a 4% of samples above the MRL. The results of the present investigation report the 0.44% of samples exciding the MRL values. Even lower the percentages reported for 2006 and 2007 of: 1% and 1.3%, respectively (PNRA, 2006; Lega Ambiente, 2008). But, two considerations must be done: first, the present investigation is addressed to evaluate risk coming from farming activity, risk from post harvesting (e.g.,

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imazalil) are deliberately neglected; afterwards, data concerning this investigation refer to a controlled citrus chain, for instance, street food is not taken in account.

*Assessment of EDI and EDI /NOAEL ratio.*

The first two simulations were aimed to:

- estimate the most probable EDI values, and their distributions, for ethyl chlorpyrifos, for methyl chlorpyrifos and the sum of two pesticides from consuming Italian *Tarocco* oranges, considering a consumption distributed on 365 and 180 days, respectively;
- estimate the EDI values as described above, but keeping in account also the probability distribution of the presence of the pesticide (qualitative analysis).

In practice, the first one describe the EDI of a consumer that is going to eat an orange having a certain level of chlorpyrifos, the second one the EDI of consumer that is going to eat an orange, without knowing if it contains or not pesticides.

When a *Tarocco* orange contain a certain level of ethyl chlorpyrifos, the most probable EDI values, calculated on 180 or 365 days of consumption, are: 0.01 mg/day or 0.006 mg/day, respectively. Moreover the EDI distributions say that, acceptable values can range from 0 up to 0.03 mg/day, for 180 days, the half for 365 days. EDI of ethyl chlorpyrifos values have a substantial decrease if we consider that this pesticide is not always present in oranges. In fact, EDI values are: $2.6*10^{-3}$ and $1.3*10^{-3}$ mg/day, calculated at 180 and 365 days, respectively. The WHO/FAO report on pesticides residues in food (2004), assign a daily intake in Europe, of ethyl chlorpyrifos from citrus, of $3.6*10^{-3}$ mg/day. This value is significantly higher, 2.8 fold, than the analogous reported above ($1.3*10^{-3}$ mg/day). The difference could be ascribed

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to the lower level found in blood oranges (Table 3) compared to other citrus (Fernandez et al., 2001).

For methyl chlorpyrifos residues found in our samples, the four simulations indicate that the most probable EDI values (on 180 or 365 days of consumption) are of 0.09 mg/day or 0.04 mg/day, respectively. Furthermore acceptable values of EDI distributions can range from 0 up to 0.23 mg/day, for 180 days, from 0 up to 0.12 for 365 days. If we consider that methyl chlorpyrifos is not always present in oranges, EDI values have a considerable reduction; in fact they are: $6.7*10^{-3}$ and $3.4*10^{-3}$ mg/day, calculated at 180 and 365 days, respectively. Data shown above highlighted that the daily intake of methyl chlorpyrifos, from blood oranges, is always higher than ethyl chlorpyrifos, up to 7.6 fold.

As methyl chlorpyrifos has been found in some samples, also the presence of, a multiple intake (sum of ethyl and methyl chlorpyrifos), was estimated. In both cases: keeping in account the distribution of the averages levels and the probability distribution that the two molecules are contemporarily in the same sample. Considering just the concentrations encountered in our samples the estimated daily intake increases significantly up to: 0.03 and 0.01 mg/day for 180 and 365 day, respectively. But, keeping in account the probability distribution that this event occurs, the co-presence in the same sample, the EDI values go down up to: $4*10^{-4}$ and $2*10^{-4}$ mg/day, respectively.

The ADI (Acceptable Daily Intake) value for chlorpyrifos is in the range 0-0.01 mg/kg bw (INCHEM, 1999). For a person of 60 kg of body weight, the highest acceptable daily intake is: 0.6 mg. This means that, in the worst case, using EDI at 180 days, the consumption of

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Italian *Tarocco* oranges can contribute to the 1.87%, 14.37% and 4.71%, of ADI as ethyl chlorpyrifos, methyl chlorpyrifos and the sum of ethyl and methyl, respectively.

Results of simulations aimed to verify the distance, in terms of distributions probability, among the EDI values and the NOAEL values, indicate that, even in the worst case, for the presence of ethyl chlorpyrifos, considering a consumption of oranges during 6 months (180 days), the ratio (EDI/NOAEL) has a 0.48 % as average value and a highest acceptable value of 1.7%. Considering the probability that chlorpyrifos may be not present, the ratio is 0.1 or 0.05% for 180 or 365 days, respectively.

For the presence of methyl chlorpyrifos, the ratio (EDI/NOAEL $_{180\ days}$) has a 3.7 % as average value and a highest acceptable value of 14%, if we considering the probability that methyl chlorpyrifos may be not present, the ratio decrease significantly to 0.29% or 0.14 % for 180 or 365 days, respectively. The cumulative analysis shows that the ratio (EDI/NOAEL $_{180\ days}$) has a 1.19 % as average value and a highest acceptable value of 4.9%; besides if we considering the probability that methyl and ethyl chlorpyrifos may be not present contemporarily in the same sample, the ratio decrease significantly to 0.02% or 0.01 % for 180 or 365 days, respectively.

The above simulations have shown that chlorpyrifos levels are always largely in the safety area.

**Conclusions**

This study highlighted the following findings:

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- The number, the associated probability and distribution of pesticides in *Tarocco* oranges;
- The most common pesticide residue for farming activity is ethyl chlorpyrifos, followed by methyl chlorpyrifos;
- All but two of the 460 analysed samples shown pesticide levels below the MRL;
- The Estimated Daily Intake of methyl and ethyl chlorpyrifos, and the sum of two, from eating *Tarocco* Blood oranges has been estimated;
- Authors believe the EDI, calculated considering a citrus eating spread of 180 days, more realistic than that calculated on 365 days;
- The percentage of ethyl chlorpyrifos, for a person of 60 kg of body weight, in comparison ADI has been calculated;
- Both for ethyl and methyl chlorpyrifos, as well as for their sum, the measured levels in oranges, and, as consequence, the estimated consumer intake is far from NOAEL value.

Moreover, data reported above, in our opinion, have shown the efficacy of performance-based (product) control systems (Cho and Hooker, 2009) and of the co-regulation approach (Martinez et al., 2007) to guarantee food safety. The acceptable level of pesticides could be ascribed also to the pressure of Public Authorities on business operators and, as a consequence, the practical capacity of citrus chain actors to control it. In fact, also when 2 of the 460 samples where outlaw they have been intercepted not representing a real risk for consumers. Studies concerning post-harvesting citrus activities, as well as, the not controlled (farmers' direct selling, street or small shops) are in progress.

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**FIGURE CAPTIONS**

**Figure 1:**

a. Probability distribution of presence - absence , 1 pesticide residue 2003-2007

b. Probability distribution of presence-absence, 2 pesticide residues 2003-2007

c. Probability distribution of presence-absence, 3 pesticide residues 2003-2007

**Figure 2:**

a. Probability distribution of presence-absence, ethyl chlorpyrifos 2003-2007

b. Probability distribution of presence-absence, methyl chlorpyrifos 2003-2007

c. Probability distribution of presence-absence, ethyl + methyl chlorpyrifos 2003-2007

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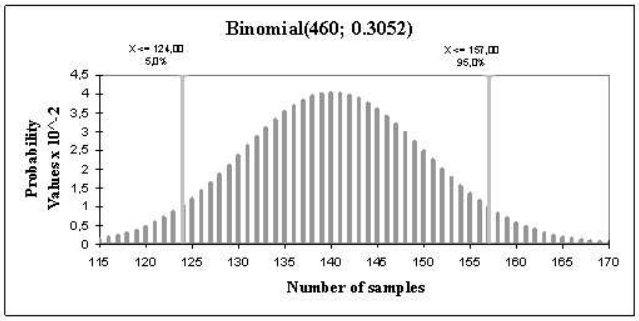


Figure 1a. Probability Distribution of presence-absence, 1 pesticide residue 2003-2007

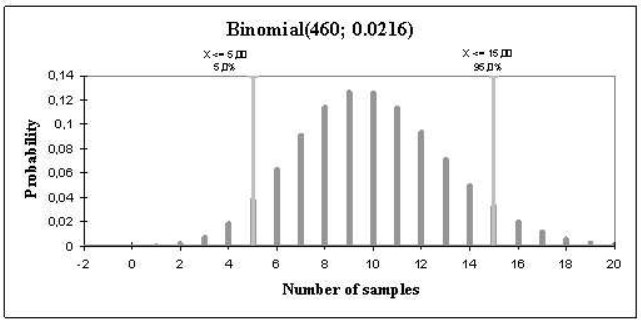


Figure 1b. Probability Distribution of presence-absence, 2 pesticide residue 2003-2007

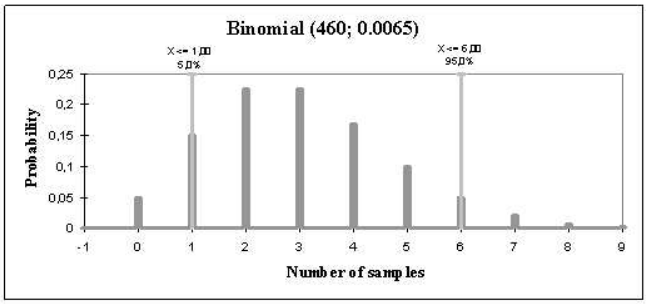


Figure 1c. Probability Distribution of presence-absence, 3 pesticide residue 2003-2007

190x254mm (96 x 96 DPI)

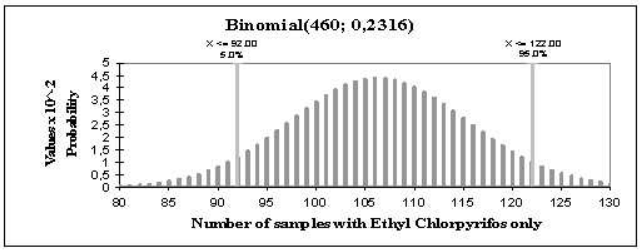


Figure 2a. Probability Distribution of presence-absence, Ethyl Chlorpyrifos 2003-2007

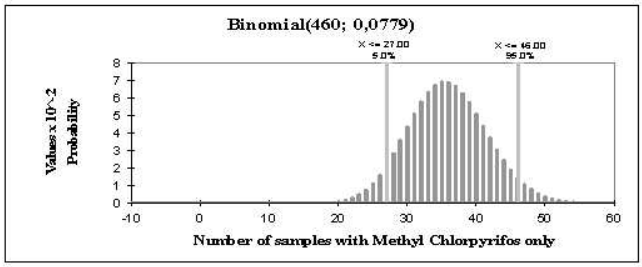


Figure 2b. Probability Distribution of presence-absence, Methyl Chlorpyrifos 2003-2007

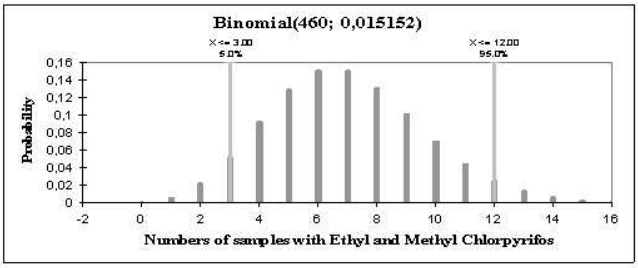


Figure 2c. Probability Distribution of presence-absence, Ethyl + Methyl Chlorpyrifos 2003-2007

190x254mm (96 x 96 DPI)

Table 1. Sample Numbering

| Year | Percentage | Samples Number |
|---|---|---|
| 2003 | 15.21 | **70** |
| 2004 | 20 | **92** |
| 2005 | 34.35 | **158** |
| 2006 | 21.74 | **100** |
| 2007 | 8.7 | **40** |
| 2003-2007 | 100 | **460** |

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Table 2. Results of qualitative analysis

| | Samples Numbers | Samples with 1 residue | Samples with 2 residues | Samples with 3 residues |
|---|---|---|---|---|
| **2003** | **70** | *11 (7-17)* *p=0.1667* | *1 (0-4)* *P=0.0278* | *1(0-4)* *p=0.0278* |
| **2004** | **92** | *31 (24-39)* *P=0.3404* | *1 (0-4)* *P=0.0213* | *0* |
| **2005** | **158** | *69 (59-79)* *p=0.4375* | *5/ (2-10)* *P=0.0375* | *0* |
| **2006** | **100** | *18 (12-25)* *P=0.1863* | *2 (0-6)* *p= 0.0294* | *1 (0-4)* *P=0.0196* |
| **2007** | **40** | *11 (7-16)* *P=0.2857* | *0* | *0* |
| **2003-2007** | **460** | *140 (124-157)* *p=0.3052* | *9 (5-15)* *p=0.0216* | *2 (1-6)* *p=0.0065* |

*(M.L. min. 5%; max 95%)*

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Table 3. Total Quantitative Analysis (2003-2007)

| | Ethyl Chlorpyrifos | Methyl Chlorpyrifos | Dicofol | Etofenprox | Fenazaquin | Fenithrotion | Imazalil | Malathion | Metalaxil-m |
|---|---|---|---|---|---|---|---|---|---|
| **$(RSD)_R$** | 8.1% | 8.0% | 18.3% | 5.2% | 15.2% | 6.2%) | 6.0%) | 12.5%) | 15.5%) |
| **Positive samples** | 106 (23.04%) | 35 (7.60%) | 3 (0.65%) | 4 (0.87%) | 2 (0.43%) | 4 (0.87%) | 5 (1.08%) | 2 (0.43%) | 3 (0.65%) |
| **MRL ( mg/Kg)** | 0.3 | 0.5 | 2 | 1 | 0.2 | 2 | 5 | 2 | 0.5 |
| **Mean of Positive Samples (mg/Kg)** | 0.07±0.07 | 0.56±0.57 | 6.69±11.53 | 0.003±0.001 | 0.03±0.03 | 0.04±0.05 | 3.30±0.90 | 0.02±0.01 | 0.033±0.003 |
| **Min. value** | 0.003 | 0.04 | 0.003 | 0.002 | 0.004 | 0.001 | 2.38 | 0.02 | 0.03 |
| **Max value** | 0.29 | 2.05 | 20 | 0.004 | 0.05 | 0.12 | 4.8 | 0.03 | 0.04 |
| **Best Distribution** | =RiskNormal (0.07;0.07) | =RiskNormal (0.56 ;0.57) | =RiskTriang (0.003;6.69;20) | * | * | * | =RiskNormal (3.30; 0.9) | * | * |

* Distribution absent for low number of samples

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