



HAL
open science

Exposure to pesticide residues from consumption of Italian blood oranges

Biagio Fallico, Mary Giuseppina d'Urso, Elena Chiappara

► **To cite this version:**

Biagio Fallico, Mary Giuseppina d'Urso, Elena Chiappara. Exposure to pesticide residues from consumption of Italian blood oranges. *Food Additives and Contaminants*, 2009, 26 (07), pp.1024-1032. 10.1080/02652030902839731 . hal-00573875

HAL Id: hal-00573875

<https://hal.science/hal-00573875>

Submitted on 5 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Exposure to pesticide residues from consumption of Italian blood oranges

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2008-330.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	18-Feb-2009
Complete List of Authors:	Fallico, Biagio; Universita degli Studi di Catania, DOFATA D'Urso, Mary Chiappara, Elena; Università di Catania, DOFATA - sez. Tecnologie
Methods/Techniques:	Chromatography - GC/MS
Additives/Contaminants:	Pesticide residues
Food Types:	Fruit, Vegetables

SCHOLARONE™
Manuscripts

1
2
3 **1 Exposure to pesticides residues from consumption of Italian blood oranges**
4

5
6 2

7
8 3 *B. Fallico*, M.G. D'Urso, E. Chiappara*
9

10 4

11
12
13 5 *DOFATA sez. Tecnologie Agro-alimentari, Università degli Studi di Catania, Italy*
14

15 6

16
17
18 7 * Email: bfallico@unict.it, Tel. 0039 957580214.
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Abstract

2 The paper reports the results of a 5-year study aimed to evaluate pesticide levels, deriving
3 from orchards activities, on the Italian most common orange cultivar (*Citrus Sinensis*, *L.*
4 *Osbeck*, *cv. Tarocco*). The study, using a Bayesian approach, allowed both the qualitative (the
5 number) and quantitative distributions (the amount) of pesticides to be determined with its
6 own probability value. Multi-residue analyses of 460 samples highlighted the presence of:
7 ethyl and methyl chlorpyrifos, dicofol, etofenprox, fenazaquin, fenitrothion, imazalil,
8 malathion and metalaxil-m. 30.5% of samples contained just one pesticide, 2.16% two
9 pesticides and 0.65% of samples had three pesticides at the same time. The most common
10 residue was ethyl chlorpyrifos followed by methyl chlorpyrifos.. The Estimated Daily Intake
11 (EDI) values for ethyl and methyl chlorpyrifos, as well as the distance from safety level
12 (NOAEL), were calculated. The risk has been differentiated in order: 1) to take account also
13 the period of real citrus consuming (180 days); 2) to differentiate the risk derived from eating
14 oranges that contain a certain level of chlorpyrifos from those unspecified. The most likely
15 EDI values for ethyl chlorpyrifos, derived from Italian blood orange consumption are: 0.01
16 and 0.006 mg/day, calculated on 180 and 365 days, respectively. Considering the probability
17 of occurrence of ethyl chlorpyrifos, the EDI values are reduced up to: $2.6 \cdot 10^{-3}$ and $1.3 \cdot 10^{-3}$
18 mg/day, respectively. Analogously for methyl chlorpyrifos, the EDI most likely values are:
19 0.09 and 0.04 mg/day, respectively. Considering its probability of occurrence the EDI values
20 are: $6.7 \cdot 10^{-3}$ and $3.4 \cdot 10^{-3}$ mg/day, respectively. The results confirmed that levels of
21 pesticides in Italian Tarocco oranges deriving from a controlled chain are safe.

22
23 **Keywords:** EDI, NOAEL, Tarocco oranges, risk assessment, safety, @ risk, Monte Carlo.

1 Introduction

2
3
4
5
6
7
8 The increase in recorded incidence of food-borne illness have driven the more industrialised
9
10 countries to develop more effective food safety control systems, based both on process
11
12 (design) and on performance-based (product) systems (Cho and Hooker, 2009). All food
13
14 operators are requested that they are managing food safety aspects correctly, developing
15
16 appropriate control procedures, analyses and monitoring activities. A risk-based approach is
17
18 strongly recommended. One of the most important aspect concerns the presence of
19
20 phytosanitary products.
21
22
23
24

25
26
27 The most comprehensive report of pesticide residues in Italy is prepared annually by an
28
29 important environmental association (Lega Ambiente, 2008). They reported the results of the
30
31 analysis of 10,048 vegetables and fruits carried out in 2007 by Italian Public Authorities
32
33 involved in food control (Chiappara and Fallico, 2008), and highlight that the number of
34
35 irregular samples of oranges declined from 1.3% to 1.0% but that 47.4% of samples contained
36
37 at least one pesticide. This improvement is significant also for Sicilian production, where of
38
39 80 samples of analysed citrus (lemons and oranges): 57 (71.2%) were pesticides free, 16
40
41 (20%) contained one pesticide, and 6 samples (7.6%) had more than one pesticide residue.
42
43
44 The number of irregular samples in the citrus chain, according to results of national
45
46 monitoring plan for pesticides residues on food products (P.N.R.A.), co-ordinated by The
47
48 General Directorate for food safety and nutrition of Italian Ministry of Health, has decreased
49
50 significantly in the last decade. In fact, it declined from 5.6% in 1993 to 1% in 2006.
51
52
53
54
55

56
57
58 The total Italian production of citrus fruit (medium value 2004-2006) is about 3.4 million
59
60 tons, with an invested area of 170.000 hectares. Sicily supplies 52.2% of orange production

1 and 50% of Italian orange production comprises blood oranges; this percentage increases up
2 to 70% in Sicily where the cultivar *Tarocco* is predominant. (Tribulato, Maccarrone, La Rosa,
3 2007). In blood oranges there are many healthy substances, with antioxidant activity
4 (Maccarrone, 2004). In fact their juice contains anthocyanins, up to about 100 mg/l in freshly-
5 squeezed *Tarocco* juices (Arena et al. 2001), ascorbic acid up to about 800 mg/l (Maccarrone,
6 2004), hydroxycinnamic acids derivatives like coumaric acid (Rapisarda et al., 1998).

7
8 The most widely used class of phytosanitary products in farming are the organophosphorus
9 compounds. They represent about the 40% of registered products. They can be very
10 dangerous as inhibit the normal action of acetylcholinesterase (AChE). The most common
11 one is chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridinyl) phosphorothionate].
12 Alternatively in post-harvest treatment thiabendazole and imazalil are currently applied to
13 control mould decay in citrus fruit (Schirra et al., 1996).

14
15 Many studies have been carried out in order to evaluate the decay kinetics of some pesticides
16 currently used on citrus fruit. Montemurro et al. (2002) found that degradation kinetics of
17 chlorpyrifos depends on commercial formulation as well as for fenitrothion (Montemurro et
18 al., 2005). Schirra et al. (2005) evaluated the residue levels, as well as persistence and storage
19 performance of citrus fruits treated with fludioxonil.

20
21 The importance of a risk-based approach to guarantee food safety/quality is strongly
22 recommended both by Regulatory Agencies (Reg. CE 852/04; FAO, 2006) and other bodies
23 (ISO 22000:05; GLOBAL/GAP). Many methods have been proposed to perform risk
24 assessment. The simplest ones, qualitative or semi-quantitative, take in account just the
25 probability and the consequences (impact) of the negative event(s). Exhaustive methods to

1 perform risk assessment, from general applications to food safety/quality, are available in
2 literature (Tennant, 1997; Vose, 2000; FAO, 2006). Risk assessment is based both on
3 *deterministic approach* when the calculated risk is based on a point estimate, usually the
4 worst-case value for each input variable; instead in a *probabilistic risk assessment model* both
5 variability and uncertainty in input variables can be taken into consideration, by using
6 probability distributions to represent the input variables instead of point estimates (Vose,
7 2000; Spanjersberg et al. 2007). Vose (1998) proposed a QRA, based on Monte Carlo
8 simulation method, to evaluate microbial food safety. The probabilistic approach has been
9 used by Slob and Pieters (1998) to estimate human acceptable exposure limits (RfD, ADI,
10 TDI) using distributions for extrapolation factors instead of single uncertainty factors, more
11 conservative; beside Slob (2006) published a study on the probabilistic dietary exposure
12 assessment taking into account variability in both amount and frequency of consumption. The
13 probabilistic model has been also used (Spanjersberg et al., 2007) for allergen risk assessment,
14 resulting in a more exhaustive risk assessment respect to traditional deterministic risk
15 assessment. Beside probabilistic modelling has been used for exposure assessment of operator
16 and residential non-dietary exposure for pesticide products (Lunchick, 2001), for cumulative
17 risk assessment of the exposure to organophosphorus and carbamate insecticides in the Dutch
18 diet (Boon et al., 2008) and also to estimate children's short-term residential exposure to lead
19 (Canales, Leckie, 2007).

20
21 The aims of this paper were: a multi-annual evaluation, both qualitative and quantitative, of
22 pesticide residues on blood oranges (*Citrus Sinensis L. Osbeck, Cl Tarocco*); to estimate the
23 daily intake of residues by consumption of blood oranges; the probability of consumers'
24 overexposing to exposure levels above the TDI.

25

1 **Materials and methods**

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

7 *Sampling and Multiresidual analysis*

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

16 The GC-MS system was a SHIMADZU GC 17A-MD 5800, equipped with a column SLB™ -
17 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
18 velocity of 36.3 cm/sec. The sampling time was of 5.0 min., the injector temperature was of
19 270.0 °C. The oven temperature program was initially set at 50.0 °C with a hold of 1 min,
20 after was ramped to 120.0 °C at 25 °C/min, with a hold of 1 min and finally was ramped to
21 300.0 °C at 5 °C/min, with a hold of 13.20 min. The total oven program was of 53.0 min. The
22 interface temperature of detector was of 280.0 °C and the range mass was from 54.00 *m/z* to
23 580.0 *m/z*, working in Selected Ion Monitoring (*SIM*) target qualifier ions were used, both for
24 the identification and quantification of each compound. Recovery (%) and reproducibility
25 ((RSD)_R %), for each of the detected compounds, were studied at four fortification levels

1
2
3 1 (0.001 – 1.00 mg/kg), for a total of 8 replicates. All but one, fenazaquin (80%), the recovery
4
5 2 was always higher than 90%. RSD values are reported in table 3.
6
7 3

8
9
10 4 Statistics and risk analysis were carried out with the software Excel 2003 (Microsoft Inc.) and
11
12 5 @Risk (ver. 4.5, Palisade Inc.), respectively. All distribution are analysed between the 5° and
13
14 6 95° percentiles.
15
16
17 7

18 19 20 8 *Qualitative Analysis*

21
22 9 For each of the studied years and for the total of 5-years, the number and kind of
23
24 10 phytosanitary products on each orange sample was evaluated. To this purpose, all analytical
25
26 11 results where reported in an excel spreadsheet, assigning: the value *one* when the chemical
27
28 12 compound was present, *zero* in all others cells. According to Vose (1998), data on
29
30 13 presence/absence (successes or failures) have been used as inputs to determine the probability
31
32 14 of an event occurs. The Beta distribution describes the true probability (p) of an event
33
34 15 occurring, given (n) and (s) obtained from spread sheets, respectively (Vose, 1998). In order
35
36 16 to estimate the number of phytochemicals present at the same time on *Tarocco* oranges (up to
37
38 17 6), and each associated probability, a Binomial distribution was used. The distributions are :

39
40
41
42
43 18 $p = \text{BETA}(n+1;n-s+1); s = \text{Binomial}(n;p)$

44
45
46 19 This qualitative analysis was carried out in order to evaluate the probability of finding: ethyl
47
48 20 chlorpyrifos, methyl chlorpyrifos, or both of them.
49

50 51 21 *Quantitative Analysis*

52
53 22 The aim of this part of the study was, for each and the five years, to evaluate the level
54
55 23 (mg/kg) of each phytochemical present in *Tarocco* oranges. Moreover for each phytochemical
56
57 24 the associated best-fitted distribution was obtained. For each sample and for each
58
59 25 phytochemical in Table 3 are reported: the RSD, the number and the percentage of positive

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

3 *Risk Assessment*

4 For the most important residues found in blood oranges the *Estimated Daily Intake (EDI) and*
5 *the Potential risk (EDI /NOAEL)* for consumers were calculated for the period 2003-2007. In
6 both cases a probabilistic approach was used (@Risk, ver. 4.5, Palisade Inc.). The EDI (Food
7 chemical concentration x food consumption) was estimated according to Douglass and
8 Tennant (1997) multiplying the distribution of the phytochemical concentration (mg/kg) in
9 *Tarocco* oranges with the distribution of orange *pro capita* consumption in Italy (ISMEA,
10 2008) in the same period. The simulations were run, as single, for ethyl chlorpyrifos and
11 methyl chlorpyrifos, as multiple intakes for the sum of ethyl and methyl chlorpyrifos.

12
13 In order to characterise the risk (FAO, 2006), the NOAEL (Non Observed Adverse Effect
14 Level), the highest tested dose on animals without adverse effect, was obtained from literature
15 (INCHEM, 1999). Analogously to other food risk assessment studies (Spanjersberg et al.,
16 2007; Zhang et al., 2008), the potential risk for consumers, the relationship between the
17 chemical intake distribution and the safety factors distribution for both ethyl and methyl
18 chlorpyrifos as single, and as multiple intakes for the sum of them, was estimated dividing the
19 two distributions (*in practice how far are the pesticides concentrations on oranges from*
20 *safety concentrations*).

21
22 In order to use the same units of EDI, mg/day, safety factor NOAEL was multiplied for 60 Kg
23 (the average weight of a person) (WHO and FAO, 2004).

24 In all risk analyses the following assumptions were done:

- 1
2
3 1 1. Pesticides analyses on oranges, according to official methods (ISTISAN, 97/23), must
4
5
6 2 be carried out on the entire fruit (peel and edible portion). In *Tarocco* oranges the ratio
7
8 3 between *flavedo* + *albedo*= peel and edible portions (pulp) is around: 10/90; while the
9
10 4 most part of pesticides remains on *flavedo*, only a small amount, usually 3-5%,
11
12 5 transfers into the *albedo* (edible portion). As the strong difference in pesticides
13
14 6 concentrations between the peel and the edible portion is largely compensated by the
15
16 7 ratio between the two parts: the data concerning pesticides have not been modified.
17
18
19
20 8 2. Usually the consumption *pro capite* of a food commodity is calculated per year (365
21
22 9 days), as the fresh oranges consumption is concentrated between December and May,
23
24 10 a 6 months (180 day) *pro capite* consumption, and relatives simulations, have been
25
26 11 also arbitrary calculated.
27
28
29 12 3. Lastly, in order to differentiate the risks by pesticides exposure of the generic
30
31 13 population by the consumer that really eat Italian *Tarocco* oranges, one more input has
32
33 14 been added. In fact, the distribution of pesticide(s) concentration has been multiplied
34
35 15 for the distribution of probability of the presence of the pesticide(s) (Output of
36
37 16 qualitative analysis).
38
39
40
41 17
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 Results and discussion

2
3
4
5
6
7
8 Multi-residue analysis of 460 samples of Italian Tarocco oranges, for the years 2003-2007,
9
10 highlighted the presence of the following pesticides: ethyl and methyl chlorpyrifos, dicofol,
11
12 etofenprox, fenazaquin, fenitrothion, imazalil, malathion, metalaxil-m.
13
14

15 *Qualitative Analysis*

16
17 Orange samples, for each year, were analysed in order to know the probability to have, at the
18
19 same time, one, two or three pesticides residues. Results of the binomial distribution, are
20
21 reported in Table 2.
22
23

24
25 Of the analysed samples, over the five studied years, 140 (30.5%) contained just one
26
27 pesticide; 9 samples two pesticides (2.16%); two samples 3 pesticides (0.65%). None of the
28
29 samples had four, or a higher number, of pesticide residues. Moreover, the probability
30
31 distributions (Figure 1a) highlighted that: although 140 is the most probable number, it must
32
33 be expected that *Tarocco* oranges with just one pesticide will fall in the range between: 124-
34
35 157 (26.95-34.13%); and between 5 and 15 (1.08-3.26%) samples with two pesticides (Figure
36
37 1b), and 1 to 6 (0.22-1.30%) with three (Figure 1c). In 2003, 11 samples (16.7%) contained
38
39 just one pesticide, with an acceptable range between 7-17 (Table 2). In this case the most
40
41 common pesticide was ethyl chlorpyrifos, followed by methyl chlorpyrifos and three samples
42
43 containing: dicofol, etofenprox and malathion, respectively. The two samples with two and
44
45 three pesticides contained ethyl and methyl chlorpyrifos; ethyl, methyl chlorpyrifos plus
46
47 fenanzaquin, respectively.
48
49
50
51
52

53
54
55 Of the 92 samples analysed in 2004, 31 contained one pesticide: ethyl chlorpyrifos. Just one
56
57 sample contained two pesticides: ethyl chlorpyrifos and dicofol at the same time. The
58
59
60

1 probability distribution, concerning the presence of one pesticide, has shown acceptable range
2 between 24 and 39 samples; and up to 4 samples for the presence of two pesticides (Table 2).

3 In 2005, 43.7% of samples contained just one pesticide, distributed as follows: 47 samples
4 ethyl chlorpyrifos, 19 methyl chlorpyrifos, 2 metalaxil-m, 1 with fenitrothion. The 3.75%
5 showed at the same time two pesticides, distributed as follows: 2 samples with ethyl and
6 methyl chlorpyrifos, one sample each: methyl chlorpyrifos and metalaxil-m; ethyl
7 chlorpyrifos and etofenprox; etofenprox and fenazaquin.

8
9 In 2006 the 18.6% of samples contained just one pesticide, distributed as follows: 14 samples
10 ethyl chlorpyrifos, two samples fenitrothion, one with methyl chlorpyrifos and etofenprox,
11 respectively. Two samples contained ethyl and methyl chlorpyrifos. One sample at the same
12 time contained methyl chlorpyrifos, dicofol and fenitrothion.

13
14 In 2007 the 28.6% of samples contained one pesticide residue of which 6 samples contained
15 ethyl chlorpyrifos and 5 samples contained imazalil.

16
17 All pesticides found in *Tarocco* oranges, in the five years which were studied conformed with
18 the legislation (Ministerial Decree of 27/08/2004).

19
20 As chlorpyrifos, both as ethyl and methyl, was the most commonly occurring residue, a
21 specific qualitative analysis was carried out in order to evaluate the probability as single or in
22 co-presence. Results are in figures 2a, 2b and 2c.

23
24 In particular, the 23.16% of samples have shown only ethyl chlorpyrifos residues, with a
25 probability of about 4.5%, with an acceptable range between 20% and 26.5% (figure 2a);

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

7 8 *Quantitative Analysis*

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

14
15 As described above, ethyl and methyl chlorpyrifos are the most commonly occurring
16 pesticides, deriving from farming activities, in blood oranges followed by etofenprox and
17 fenitrothion. The presence of imazalil (Table 3) must be discussed separately. In fact, it is
18 present in a very low number of samples compared to literature data (Fernandez et al., 2001).
19 This is normal if we consider that it is not used during farming. But, is the most used post-
20 harvest fungicide in citrus fruit, given with the waxing of product. The presence of this
21 molecule at this stage of the chain may indicate that a few lots are not supplied directly by
22 farmers but from other citrus business operators.

23
24 The average level of ethyl chlorpyrifos was: 0.07 mg/kg of oranges, with the lowest value, in
25 positive samples, of 0.003 mg/kg and the highest value of 0.29 mg/kg. The concentration was

1
2
3 1 always lower than the MRL and was normal distributed. The average level of methyl
4
5 2 chlorpyrifos was 0.56 mg/kg, with the highest value, measured in 2005, of 2.05 mg/kg. The
6
7 3 last value was four fold higher the MRL (Table 3). Also methyl chlorpyrifos was normally
8
9 4 distributed. Dicofol was present in three samples over the five studied years with an average
10
11 5 value of 6.69 mg/kg; but, in 2003, one sample showed, with 20 mg/kg, the highest measured
12
13 6 concentration of pesticide; this value was ten fold higher than the MRL value; instead, in
14
15 7 2004, one sample showed, with 0.07 mg/kg, a concentration twenty-eight times lower than
16
17 8 MRL, and in 2006 seventy-four times lower. All but one of the remaining pesticides have
18
19 9 shown very low concentrations and far from MRL. The only exception was imazalil with an
20
21 10 average value of 3.3 mg/kg and the highest measured value, 4.8 mg/kg, next to the MRL
22
23 11 (Table 3).
24
25
26
27
28
29
30
31
32
33

34 13 Data shown above are qualitatively similar to pesticides residues found in Spanish oranges
35
36 14 (Fernandez et al., 2001), but quantitatively shown some differences. In fact, without
37
38 15 considering differences of imazalil, explained above, it is possible to see that: ethyl
39
40 16 chlorpyrifos is largely used in Italian samples than in Spanish ones, but at lower levels, 0.07
41
42 17 mg/kg and 0.16 mg/kg, respectively; Dicofol was present in 18.7% of the Spanish with an
43
44 18 average value of 0.28 mg/kg, while in Italian *Tarocco* oranges was present in the 0.65% of
45
46 19 samples with an average value, excluding the sample with 20 mg/kg, of: 0.04 mg/kg.
47
48 20 Fernandez et al. (2001) report a 4% of samples above the MRL. The results of the present
49
50 21 investigation report the 0.44% of samples exciding the MRL values. Even lower the
51
52 22 percentages reported for 2006 and 2007 of: 1% and 1.3%, respectively (PNRA, 2006; Lega
53
54 23 Ambiente, 2008). But, two considerations must be done: first, the present investigation is
55
56 24 addressed to evaluate risk coming from farming activity, risk from post harvesting (e.g.,
57
58
59
60

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

3
4
5
6
7
8
9
10
11 *Assessment of EDI and EDI/NOAEL ratio.*

12 The first two simulations were aimed to:

- 13
14
15 • estimate the most probable EDI values, and their distributions, for ethyl chlorpyrifos,
16 for methyl chlorpyrifos and the sum of two pesticides from consuming Italian
17 *Tarocco* oranges, considering a consumption distributed on 365 and 180 days,
18 respectively;
19
20 • estimate the EDI values as described above, but keeping in account also the
21 probability distribution of the presence of the pesticide (qualitative analysis).

22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
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: $2.6 \cdot 10^{-3}$ and $1.3 \cdot 10^{-3}$ 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 $3.6 \cdot 10^{-3}$ mg/day. This value is significantly higher, 2.8
fold, than the analogous reported above ($1.3 \cdot 10^{-3}$ mg/day). The difference could be ascribed

1
2
3 1 to the lower level found in blood oranges (Table 3) compared to other citrus (Fernandez et al.,
4
5 2001).
6
7
8
9

10 4 For methyl chlorpyrifos residues found in our samples, the four simulations indicate that the
11
12 5 most probable EDI values (on 180 or 365 days of consumption) are of 0.09 mg/day or 0.04
13
14 6 mg/day, respectively. Furthermore acceptable values of EDI distributions can range from 0
15
16 7 up to 0.23 mg/day, for 180 days, from 0 up to 0.12 for 365 days. If we consider that methyl
17
18 8 chlorpyrifos is not always present in oranges, EDI values have a considerable reduction; in
19
20 9 fact they are: $6.7 \cdot 10^{-3}$ and $3.4 \cdot 10^{-3}$ mg/day, calculated at 180 and 365 days, respectively.
21
22
23
24 10 Data shown above highlighted that the daily intake of methyl chlorpyrifos, from blood
25
26 11 oranges, is always higher than ethyl chlorpyrifos, up to 7.6 fold.
27
28
29
30
31

32 13 As methyl chlorpyrifos has been found in some samples, also the presence of, a multiple
33
34 14 intake (sum of ethyl and methyl chlorpyrifos), was estimated. In both cases: keeping in
35
36 15 account the distribution of the averages levels and the probability distribution that the two
37
38 16 molecules are contemporarily in the same sample. Considering just the concentrations
39
40 17 encountered in our samples the estimated daily intake increases significantly up to: 0.03 and
41
42 18 0.01 mg/day for 180 and 365 day, respectively. But, keeping in account the probability
43
44 19 distribution that this event occurs, the co-presence in the same sample, the EDI values go
45
46 20 down up to: $4 \cdot 10^{-4}$ and $2 \cdot 10^{-4}$ mg/day, respectively.
47
48
49
50
51

52 22 The ADI (Acceptable Daily Intake) value for chlorpyrifos is in the range 0-0.01 mg/kg bw
53
54 23 (INCHEM, 1999). For a person of 60 kg of body weight, the highest acceptable daily intake
55
56 24 is: 0.6 mg. This means that, in the worst case, using EDI at 180 days, the consumption of
57
58
59
60

1
2
3 1 Italian *Tarocco* oranges can contribute to the 1.87%, 14.37% and 4.71%, of ADI as ethyl
4
5 2 chlorpyrifos, methyl chlorpyrifos and the sum of ethyl and methyl, respectively.
6
7 3
8
9

10 4 Results of simulations aimed to verify the distance, in terms of distributions probability,
11
12 5 among the EDI values and the NOAEL values, indicate that, even in the worst case, for the
13
14 6 presence of ethyl chlorpyrifos, considering a consumption of oranges during 6 months (180
15
16 7 days), the ratio (EDI/NOAEL) has a 0.48 % as average value and a highest acceptable value
17
18 8 of 1.7%. Considering the probability that chlorpyrifos may be not present, the ratio is 0.1 or
19
20 9 0.05% for 180 or 365 days, respectively.
21
22
23
24
25
26

27 11 For the presence of methyl chlorpyrifos, the ratio (EDI/NOAEL_{180 days}) has a 3.7 % as
28
29 12 average value and a highest acceptable value of 14%, if we considering the probability that
30
31 13 methyl chlorpyrifos may be not present, the ratio decrease significantly to 0.29% or 0.14 %
32
33 14 for 180 or 365 days, respectively. The cumulative analysis shows that the ratio (EDI/NOAEL
34
35 15_{180 days}) has a 1.19 % as average value and a highest acceptable value of 4.9%; besides if we
36
37 16 considering the probability that methyl and ethyl chlorpyrifos may be not present
38
39 17 contemporarily in the same sample, the ratio decrease significantly to 0.02% or 0.01 % for
40
41 18 180 or 365 days, respectively.
42
43
44
45
46
47

48 20 The above simulations have shown that chlorpyrifos levels are always largely in the safety
49
50 21 area.
51
52
53
54

55 23 **Conclusions**

56
57 24 This study highlighted the following findings:
58
59
60

- 1
- 2
- 3 1 • The number, the associated probability and distribution of pesticides in *Tarocco*
- 4 oranges;
- 5
- 6 2
- 7
- 8 3 • The most common pesticide residue for farming activity is ethyl chlorpyrifos,
- 9 followed by methyl chlorpyrifos;
- 10
- 11 4
- 12
- 13 5 • All but two of the 460 analysed samples shown pesticide levels below the MRL;
- 14
- 15 6 • The Estimated Daily Intake of methyl and ethyl chlorpyrifos, and the sum of two,
- 16 from eating *Tarocco* Blood oranges has been estimated;
- 17
- 18 7
- 19
- 20 8 • Authors believe the EDI, calculated considering a citrus eating spread of 180 days,
- 21 more realistic than that calculated on 365 days;
- 22
- 23 9
- 24
- 25 10 • The percentage of ethyl chlorpyrifos, for a person of 60 kg of body weight, in
- 26 comparison ADI has been calculated;
- 27
- 28 11
- 29
- 30 12 • Both for ethyl and methyl chlorpyrifos, as well as for their sum, the measured levels in
- 31 oranges, and, as consequence, the estimated consumer intake is far from NOAEL
- 32 value.
- 33
- 34 14
- 35
- 36

37 15 Moreover, data reported above, in our opinion, have shown the efficacy of performance-based

38 (product) control systems (Cho and Hooker, 2009) and of the co-regulation approach

39 (Martinez et al., 2007) to guarantee food safety. The acceptable level of pesticides could be

40 ascribed also to the pressure of Public Authorities on business operators and, as a

41 consequence, the practical capacity of citrus chain actors to control it. In fact, also when 2 of

42 the 460 samples where outlaw they have been intercepted not representing a real risk for

43 consumers. Studies concerning post-harvesting citrus activities, as well as, the not controlled

44 (farmers' direct selling, street or small shops) are in progress.

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

1 **References**

- 2 Arena E, Fallico B, Maccarrone E. 2001. Evaluation of antioxidant capacity of blood orange
3 juices as influenced by constituents, concentration process and storage. *Food Chemistry*
4 74: 423-427.
- 5 Boon Pe, Van der Voet H, Van Raaij Mtm, Van Klaveren Jd. 2008. Cumulative risk
6 assessment of the exposure to organophosphorus and carbamate insecticides in the
7 Ducht diet. *Food and chemical toxicology* 46: 3090-3098.
- 8 Canales R, Leckie JO. 2007. Application of a stochastic model to estimate children's short-
9 term residential exposure to lead. *Stoch. Environ. Res. Risk Assess.* 21: 737-745.
- 10 Chiappara E, Fallico B. 2008. Analysis of the Italian Public Food Control System and its
11 relationship to European hygiene and safety legislation. *Italian Journal of Food Science*
12 vol. 20: 297-320.
- 13 Cho B, Hooker N. 2009. Comparing food safety standards. *Food Control* 20: 40-47.
- 14 Douglass J.S., Tennant D.R.1997. Estimation of dietary intake of food chemicals. In
15 Chapman & Hall (Ed.), *Food Chemical Risk Analysis* (pp.212-215), chap. 8.
- 16 FAO and WHO. 2006. Food safety risk analysis. A guide for national food safety authorities.
17 FAO FOOD AND NUTRITION PAPER 87.
- 18 INCHEM 1999 available from: <http://www.inchem.org/>
- 19 ISTISAN 97/23. National Health Institute. 1997. Multiresidue analytical procedures for
20 pesticides residues in vegetable products. Sections: A (pp. 3-5, C.2.3. (pp 32-33).
21 <http://www.iss.it/publ/rapp/cont.php?id=656&lang=1&tipo=5&anno=1997>
- 22 Fernández M, Picó Y, Mañes J. 2001. Pesticides residues in oranges from Valencia (Spain).
23 *Food Additives and Contaminants* 18: 615-624.

- 1
2
3 1 Italian Ministry of Health. Ministerial Decree 27 August 2004. Phytochemicals: Maximum
4
5 2 residue levels of active substances on food products. Italian Republic Official Gazette
6
7 3 No 292, 14/12/2004, O.S. No. 179.
8
9
10 4 Italian Ministry of Health. 2007. Official Control of the Phytosanitary Products Residues in
11
12 5 Fruit and Vegetables. Results for 2006. Available from:
13
14 6 http://www.ministerosalute.it/alimenti/resources/documenti/sicurezza/anno_2006.pdf.
15
16
17 7 ISMEA. April 2008. Citrus Consumption in Italy. Available from:
18
19 8 <http://www.ismea.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/3051>
20
21
22 9 Lega Ambiente: Dossier Pesticidi nel Piatto 2008. 23 Maggio 2008. Available from: <http://>
23
24 10 www.legambiente.eu/documenti/2008/0526_pesticidi_nel_piatto_2008/0526_Pesticidi2
25
26 11 008.pdf
27
28
29 12 Lunchick C. 2001. Probabilistic Exposure Assessment of operator and residential non-dietary
30
31 13 exposure. *Ann. Occup. Hyg.*, 45: S29-S42.
32
33
34 14 Maccarrone E. 2004. Composition and healthy value of Sicilian blood oranges juice. *Rivista*
35
36 15 *di frutticoltura e di ortofloricoltura*. Volume LXVI – N.3 – Marzo 2004 (25:27).
37
38
39 16 Martinez M.G, Fearne A, Caswell J.A, Henson S. 2007. Co-regulation as a possible model for
40
41 17 food safety governance: Opportunities for public-private partnerships. *Food Policy* 32:
42
43 18 299-314.
44
45
46 19 Montemurro N, Grieco F, Lacertosa G, Visconti A. 2005. Persistence of Fenitrothion in
47
48 20 oranges and clementines after treatment with emulsifiable concentrate and
49
50 21 microencapsulate formulations. *Food Additives and Contaminants* 22: 39-47.
51
52
53 22 Rapisarda P, Carollo G, Fallico B, Tomaselli F, Maccarrone E. 1998. Hydroxycinnamic acids
54
55 23 as markers of Italian blood orange juices. *Journal of Agriculture and Food Chemistry*
56
57 24 46: 464-470.
58
59
60

- 1
2
3 1 Schirra M, Cabras P, Angioni A, Melis M. 1996. Residue level of Imazalil fungicide in
4
5 2 lemons following prestorage dip treatment at 20 and 50 ° C. Journal of Agricultural and
6
7 3 Food Chemistry 44: 2865-2869.
8
9
10 4 Schirra M, D'Aquino S, Palma A, Marceddu S, Angioni A, Cabras P, Scherm B, Migheli Q.
11
12 5 2005. Residue level, persistence and storage performance of citrus fruit treated with
13
14 6 fludioxonil. Journal of Agricultural and Food Chemistry 53: 6718-6724.
15
16
17 7 Slob W. 2006. Probabilistic dietary exposure assessment taking into account variability in
18
19 8 both amount and frequency of consumption. Food and Chemical Toxicology 44: 933-
20
21 9 951.
22
23
24 10 Slob W, Pieters M.N. 1998. A probabilistic approach for deriving acceptable human intake
25
26 11 limits and human health risks from toxicological studies: general framework. Risk
27
28 12 Analysis 18: 787-798.
29
30
31 13 Spanjersberg M.Q.I, Kruizinga A.G, Rennen M.A.J, Houben G.F. 2007. Risk assessment and
32
33 14 food allergy: the probabilistic model applied to allergens. Food and Chemical
34
35 15 Toxicology 45: 49-54.
36
37
38 16 Tennant D.R. 1997. Food Chemical Risk analysis. First Edition. England. Chapman & Hall
39
40 17 (Ed.)
41
42
43 18 Tribulato E, Maccarrone E, La Rosa G. 2007. Italian citrus cultivation. The present time and
44
45 19 perspectives. In Gruppo Perdisa Edition, *New frontiers of Italian arboriculture* (pp. 205-
46
47 20 221).
48
49
50 21 Vose D.J. 1998. The Application of quantitative risk assessment to microbial food safety.
51
52 22 Journal of Food Protection 61: 640-648.
53
54
55 23 Vose D.J. 2000. Risk Analysis. A quantitative guide. 2nd ed. England. John Wiley & Sons,
56
57 24 Ltd.
58
59
60 25 WHO and FAO. Pesticides residues in food-2004. Report 2004. FAO, Plant Production and

- 1 protection paper 178. Rome, Italy, 20-29 September 2004. Available from:
2 <http://www.fao.org/ag/agp/agpp/Pesticid/p.htm>
3 Zhang XF, Driver JH, Li YH, Ross JH, Krieger RI. 2008. Dialkyl phosphates (DAPs) in fruits
4 and vegetables may confound biomonitoring in organophosphorus insecticide exposure
5 and risk assessment. *Journal of Agricultural and Food Chemistry* 56: 10638-10645.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

22 **FIGURE CAPTIONS**

23 **Figure 1:**

- 24
25
26
27 a. Probability distribution of presence - absence , 1 pesticide residue 2003-2007
28
29 b. Probability distribution of presence-absence, 2 pesticide residues 2003-2007
30
31 c. Probability distribution of presence-absence, 3 pesticide residues 2003-2007
32
33

34 **Figure 2:**

- 35
36
37
38 a. Probability distribution of presence-absence, ethyl chlorpyrifos 2003-2007
39
40 b. Probability distribution of presence-absence, methyl chlorpyrifos 2003-2007
41
42 c. Probability distribution of presence-absence, ethyl + methyl chlorpyrifos 2003-2007
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

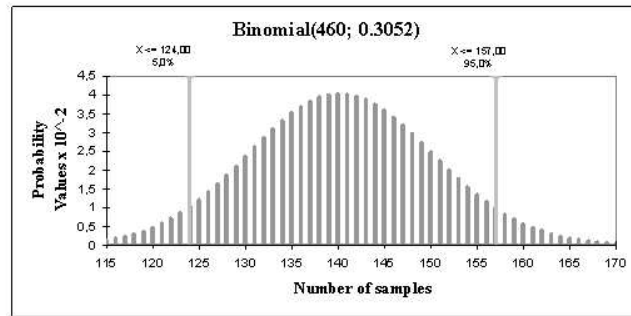


Figure 1a. Probability Distribution of presence-absence, 1 pesticide residue 2003-2007

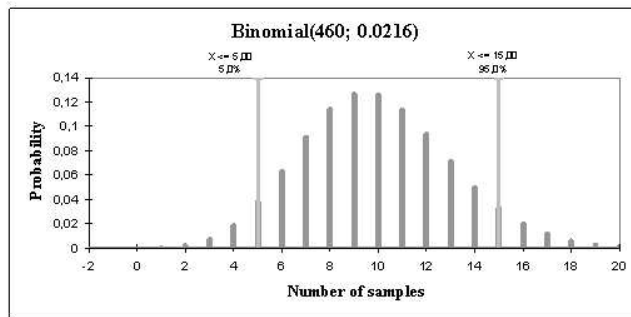


Figure 1b. Probability Distribution of presence-absence, 2 pesticide residue 2003-2007

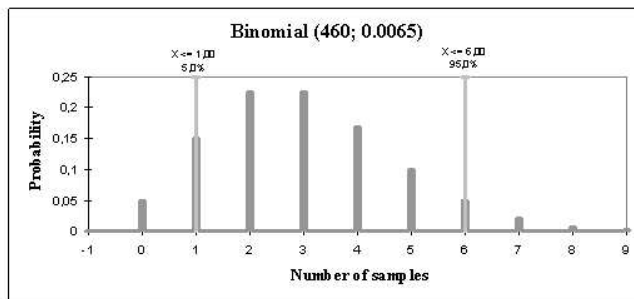


Figure 1c. Probability Distribution of presence-absence, 3 pesticide residue 2003-2007

190x254mm (96 x 96 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

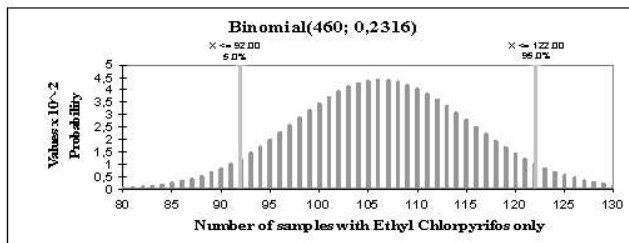


Figure 2a. Probability Distribution of presence-absence, Ethyl Chlorpyrifos 2003-2007

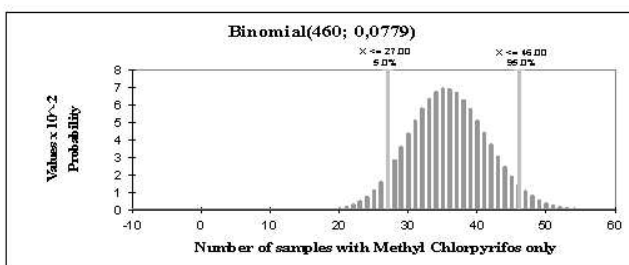


Figure 2b. Probability Distribution of presence-absence, Methyl Chlorpyrifos 2003-2007

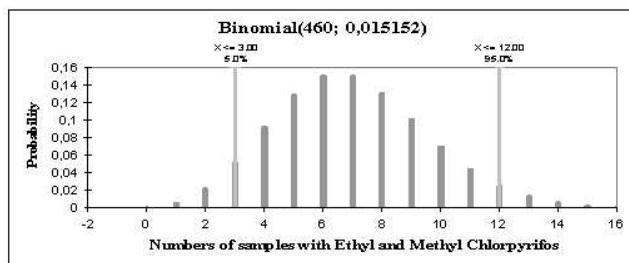


Figure 2c. Probability Distribution of presence-absence, Ethyl + Methyl Chlorpyrifos 2003-2007

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

Table 2. Results of qualitative analysis

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

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

Table 3. Total Quantitative Analysis (2003-2007)

	Ethyl Chlorpyrifos	Methyl Chlorpyrifos	Dicofol	Etofenprox	Fenazaquin	Fenitrothion	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