

# Association between oxidative stress biomarkers and PON and GST polymorphisms as a predictor for susceptibility to the effects of pesticides

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**Abstract.** Low levels of pesticides persist in the environment and can affect the health of exposed subjects. Oxidative stress is considered as one of the mechanisms responsible for the adverse effects on human health and some molecules may represent useful biomarkers for the evaluation of this physiological balance. This study investigated the role of these biomarkers, such as advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and reactive oxygen metabolites (ROMs) in relation to genetic polymorphisms of paraoxonase (PON)1, PON2, glutathione S-transferase pi 1 (GSTP1), glutathione S-transferase theta 1 (GSTT1) and glutathione S-transferase mu 1 (GSTM1). An increase in the levels of these biomarkers is usually inversely associated with the depletion of the biological antioxidant potential (BAP). The results revealed a statistically significant difference in the sex-dependent variation of AGE, BAP, AOPP and ROM protein levels. Furthermore, an association between the PON2 S331C gene polymorphism and the serum levels of AOPP, ROMs and BAP was found. Thus, compared with AGE, the levels of AOPP and ROMs provided a more sensitive biomarker, with an improved association with the PON2 genotype. Such an association strengthens the importance of PON in the occurrence of oxidative stress. According to these results, an individual's genetic background may be taken into account for the health surveillance of individuals occupationally exposed to pesticides, in order to define a cluster of highly susceptible workers so as to guarantee greater protection.

## Introduction

The term pesticide is generic, including a wide and miscellaneous category of chemicals conceived to prevent and defeat weeds or pests, with different targets, chemical structures and biological effects. Although their efficacy has contributed to their wide use, occupational and environmental exposure is a threat to the public health (1). Low levels persist in the environment; however, certain categories of workers (e.g., greenhouses workers) may be exposed to high concentrations of pesticides with potential health consequences. Even though 'pesticide exposure' may appear to be a very unspecific statement, epidemiological studies are often biased by the lack of a proper assessment of exposure and cannot consider exposure to specific pesticides, since they are almost invariably used as mixtures (2).

The symptoms of acute exposure are easily identified; however, chronic exposure can contribute to the development of chronic pesticide-related illness (3-12). Chronic exposure to pesticide has been associated with both genetic and epigenetic alterations underlying the development of different diseases (13). Some classes of pesticides are recognized as causative factors of gene mutations and polymorphisms affecting key factors involved in the regulation of toxic agents and xenobiotic metabolisms (14). In addition, it has been demonstrated that pesticides are able to modulate the expression levels of microRNAs (miRNAs or miRs) (15,16), known to be involved in the development of different chronic degenerative diseases (17-19).

Recent studies have suggested oxidative stress as one of the mechanisms for the adverse health effects of pesticides; moreover, pesticides can act as endocrine disruptors, particularly if used in mixtures (20). Oxidative stress results from the inability of the cell to neutralize an excess of oxidative species, altering the physiological balance between the production and the elimination of oxidant chemical species by antioxidant enzymes. The production of free radicals can disrupt all the components of the cell, including proteins, lipids and DNA (21,22). Furthermore, oxidative stress enhances the inflammatory response, and thus contributes to the pathophysiology of several illnesses (23,24). Also the diet can alter

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the oxidative status because of the daily intake of substances with antioxidant properties, such as polyphenols (25,26). These mechanisms form several molecules which could represent useful biomarkers for the evaluation of oxidative stress in workers exposed to pesticides, such as advanced oxidation protein products (AOPP), advanced glycation end-products (AGE) and reactive oxygen metabolites (ROMs). An increase in the levels of these biomarkers is usually inversely associated with the depletion of the biological antioxidant potential (BAP) (27). The main challenge in occupational exposure assessment originates from the variability in individual response and genetic susceptibility, revealing diverse sensitivity to xenobiotics. In fact, certain individuals seem to be at a higher risk of pesticide-induced oxidative stress due to the presence of genetic polymorphisms which influence the metabolism of such xenobiotics (28,29). Some of these genetic polymorphisms have been widely studied. Glutathione S-transferases (GSTs) are enzymes involved in phase II metabolism participating in the detoxification of xenobiotics or endogenous compounds. GSTs play a key role in the interaction between glutathione (GSH) and the substrate. Genetic differences in the expression and activity of these enzymes are due to polymorphic alleles. These polymorphisms alter GST activity and consequently, the susceptibility to toxic compounds (30). Paraoxonase (PON) family genes are located in region 21.3-22.1 (7q21.3-22.1) of chromosome 7. The family includes 3 proteins (PON1, PON2 and PON3) exhibiting different activities. PON1 plays a defensive role against atherosclerosis due to its esterase and lipoprotein antioxidant activity. The functions of PON2 and PON3 are less clear than those of PON1. It has been demonstrated that they exhibit antioxidant and anti-atherosclerotic properties, similar to those of PON1 (31).

The genotypic characterization of these genes may be used as predictor for susceptibility to pesticides. The present study aimed to assess the contribution of genetic polymorphisms of some pesticide-metabolizing enzymes on the serum levels of AOPP, AGE, ROMs and BAP as biomarkers of oxidative stress in a cluster of occupationally exposed farmers.

## Materials and methods

**Subjects.** In total, 62 subjects, working as farmers in Eastern Sicily, Italy, were enrolled in this study. All farmers were Caucasian, with a mean age of  $46.48 \pm 12.72$  years and had been employed for  $21.97 \pm 12.44$  years. The subjects, included in a compulsory medical surveillance program, provided written informed consent for this survey. Workers included in the study were those who accepted voluntary enrollment between 120 subjects and are representative of a population occupationally exposed to pesticides. A questionnaire was used to obtain information regarding the sociodemographic characteristics and lifestyle of the subjects.

**Genotyping.** Briefly, 3 ml peripheral blood samples were collected from each participant in vacuum tubes containing K<sub>3</sub>-EDTA. Genomic DNA was then isolated from peripheral blood using the Gentra PureGene DNA Purification system (Qiagen). Following qualitative analysis, DNA was quantified spectrophotometrically. The genotyping of polymorphisms was performed using real-time polymerase chain reaction (PCR)

allelic discrimination technique on a 7500 Real-time PCR instrument (Applied Biosystems), using Pre-Designed TaqMan SNP Genotyping assay 5 (Applied Biosystems). The homozygous wild-type genotype was recognized on the basis of a VIC fluorescent signal, heterozygous genotype on the basis of a VIC/FAM fluorescent signal, and homozygous mutated genotype on the basis of a FAM fluorescent signal.

**Quantification of AGE.** AGE levels were determined as previously described (27). The fluorescence intensity with  $\lambda_{exc}=350$  nm and  $\lambda_{em}=440$  nm was measured using a Sinergy HT microplate absorbance reader (Biotek Instruments, Inc.) and expressed as AU/ml.

**Quantification of AOPP.** The serum concentrations of AOPP were determined as previously described by Costa *et al* (27). The absorbance was measured using a Sinergy HT microplate absorbance reader, with a calibration curve of 0-128  $\mu$ M chloramine T.

**Quantification of ROMs and BAPs.** In order to assess reactive oxygen metabolite levels and biological antioxidant potential in serum, the d-ROMS test and BAP test were used (Diacron International). The absorbance at 505 nm was recorded and measurements were expressed as Carr Units (U CARR) and as nmol/ml respectively (32,33).

**Statistical analysis.** The normality of the variables was assessed using the D'Agostino and Pearson test. Mann-Whitney test was used to evaluate a possible influence of sex in AOPP, AGE and BAP while Student's t-test was used for ROMs. The stratification of workers in 3 risk classes, male vs. female, was performed using the Chi-square contingency test. The Kruskal-Wallis test followed by Dunn's multiple comparison test was used to assess differences between the AOPP, AGE and BAP levels between subjects with different polymorphic alleles and between risk classes, while for the for ROM levels, one-way ANOVA followed by Tukey's multiple comparison test was used. Grouped analysis of biomarker levels related to sex and risk class was performed using two-way analysis of variance. All analyzes were performed using Prism version 6.01 (GraphPad software). A value of  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Characteristics of the study population.** The sociodemographic and lifestyle characteristics of the study population are presented in Table I. The majority were smokers (61.3%), and only 9.7% subjects consumed  $>2$  glasses of wine or beer or one serving of liquor/day. All subjects included used personal protective equipment provided by the employer. Other information collected concerned occupational features and the absence of known disorders or diseases in the 3 months preceding the survey. Data regarding the demographic and lifestyle characteristics of the exposed subjects are presented in Table I. The subjects were exposed to a mixture of pesticides (including imidacloprid, cypermethrin, pirimethanil, dimetomorf and carbendazim), with the prevalent use of chlorpyrifos, which is an organophosphate (OP). No cases of exposure to pesticides

Table I. Sociodemographic characteristics and lifestyle of subjects.

Characteristic	No. (%)
Number of subjects	62 (100)
Age (years)	46.48±12.72
Sex	
Male	43 (69.3)
Female	18 (29.0)
Missing data	1 (1.7)
Occupational seniority (years)	21.97±12.44
Ethnicity	Caucasian 62 (100)
Smoking status <sup>a</sup>	
Smoker	38 (61.3)
Non smoker	24 (38.7)
Alcohol consumption <sup>b</sup>	
Yes	6 (9.7)
No	56 (90.3)
Personal protective equipment	
Yes	62 (100)
No	0 (0)

<sup>a</sup>Smoker >10 cigarettes/day; <sup>b</sup>>2 glasses of wine or beer or one serving of liquor/day.

from non-occupational sources were registered. No infectious or inflammatory diseases and no drug use was reported in the subjects in the 3 months preceding the survey. The frequency of polymorphisms and the levels of AGE, AOPP, ROMs and BAP are presented in Table II.

The D'Agostino and Pearson normality test revealed that the AGE, AOPP and BAP levels did not follow a normal distribution. Therefore, non-parametric tests were performed on these biomarkers. Conversely, the ROM serum levels seemed to follow a normal distribution; thus, parametric tests were used for this variable (Fig. 1).

Since some data regarding the polymorphic profile of the studied genes were missing, all the subjects whose genetic profile was not complete were excluded to proceed with a grouping according to different hypothetical risk classes, in order to avoid statistical bias. The 32 resulting subjects were subsequently grouped into 3 risk classes, assigning the score 0 to the wild-type profile, 1 to the heterozygote profile and 2 to the homozygous profile for the polymorphisms of PON1, PON2 and glutathione S-transferase Pi 1 (GSTP1); mutations of glutathione S-transferase mu 1 (GSTM1) and glutathione S-transferase theta 1 (GSTT1) were assigned scores 0 where there was no deletion, and 1 where the deletion was present. The sum of the scores resulting from polymorphic alterations ranged from 0 to 7. The 3 risk classes were thus constituted: Class 1 (score 0-2, low risk); Class 2 (score 3-4, medium risk); and Class 3 (score 5-7, high risk). The Chi-square test performed on these datasets was statistically not significant. The data contingency analysis revealed that no female individual belonged to the high-risk group (Fig. 2).

**Oxidative stress biomarkers.** There was a statistically significant difference in the sex-dependent variation of the AGE, BAP, AOPP and ROMs levels. Statistical significance was calculated using the Mann-Whitney non-parametric test, apart from the ROM data, where the Student's t-test was used (Fig. 3). Conversely, the same analysis revealed that alcohol and smoking do not influence oxidative stress biomarkers (data not shown).

Statistically significant differences in the AOPP and ROM levels in subjects with the PON2 S331C polymorphism were highlighted by Kruskal-Wallis and ANOVA tests, respectively; analyzing the individual groups by Mann-Whitney and t-test (data not shown), respectively, for AOPP (Fig. 4), although the Kruskal-Wallis test revealed an overall statistical difference among the three groups ( $P=0.0376$ ), Dunn's multiple post-hoc test comparisons test did not reveal any statistical differences between individual groups (Fig. 4). The only statistically significant difference found was between the wild-type subjects compared to the heterozygous subjects for ROMs (Fig. 5). A similar trend was observed for the BAP levels (significant difference between wild-type vs. heterozygous), but with an inverse association (BAP levels were higher in wild-type compared to both homo- and heterozygous groups) (Fig. 6).

Following the stratification of the subjects exposed to pesticides into 3 filtered classes, statistically significant differences in AOPP levels were observed (Fig. 7A) in the subjects who were at low-risk compared to those at medium-risk (Fig. 7B). A similar trend was observed with an inverse association, for the BAP levels. The Kruskal-Wallis test followed by Dunn's comparison test revealed significant differences between the low-risk and medium-risk individuals and between the low-risk and high-risk individuals (Fig. 8).

No statistically significant differences were observed with regards to AGE and ROMs protein levels taking into consideration the filtered risk classes of subjects exposed to pesticides. By performing the t-test there was no statistically significant difference between the individual groups.

By performing a grouped analysis using two-way ANOVA, statistically significant differences were found for AOPP and BAP between medium-risk male and female workers (Fig. 9). In particular, for AOPP, statistically significant differences were identified between the male medium risk vs. the female medium risk groups ( $P=0.02645$ ), the male medium risk vs. female low risk groups ( $P=0.01974$ ), the male high risk vs. female low risk groups ( $P=0.02816$ ) and between the male high risk vs. female medium risk groups ( $P=0.03654$ ) (Fig. 9A). For BAP, significant differences were observed between the male the low risk vs. male medium risk groups ( $P=0.011$ ), the male low risk vs. male high risk groups ( $P=0.0028$ ) and between the male medium risk vs. female low risk groups ( $P=0.0187$ ).

## Discussion

The results of this study associating the PON2 S331C polymorphism with altered levels of serum oxidative stress biomarkers associated with a low antioxidant potential of the organism leads to the hypothesis of a possible role of this PON family in the metabolism of pesticides. In fact, the AOPP and ROMs levels were higher in individuals who had

Table II. Frequency and effect of genotype on oxidative stress biomarkers levels in farmers exposed to pesticides.

Gene	Polymorphism	Genotype	N (%)	AGE (AU/ml)	AOPP (nmol/ml)	ROMs (U CARR)	BAP nmol/ml
PON1	Q192R	Wild-type (QQ)	31 (50.0)	147.20±38.82	1.60±0.98	314.90±96.13	2,101.16±649.55
		Heterozygote (QR)	27 (43.5)	150.05±26.02	1.69±1.27	311.55±99.01	1,944.63±631.53
		Mutant homozygote (RR)	1 (1.7)	189.20	0.73	346.00	1,867.00
		Missing data	3 (4.8)				
PON2	S331C	Wild-type (CC)	37 (59.6)	153.49±35.80	1.29±0.76	279.65±82.15	2,276.11±563.21
		Heterozygote (CG)	17 (27.4)	140.41±29.44	2.14±1.48	350.76±99.94	1,700.88±628.05
		Mutant homozygote (GG)	7 (11.3)	146.60±20.08	1.96±1.06	398.57±62.37	1,672.71±477.26
		Missing data	1 (1.7)				
GSTP1	A148G	Wild-type (CC)	26 (41.9)	152.75±38.95	1.42±0.84	297.88±84.48	2,119.58±631.99
		Heterozygote (CG)	7 (11.3)	156.68±20.54	1.92±0.66	336.00±60.83	1,753.00±475.63
		Mutant homozygote (GG)	6 (9.7)	121.17±26.98	1.29±1.28	289.17±125.96	2,280.67±731.40
		Missing data	23 (37.1)				
GSTP1	Ile105Val	Wild-type	33 (53.2)	154.11±35.14	1.60±0.98	323.15±88.44	1,998.00±628.86
		Heterozygote	21 (33.8)	146.88±29.61	1.59±1.29	291.38±101.80	2,116.67±648.94
		Mutant homozygote	7 (11.3)	132.23±26.39	1.59±1.17	317.57±109.58	2,036.28±632.87
		Missing data	1 (1.7)				
GSTP1	Ala114Val	Wild-type	57 (91.9)	148.98±33.88	1.63±1.13	314.03±96.66	2,039.37±651.59
		Heterozygote	2 (3.2)	155.90±13.70	0.73±0.30	184.00±30.00	2,469.50±235.50
		Mutant homozygote	1 (1.7)	169.80	1.78	374.00	1,534.00
		Missing data	2 (3.2)				
GSTM1		Deleted	28 (45.1)	148.94±32.89	1.59±1.10	324.11±103.35	1,902.32±662.06
		Non-deleted	24 (38.7)	153.67±35.77	1.48±0.47	307.96±90.45	2,098.04±588.16
		Missing data	10 (16.2)				
		Deleted	34 (54.8)	147.59±37.08	1.58±1.30	311.50±101.45	2,066.65±634.68
GSTT1		Non-deleted	18 (29.0)	158.82±26.19	1.79±0.82	326.39±90.14	1,852.89±616.30
		Missing data	10 (16.2)				

AGE, advanced glycation end-products; AOPP, advanced oxidation protein products; BAP, biological antioxidant potential; ROMs, reactive oxygen metabolites; PON, paraoxonase; GSTP1, glutathione S-transferase pi 1; GSTM1, glutathione S-transferase mu 1; GSTT1, glutathione S-transferase theta 1.

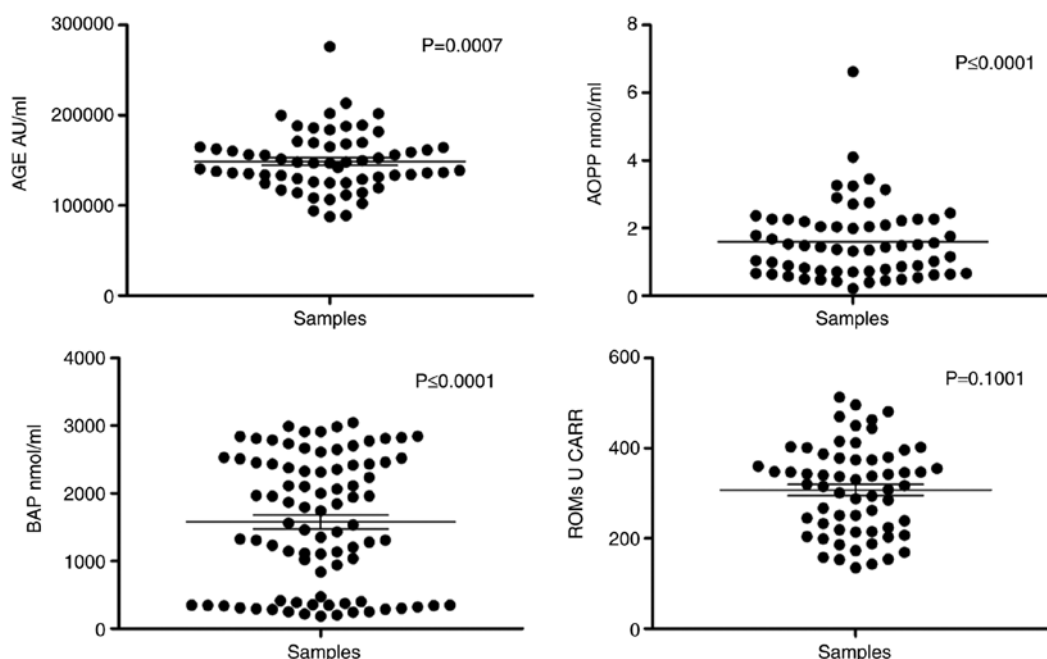


Figure 1. Distribution of the AGE, AOPP, BAP and ROMs levels according to the D'Agostino and Pearson normality test. AGE, advanced glycation end-products; AOPP, advanced oxidation protein products; BAP, biological antioxidant potential; ROMs, reactive oxygen metabolites.

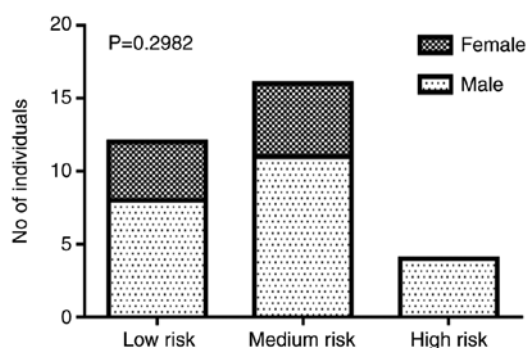


Figure 2. Chi-square contingency test on risk classes assessing the distribution of population stratified for sex.

the heterozygous profile of the polymorphism with respect to the wild-type profile, and coherently, the levels of BAP followed an inverse trend with respect to the first two markers. These oxidative biomarkers have been linked to the toxicity of pesticides due to their pathogenetic mechanisms (34). Thus, compared with AGE, the levels of AOPP and ROMs provided a more sensitive biomarker, with an improved association with the PON2 genotype.

The findings of this study are in contrast to the results of a previously study conducted by the authors (27), in which no significant associations were found regarding the polymorphism of the PON1 (Q192R) gene investigated. This divergence could be justified by differences concerning the characteristics of the population and above all, the class of pesticides handled by the workers examined. In the previous study, the workers were exposed to organophosphates and this would explain the positive association with the PON1 polymorphism, with PON1 being a key enzyme in the metabolism of these xenobiotics; by contrast, this study population was not

only exposed to organophosphorus, but to a mixture of pesticides which also contained other classes of compounds. This may provide an explanation for the contrasting data derived from the two studies. The second explanation could be the different composition of the study population. In the previous study, the sample consisted of only male subjects, while the sample under examination in this study was almost 30% female individuals. A difference was also observed between male and female individuals, having the latter lower values of AGE, AOPP and ROMs and consistently a higher BAP value. This suggests that women have a better response to oxidative damage from exposure to pesticides.

The allele frequency is coherent with most studies conducted on populations from different geographical areas (35). There are a number of studies in the literature concerning the role of PON1 and the metabolism of pesticides; however, the role of PON2 has not yet been fully clarified. A recent study demonstrated the increased expression of PON1 and PON2 related to exposure to toxicants (36). Another study suggested PON2 polymorphisms to play a role in male infertility (37). PON2 has also been suggested as an alternative potential treatment for Parkinson's disease, functioning as an endogenous antioxidant system (38), or playing a role in the etiology of noise-induced hearing loss (39). The antioxidant properties of PONs seem to be useful in the prevention and treatment of cardiovascular diseases and related disorders, as PON2 reduces oxidative stress in vascular cells protecting against cell-mediated oxidation of LDL (40). Others also have investigated the role of PON2, although with weak results (41). Two polymorphic sites of PON2, S331C and G148A, have been investigated in several disorders, both cardiovascular and neurological, such as Alzheimer's disease, diabetes and coronary artery disease (42-48).

The whole population is exposed on daily basis to pesticide residues through food, water and other commercial

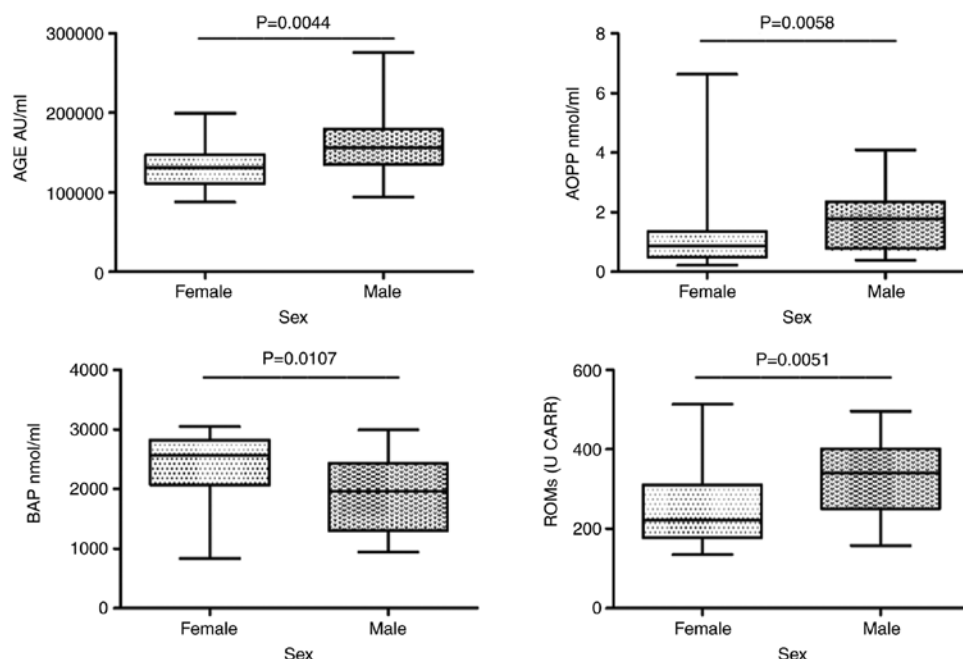


Figure 3. Oxidative stress biomarkers levels in serum of pesticide-exposed workers, in relation to sex. AGE, advanced glycation end-products; AOPP, advanced oxidation protein products; BAP, biological antioxidant potential; ROMs, reactive oxygen metabolites.

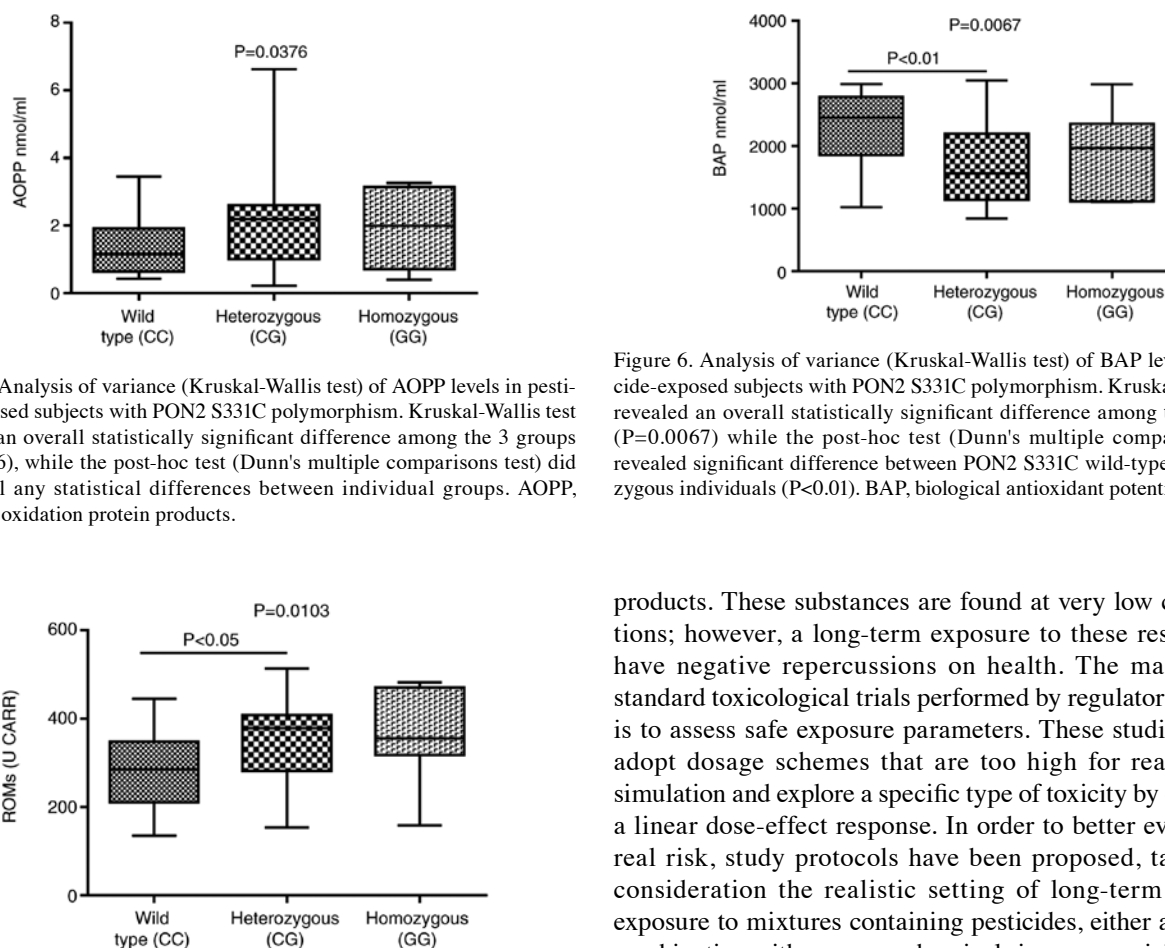


Figure 4. Analysis of variance (Kruskal-Wallis test) of AOPP levels in pesticide-exposed subjects with PON2 S331C polymorphism. Kruskal-Wallis test revealed an overall statistically significant difference among the 3 groups ( $P=0.0376$ ), while the post-hoc test (Dunn's multiple comparisons test) did not reveal any statistical differences between individual groups. AOPP, advanced oxidation protein products.

Figure 6. Analysis of variance (Kruskal-Wallis test) of BAP levels in pesticide-exposed subjects with PON2 S331C polymorphism. Kruskal-Wallis test revealed an overall statistically significant difference among the 3 groups ( $P=0.0067$ ) while the post-hoc test (Dunn's multiple comparisons test) revealed significant difference between PON2 S331C wild-type and heterozygous individuals ( $P<0.01$ ). BAP, biological antioxidant potential.

Figure 5. Analysis of variance (one-way ANOVA test) of ROM levels in pesticide-exposed subjects with PON2 S331C polymorphism. One-way ANOVA test revealed an overall statistically significant difference among the 3 groups ( $P=0.0103$ ), while the post-hoc test (Tukey's multiple comparisons test) revealed significant difference between PON2 S331C Wild-type and heterozygous individuals ( $P<0.05$ ). ROMs, reactive oxygen metabolites.

products. These substances are found at very low concentrations; however, a long-term exposure to these residues can have negative repercussions on health. The main aim of standard toxicological trials performed by regulatory agencies is to assess safe exposure parameters. These studies usually adopt dosage schemes that are too high for real-life risk simulation and explore a specific type of toxicity by evaluating a linear dose-effect response. In order to better evaluate the real risk, study protocols have been proposed, taking into consideration the realistic setting of long-term low-dose exposure to mixtures containing pesticides, either alone or in combination with common chemicals in commercial products, in order to establish a standardized method to evaluate the daily exposures of the general population. This could lead to a new cumulative risk assessment and no longer to the current single risk (49,50). Clearly, it is improbable to test thousands of substances; however, if the hypothesis of an increase in the



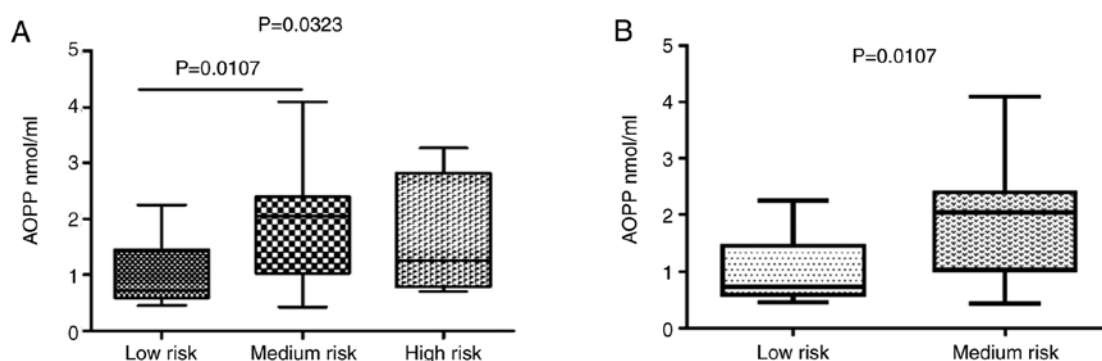


Figure 7. Analysis of variance [Kruskal-Wallis test (A) and Dunn's comparison test (B)] of AOPP levels in serum of pesticide-exposed workers stratified for risk class. (A) Significant difference was globally observed (Kruskal-Wallis P-value of  $P=0.0323$ ). (B) Statistical difference was observed from the individual comparison between low-risk and medium-risk individuals (Dunn's comparison test P-value of  $P=0.0107$ ). AOPP, advanced oxidation protein products.

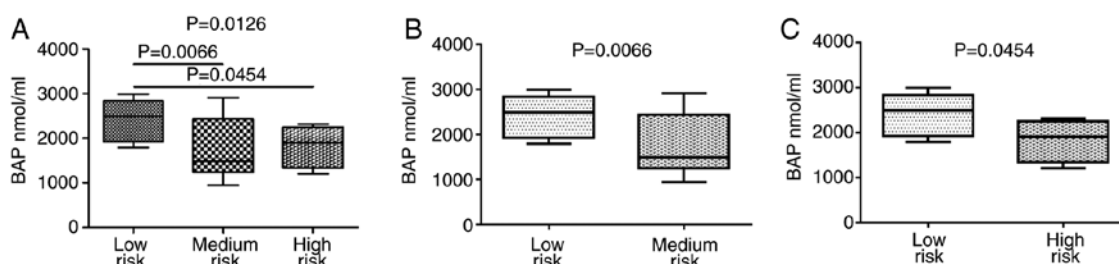


Figure 8. Analysis of variance [Kruskal-Wallis test (A) and Dunn's comparison test (B and C)] of BAP levels in serum of pesticide-exposed workers stratified for risk class. (A) A significant difference was globally observed (Kruskal-Wallis P-value  $P=0.0126$ ); (B and C) Statistically significant differences were observed between low-risk and medium-risk individuals (B) and between low-risk and high-risk individuals (C) (Dunn's comparison test P-value of  $P=0.0066$  and  $P=0.0454$ , respectively). BAP, biological antioxidant potential.

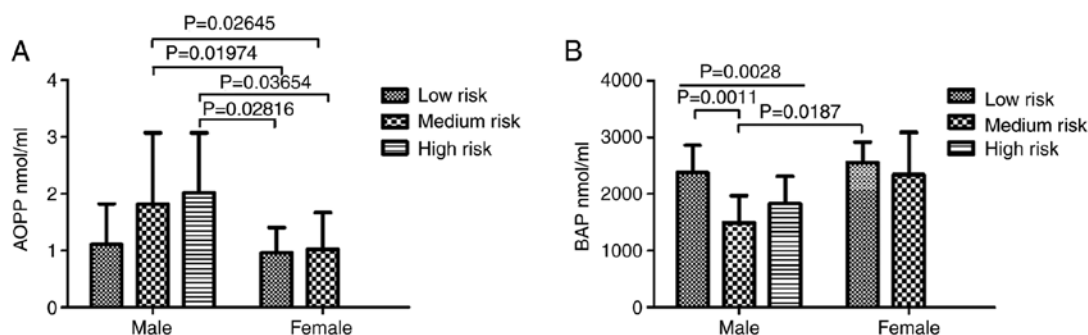


Figure 9. Two-way ANOVA grouped analysis of (A) AOPP and (B) BAP levels. AOPP, advanced oxidation protein products; BAP, biological antioxidant potential.

cumulative risk or even of a different identification of the risk is demonstrated at doses around the regulatory limits, then a new step supporting the effort to pass to the era of low-dose cumulative risk assessment is necessary.

The PON family is one of the key enzymes involved in organophosphate compound metabolism and is believed to be associated in oxidative stress-related pathology. Genetic studies on PON aim to associate the most common polymorphisms with disease occurrence. This study revealed an association between the PON2 S331C polymorphism and the serum levels of some oxidative stress biomarkers, although the direct and indirect effects on health status have not yet been well established. According to these results, the above-mentioned genotypes could be considered for the health surveillance

of individuals occupationally exposed to pesticides, in order to define a cluster of susceptible workers so as to guarantee greater protection.

Evidence on the equivalence of human versus experimental pesticide exposure is not easy to achieve, as pesticide levels in environment are influenced by climate and a variety of other factors, e.g., the design of the present study did not allow the evaluation of possible exposure to minor amounts of pesticides from food and water.

Simulating a representative scenario of long-term exposure to doses lower than regulatory limits of mixtures containing different pesticides, could help the researchers to collect more information from a single chronic toxicity study, adopting a more effective approach and taking a step forward in risk

assessment switching from the single to the cumulative risk assessment (51,52).

In conclusion, the results of this study indicated that a chronic OP pesticide exposure may result in enduring oxidative stress, and polymorphic genes encoding PON can enhance pesticide toxicity. The gene-environment interaction and pesticide exposure seem to be important in the development of several chronic and degenerative disease. The early identification of these chemical biomarkers is warranted in order to implement health prevention programs for workers who are more susceptible to the adverse effects of pesticide exposure.

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### Availability of data and materials

All data generated or analyzed during this study are included in this published article or are available from the corresponding author on reasonable request.

### Authors' contributions

CC, DC, CF, GB and MT made substantial contributions to the conception and design of the study. DC, CC, FG and RC were involved in data acquisition, analysis and interpretation. GB and MT drafted the article or critically revised it for important intellectual content. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The subjects included in this study were healthy workers included in a compulsory medical surveillance program and participation to the study did not expose them to any additional danger. They were neither administered any drug or other compound, nor were submitted to any invasive procedure. Biological samples used for the study were not primarily collected for the study itself, but for mandatory biological monitoring and medical surveillance of occupational hazards. Workers included in the study were those who accepted voluntary enrollment and provided written informed consent for this survey, formulated according to the International Declaration of Helsinki. Consequently, as safety and well-being of subjects could not be affected, approval from ethics committee was not requested.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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