

Research Article

The Taste of Pesticides in Wines

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Abstract

A very first description of the tastes of 11 pesticides is proposed. They are detected first in water, diluted freshly at the levels found in wines, by 36 professionals from wine or cooking in 195 blind tests at different periods. They are the most frequently found pesticides in wines in our experiment. Some animals can detect pesticides and change their behaviour in response. In order to find out if humans can also detect pesticides by their taste in wines, a three-step experiment was conducted. First, 16 pairs of organic and non-organic bottles of wine were identified in 7 regions. The same varieties of grapes in each pair were grown on the same soils (in neighbouring vineyards), in the same climate and in the same year. The resulting wines were assessed for over 250 pesticides. Traces were present only in one organic bottle. In contrast, 4686 ppb were detected in total in non-organic bottles, with only 2 samples at 0 and a mean of 293 ± 270 [0-1144] ppb reached by up to 6 pesticides—mostly fungicides and one glyphosate-based herbicide. Secondly, 195 blind tests with 71 different professionals were conducted at different periods. In 77% of the cases, organic wines were preferred. The same pesticides alone or in mixtures were diluted in water at the levels present in wines. At least one pesticide of the mixture was identified as such because it was judged to taste different from water in blind tests: this held true in 85% of cases in which answers (147) were offered by the professionals, and 58% recognized them all. Among the experts who detected pesticides, 57% identified the wine containing them out of the pair of bottles. To our knowledge, this experiment is the first where humans can identify pesticides by taste.

Introduction

The detection of pesticides by smell is known and documented in the animal world [1]. Several species in different phyla, including fish [2], mammals [3], and insects [4] may avoid toxicants after a first exposure, but this does not prevent the global loss of biodiversity, especially for pollinators [5]. Humans have lost most of their capacity of smell in comparison to other mammals [6], but some “noses” have refined their capacity to detect perfumes, food aromas or herbs [7], wines [8], even pollutants [9]. There are even schools that teach people how to refine their sense of smell. To our knowledge, the potential detection of pesticides in food or drinks is not taught.

Numerous pesticides have been classified as endocrine or nervous disruptors [10,11]. The receptors for these aromatic and/or steroid-like compounds may resemble each other, at least in the capacity to bind to their active site, whether irreversibly or not. Therefore, we examined whether the taste of the pesticides found

in wines could be detected when present in water, in isolation, at the same levels as in the wines.

In a secondary way, this experiment allowed testing of the potential contamination by chemical pesticides of organic wines when the neighbour vineyards are treated with pesticides. This initial investigation utilized 16 samples spread over 6 regions in France, and one in Italy. It also indicated the amount and nature of the recent contamination of wines by pesticides in these samples.

Materials and Methods

Selection of Wines

High quality organic wines were selected (JD) and their close neighbour producers of non-organic wines were identified in all regions studied. In cases where the same varieties of grapes were cultivated on the same soil, during the same period of the year and in the same climatic conditions, bottles were acquired, and the wines were assessed for pesticides. These measures were taken in

order to limit variable factors as much as possible. However, we did not control for the varying practices of the winegrowers, which could modify the final taste. After extensive research, 16 pairs were identified.

Pesticide Analysis

Regulatory-approved methods were used to carry out the pesticide analysis. After thorough mixing of the bottle contents to ensure homogeneity, a 10g sample was taken from each bottle, and an extract prepared from it. Residues of over 250 pesticides (see Table1 for the detailed list).

Analyzed by GC/MS: acrinathrin, aldrin, bifenthrin, bromophos ethyl and methyl, bromopropylate, CHB 26, CHB 50, CHB 62, chinomethionat, chlordan, chlorfenapyr, chlorfenson, chlormephos, chlorobenzilate, chloroneb, chlozolate, cyfluthrin, cypermethrin, DDD (o,p' and p, p'), DDE (o,p' and p, p'), DDT (o,p' and p, p'), deltamethrin, dichlobenil, dicloran, dicofol, dieldrin, endosulfan (sulphate, alpha and beta), endrin, etridiazole, fenclorophos, fenitrothion, fenson, fenpropathrin, fenvalerate, fipronil, flucythrinate, HCH (alpha, beta, delta), lindane, heptachlor epoxide (endo and exo), hexachlorobenzene, iprodione, isodrin, isoprothiolane, lambda-cyhalothrin, methoxychlor, nitrofen, nonachlor (cis and trans), parathion (methyl and ethyl), pendimethaline, pentachlorobenzene, permethrin, phenothrin, phorate, procymidone, profluralin, quintozone, resmethrin, tau-fluvalinate, tecnazene, tetradifon, tetramethrin, toclofos methyl, trifluralin, vinclozolin.

Analyzed by LC/MS-MS: acephate, acetamiprid, acclonifen, alachlor, aldicarb, aldicarb sulfone, amitraz, AMPA, atrazine, azinphos (ethyl and methyl), azoxystrobin, benalaxyl, bendiocarb, bifenox, bitertanol, boscalid, bromacil, bromuconazole, bupirimate, buprofezin, carbaryl, carbendazim, carbofuran, carbophenothion, carboxin, chlorfenvinphos, chloridazon, chlorimuron ethyl, chlorpyrifos (methyl and ethyl), chlorthiophos, cinosulfuron, clodinafop-propargyl, clothianidin, coumaphos, cyanazine, cyanofenphos, cyazofamid, cycloxydim, cymoxanil, cyproconazole, cyprodinil, demeton-S-methyl (and sulfone), diallate, diazinon, dichlofenthion, dichlorvos, diclofop methyl, dicrotophos, diethofencarb, difenoconazole, diflufenican, dimethachlor, dimethoate, dimethomorph, dioxathion, disulfoton, ditalimphos, EPN, epoxiconazole, ethiofencarb, ethion, ethofumesate, ethoprophos, etofenprox, etrimfos, famoxadone, fenamiphos, fenarimol, fenazaquin, fenbuconazole, fenhexamid, fenoxycarb, fenpropidin, fenpropimorph, fenpyroximate, fenthion (sulfone and sulfoxide), flufenacet, flufenoxuron, fluquinconazole, flurtamone, flusilazole, folpet, fomesafen, fonofos, glyphosate, heptenophos, hexaconazole, hexazinone, hexythiazox, imazalil, imazosulfuron, imidacloprid, indoxacarb, iprovalicarb, isofenphos, isoproturon, kresoxim-methyl, linuron, lufenuron, malaaxon, malathion, mecarbam, mepanipyrim, metalaxyl, metamidon, metazachlor, methabenzthiazuron, methamidophos, methidathion, methiocarb, methomyl, methoxyfenozide, metobromuron, metolachlor, metribuzin, metsulfuron-methyl, mevinphos, monocrotophos, myclobutanil, nuarimol, omethoate, oxadixyl, oxamyl, paclobutrazol, paraoxon (ethyl and methyl), penconazole, phenthoate, phosalone, phosmet, phosphamidon, phtalimide, picoxystrobin, piperonyl butoxide, pirimicarb, pirimiphos-ethyl and methyl, prochloraz, profenofos, promecarb, prometryn, propamocarb, propargite, propazine, propiconazole, propoxur, propyzamide, prosulfuron, prothiofos, pymetrozine, prosulfuron, prothiofos, pymetrozine, pyraclostrobin, pyrazophos, pyridaben, pyridaphenthion, pyrifenoxy, pyrimethanil, pyriproxyfen, quinalphos, quinoxifen, simazine, spinosad, spiroxamine, sulfosulfuron, sulfotep, tebuconazole, tebufenozide, tebufenpyrad, terbacil, terbufos, terbuthylazine, terbutryn, tetrachlorvinphos, tetraconazole, thiabendazole, thiachloprid, thiamethoxam, thifensulfuron-methyl, thiofanox, thiometon, triadimefon, triadimenol

Table 1: Wine contaminants measured in this study. For techniques, see materials and methods. All measurements were performed in accredited laboratories.

Were measured in samples by a multi-residue GC-MS and/or LC-MS/MS method following acetonitrile extraction/partitioning and clean-up by dispersive solid-phase extraction - QuEChERS-method [12], with European and French Standard NF EN 15662 of January 2009 for foods or drinks of plant origin. Limits of Quantification (LOQ) varied from 1 to 10 ppb (20 in 2 cases) according to each pesticide; Limits of Detection (LOD) were one-third of the LOQ. Glyphosate (G) and its degradation product Aminomethyl Phosphonic Acid (AMPA) were determined by isotope dilution and solid-phase extraction and LC-MS/MS. They were extracted (5g) with water after addition of internal standards of stable C13 -isotopes. Aliquots were derivatized using 9-Fluorenylmethyl Chloroformate (Fmoc), then purified and concentrated on solid-phase extraction cartridges (C18). After filtration, the extracts were injected in LC-MS/MS with electrospray ionization in negative-ion mode using multiple reaction monitoring. Analyses were performed "one-shot", as recommended for regulatory methods. The LOD for glyphosate and AMPA were 10 ppb. Fidelity criteria had been defined previously, during validation. Uncertainties of measurement (including SDs) were calculated from the Horwitz equation, and ranged from 16 to 32%.

Pesticides Taste Detection

Seventy-one volunteer professionals accustomed to drinking wines were recruited for the experiment. They included renowned chefs, wine makers, advisors, and retailers. Out of these professionals, independent groups were formed at different periods according to the individuals' availability and testing was processed silently and independently, with the results being recorded in writing. All tests were conducted blind. A total of 119 preliminary tests (one test was conducted for one pair of wines, using one professional) consisted of asking each professional which glass of wine she or he preferred out of one unidentified pair. The goal was not to describe the wines, but to explain the reasons for their preference in a few key words. 16 wine pairs were tested in total.

In the second step, the glasses of wine were placed to one side, and 3-8 glasses of water were presented to the professionals, each containing one isolated pesticide that had been found as part of a mixture in the non-organic bottle, diluted at the same level as in the wine, plus, in a separate glass of water, the corresponding mixture. All pesticides were diluted in pure mineral water and were presented together with 1-3 non-differentiable glasses of the same water as controls. All glasses were similar, with a different little random mark made by one organizer to note the results, and were filled with around 10 ml. They fully resembled glasses of water; 1 ml was the mean sufficient consumption for this first detection. In addition, the same mineral water was given ad libitum to each person in a different glass to rinse the mouth, plus small pieces of organic bread if necessary. The purified pesticides detected in wines were purchased from Sigma-Aldrich (USA) and diluted according to the manufacturer's instructions to ensure solubility. Fresh dilutions were prepared for each blind test. The professionals took 10-20 min to write their comments silently and independently on cards. Out of 195 tests, 147 were judged by 36 professionals as demonstrating a marked difference between the wines of the pair. In these cases, the professionals then described the tastes of the pesticides present (Table 2). This was not organized as a classical sensory test because the tastes of these types of products were previously unknown by the participants; this is called a primary test.

In a third step, the professionals wrote which one of the wines contained the same tastes according to their perception; they could taste the pair of wines again. The cards were picked up when everybody had finished.

Results

Pesticides in Wines

In total, 15 pairs in France and 1 in Italy (Figure 1) were selected because in each pair the same varieties of grapes, during the same year and on the same soil (neighbour producers) were grown, one as organic without pesticides (official label), the other one as non-organic. The organic wines (A in frame, Figure 1) had no detectable levels of pesticides or (in one case) traces in a Bordeaux below 10 ppb ($\mu\text{g/l}$). Non-organic wines had a total of 4686 ppb (B in frame) of pesticides, distributed as shown by the numbers in ppb in the glasses.

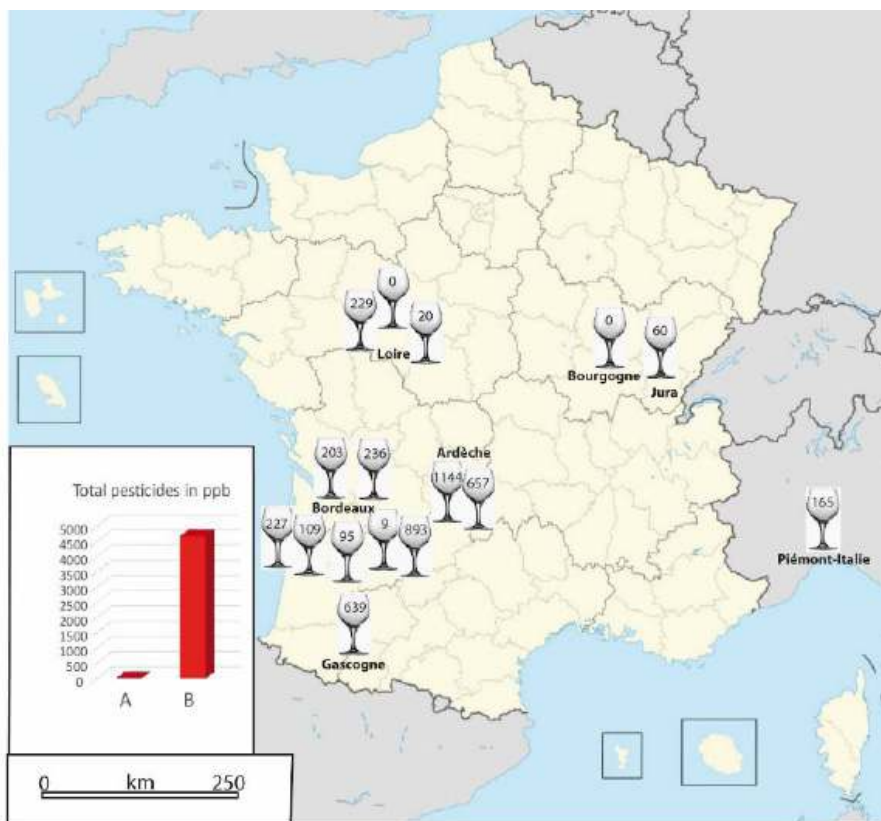


Figure 1: Locations of 16 pairs of wines assessed for 250 pesticides. In non-organic wines, the pesticides detected are indicated in glasses in ppb or $\mu\text{g/l}$; only traces in one case have been found in organic wines (A in frame), 4686 ppb in total in non-organic ones (B in frame).

The difficult aspect of this experiment was to find equivalent pairs of vineyards treated or not with pesticides, in order to limit, as much as possible, the numerous variables potentially affecting the wine quality. The same climates, same soils (on neighbours' land) and varieties were found in 16 cases after one year of research, for 7 red and 9 white wines. The winemakers did not know about the experiment and the authors of this study did not know the pesticides used in the fields. It was surprising to us to note the huge difference in pesticide residue levels between the treated and non-treated groups, whatever the climate or variety, from traces just in one case for the labelled organic wines, up to 4686 ppb in total pesticide residues in the other 16 bottles, with a mean of 293 ± 270 [0-1144] ppb. However, 2 conventional wines out of the 16 had 0 ppb of measured pesticides. These minority cases were produced in Burgundy and Loire (Figure 1). To find out if this was common, in total 36 organic bottles were measured (16 were in pairs, as indicated): 97% were not contaminated (at the threshold of 10 ppb), while out of 36 conventional wines, 89% contained detectable pesticides (period 2011-2015, in France). In our 16 conventional bottles from the taste experiment, the pesticides identified were (among 250 measured): boscalid, cyprodinil, dimethomorph, fenhexamide, folpet and its metabolite phtalimid, glyphosate and its metabolite AMPA, iprodione, iprovalicarb, and pyrimethanil. The 3 most frequently identified pesticides were (number of times detected in parenthesis): folpet (10), fenhexamide (7), and iprovalicarb (6). Folpet represented 42% of the total quantities of pesticide residues assessed, fenhexamide 35%, and pyrimethanil 9%. One contaminated bottle contained 1 to 6 pesticides.

Moreover, to find out the extent of contamination, 7 other bottles of the well-known wine Pomerol were bought in stores, being recommended by 3 retailers; the cost was between 40 and 400 euros. Only one was labelled as organic. In total, 5 bottles were found to contain a total of 1046 ppb of pesticides. Two contained no pesticides, including the one labelled as organic, and the most contaminated contained 333 ppb. Fenhexamid, folpet and boscalide were present in the majority. The most expensive wine was non-organic, from 2009 and very well known: it was marked 17/20 by wine critics Bettane and Dessauve, 18/20 by Gault and Millau, 97/100 by Wine Spectator, and was given the supreme ranking in the Parker guide: 100/100. It contained 146 ppb of boscalid, recognizable by taste. These classifications do not consider pesticide content.

Taste Preference for Wines

When 71 wine professionals or chefs, in total, tasted at least one pair of wines, in tests conducted separately at different times in a blinded manner, 77% expressed within 10-30 min their written preference for the glass that was revealed to come from the organic bottle, by the end of the experiment (Figure 2A). We ensured that it

was not possible for the professionals to influence each other. The glasses were differently marked each time. Although a description was not requested, they indicated a longer and deeper taste in the mouth, with less artificial aromas. This was an important spontaneously expressed criterion for most of them.

Taste Detection of Pesticides in Water and Wines

Again, this primary detection of pesticides in water was not conducted as a usual sensory taste: it was a preliminary trial to know the feasibility of the detection of pesticides in isolation by humans, at the levels found in wines; and to find out if the tastes of pesticides, including their smell, were describable at all. It was astounding to observe the repeatability of the results. In the 147 blind tests in which an answer was offered, the "noses" detected at least one pesticide or pesticide mixture in water (in comparison to mineral water) at the levels present in wines in 85% of cases (Figure 2B); and in 58% of cases all the pesticides were detected (Figure 2C). We examined the cards of the professionals recognizing the pesticides in each test, and 57% of them identified correctly by taste the wine containing the pesticides out of the pair of glasses (Figure 2D).

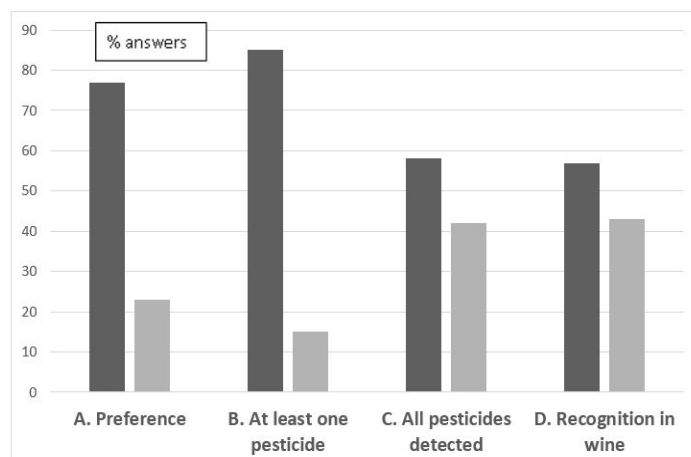


Figure 2: Preference to organic wines and detection of pesticides by taste by professionals. **A.** In black, organic wines were preferred by 77% of professionals of wine or cooking. In grey, 23% chose the non-organic wines in the pair by blind test. **B.** First detection of pesticides by taste in water, at least once in black; not detected in grey. **C.** Detection of pesticides in all cases, success in black, not in grey. **D.** Recognition of the same pesticides in wines in black; failure to do so in grey.

Taste Description

We asked the professionals to describe the tastes detected in water in a few key words, and gathered the remarks on the cards on each pesticide in the experiment. The results are presented (Table 2).

Pesticide	Taste described
Boscalid	chlorine or burning
Cyprodinil	drying, astringency, bitterness
Dimethomorph	cardboard, rag, drying, biting
Fenhexamid	chemical sweet, artificial strawberry
Folpet	alcohol, medical drug, drying, bitterness
Glyphosate	strong dryness, acid, acrid, limestone
Iprodione	irritant, bleach, old burned plastic
Iprovalicarb	astringency, mouldy nut
POEA	drying and papilla blockade, acerbity
Pyrimethanil	soil, dust, detergent
Roundup	putrefied wood, drying, bitterness
All - synthesis	drying, papilla blockade

Table 2: Description of the tastes of pesticides in water, diluted freshly at the levels found in wines, by 36 professionals from wine or cooking in 195 blind tests at different periods.

New tastes were marked forever in their memories, as they testified afterward. Some of them wanted to repeat the testing after the experiment in order to learn, a process that they said was possible. At these concentrations, tasting 1 ml exposed them to 50,000 times less than the acute toxicity regulatory limit for these pesticides, and 20-30 times less than drinking a conventional contaminated glass of wine. In general, a dryness at different locations on the tongue was described, with papilla blockade lasting (or after) a few minutes. The most original description was chemical sweetener or artificial strawberry flavour for fenhexamid for the professionals who detected it.

Discussion

Many variables can change the tastes of wines - including leaf removal during growth or time of harvest, seeding by aromatic yeasts, or the aging and processing in barrels. An original aspect of this work was that for the first time we tried to control the environmental conditions and grape variety, which can influence the taste, by choosing similar wines in pairs. However, when eco-labelled and regular wines were tasted without taking these criteria into account in a large study using 74,148 bottles from 3,842 Californian vineyards, the organic wines were significantly preferred [13]. This confirms our results with French wines. The tastes of organic wines in our experiment were judged to be less artificial and to last longer, and the over expression of artificial aromatic yeasts is avoided in natural wines. Natural yeasts could however be more difficult to control, with a greater year-specific variation.

In accord with our focus on the taste of pesticides, we precisely quantified them. The processing factor, from the pesticides used in the field or the barrel to the ones found in the

wines, may vary depending on the compound [14]. Therefore, we measured at the lowest threshold of regulatory analytical detection possible the largest available range of 250 pesticides, using accredited methods. This allowed us to consider also any potential adventitious environmental contamination of organic wines. Another recent study measured residues of 187 pesticides [15]. The pesticides detected in wines from chemically treated grapes in Canada [15] were different in identity but present at similar levels to those found in Spain [16], but at lower levels than in a recent study in China [17]. The difference in pesticide contamination in organic-labelled wines and others showed not only that organic standards were respected, but also that the cross-contamination from the environment was very limited in these cases selected at random. The relatively high contamination of renowned appellations indicates that their classification does not consider this criterion, and that the taste of pesticides may be confounded with other factors by oenologists.

Moreover, the pesticides detected were among the most frequently used ones at this period, but were still several thousand times above the admissible level in tap water (0.1 ppb). The consumption of pesticides in water proposed here to professionals to determine their tastes was largely below (50,000 times) regulatory established acute toxic levels. However, the chronic consumption of these contaminating levels (Figure 1) may be not encouraged, because they may cause or exacerbate liver steatosis and kidney damage [18,19] as well as mammary tumours [20].

Most of the 11 pesticides detected have been proposed or classified as endocrine or nervous disruptors, or even as carcinogens. The highly debated case of glyphosate has shed a new light on this molecule worldwide. Glyphosate-based herbicides are in fact the most-used pesticides of the world and are extensively used in vineyards. They were detected in the wines in this study and were found by the professionals to impart an unpleasant taste in liquids. Glyphosate is detected in tap water and at far higher levels in wines. Its toxicity below regulatory thresholds are considerably amplified by the common formulants of glyphosate-based herbicides [21], which are known to be petroleum derivatives [22] with heavy metals; all these chemicals could thus well be present in non-organic wines. Our group also demonstrated the presence of an important number of pollutants in industrial food even for laboratory animals [23]. If the chronic toxicity of these formulants present in almost all glyphosate-based herbicides and other pesticides is considered, there is a serious risk of the whole product being carcinogenic, and of causing or exacerbating kidney, liver, and hormonal diseases [24]. The analyses of toxicity based on experiments with glyphosate alone, as generally assessed by regulators, may thus be insufficient, because glyphosate occurs in a mixture, not only with other pesticides as shown in this work, but also, in common with all pesticides, with formulants. The tastes of formulants in food or drinks, as well as other pollutants, are a topic

for future study.

It is possible that these primary tests by professionals and their descriptions of the tastes of pesticides have served to develop new taste perceptions [25], since several of them said they were indelibly registered in their memories. For instance, most experts know the famous and potent sotolon aroma characteristic of yellow wines from Jura, with its fenugreek and caramel flavours. These yellow wines typically present 120-1020 ppb of sotolon [26], which is fully comparable with the levels of fenhexamid found in this study, up to 500 ppb. Thiols are other recognizable well-known aromas, giving flavours of blackcurrant, passion fruit or grapefruit to some wines. They can be detected by experts below 0.07 ppb [27]. Boscalid and folpet were found in this study to be present in some bottles at levels up to 9,000 times higher. Our tests also indicated that people could practice and learn to recognize the tastes of pesticides in drinks or possibly food. We demonstrate here that there is no scientific reason why this is not feasible. Therefore, it was interesting to collect very similar results from a relatively large number of experts on wines or aromas (Table 2). The common papilla blockade and drying effect, or the artificial aroma taste, especially in the case of fenhexamid, could be viewed in the context of the chemical structure of the pesticides. Pesticides often present broken aromatic cycles, which arise from petroleum chemistry. Like aromatic cycles from aromas, pesticides could possibly enter and bind to nervous nasal receptors, as they do in steroid or other endocrine receptors, and disrupt cellular signalling [28]. This should be studied in further detail.

A larger study could also be envisaged, not only to confirm the presence and distribution of pesticides in foods and beverages, but also to progress from this primary test of feeling to sensory tests on a wider range of pesticides and a larger number of volunteers.

To our knowledge, this experiment is the first where humans can identify pesticides by taste. It is also the largest measurement of pesticides in non-organic and organic wines from the same locations, years, and varieties.

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