



Sulfur in Wines and Vineyards: Taste and Comparative Toxicity to Pesticides

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Abstract

Vines are among the crop plants that are the most heavily treated with pesticides. They may also be treated mainly with copper (Cu) and sulfur (S), which are commonly used in organic cultures instead of chemical pesticides but at lower doses. However, in common with synthetic pesticides, Cu or S may also contain petroleum residues in formulations marketed mainly for non-organic treatments. We have already reviewed the taste and toxicity of pesticides and Cu in vineyards and wines. As a part of this trilogy, in this paper we summarize data on the taste and potential toxicity of sulfur in wines, as well as on its use and role. We underline here that it is protective for life at physiological levels, is produced at low levels by yeasts and raisins, and is toxic by saturation of the capacity of biological processes.

Sulfur is used in many forms, including mineral forms, and sulfur dioxide or sulfates in vineyards, and for instance sulfites, such as salts like potassium metabisulfite, in wines. Used at high levels (up to 450 mg/L is authorized in some countries in wines), sulfites become major fungicides and bactericides. They kill microorganisms, for instance those that do not have sulfite oxidase. Sulfites and Cu salts are also authorized for use, differently and in general to a lesser extent in organic wines. Today, S is the major additive in different forms in wines.

Here we characterize the taste of sulfite additives and the sensations that they evoke in volunteer tasters in comparison to the literature, to complete the comparative data. In the experiments, we tested sulfite additives in water and wines at similar levels. We also compared them with synthetic pesticides and Cu. Sulfites are irritants from 10-30 mg/L in water. Tasters were able to detect sulfites in a wine spiked in a blinded manner to a level of 30-90 mg/L. This was found to clearly modify the taste of wine. Sulfites at high levels break the complexity of nose and mouth sensations, according to specialists, especially for red wine. They dry and acidify the tastes of wines. As for Cu, natural sulfites cannot be considered as comparable to the petroleum-derived synthetic pesticides present in non-organic wines from any point of view. By contrast, high levels of sulfites do have grossly acute effects comparable to those of synthetic pesticides, taste-wise and toxicity-wise; however, the classical chemical pesticides have more chronic toxic effects. We were able to differentiate Cu and S toxicity levels in organic and non-organic treatments, due to the combined effects of petroleum derivatives in formulations in the latter case. The environmental impact of high sulfur treatments in non-organic vineyards and wines today appears to affect biodiversity, drinkability, taste, and health. Some tastes of Cu, S, or chemical pesticides may have been previously attributed to other characteristics of wines. The chronic toxicity of chemical pesticides is not negligible in comparison to that of alcohol, it could enhance its effect. Finally, taste could serve as a toxicity detector in wine.

Keywords: Copper; Pesticides; Sulfates; Sulfites; Sulfur; Wine

Introduction

Abbreviations: ADI: Acceptable Daily Intake; Bw: Body weight; Cu: Copper; EU: European Union; S: Sulfur

Sulfur is used in many interconvertible forms, including mineral forms, and sulfur dioxide as a gas or sulfates in vineyards, and for instance sulfites, such as salts like potassium metabisulfite,

in wines or other drinks, fermented or not, or foods, to try to control microorganisms. The measurements and regulations may write sulfur, SO₂ or sulfur dioxide, or sulfite (SO₃²⁻) in an interchangeable way. They include the free and combined forms to other molecules, combined also in a reversible way or by contrast into more stable compounds. Sulfites have been used since antiquity as a natural insecticide, a biocide against parasites, a fungicide on the plant, or as a wine preservative and bactericide at high levels, as well as an antioxidant. Today, sulfites are among the major food and drink additives authorized in different forms in Europe as E220-E228, varying from sulfur dioxide to sulfites of different salts such as sodium, calcium, and potassium [1]. S is most often obtained from petrochemistry or gas extraction, and only rarely from minerals. Natural S is extracted from the bases of volcanos or from mines. It is a wettable form acting by little evaporating doses through sublimation, while synthetic sulfur forms are used with products of formulations sometimes called adjuvants, which are mostly the petroleum oily residues allowing the formulations to better stick to leaves and fruits. These are not real “adjuvants” since they do not only help or are added (etymological meaning) but can be more toxic than S itself. They should rather be called formulants. Their toxicity requires further studies; however, their full composition is not public.

Petroleum-derived pesticides have been developed more recently, from toxic chemicals developed for warfare. Vines have been among the crop plants that are most heavily treated with these pesticides especially since 1950, as we underlined in a previous study on copper (Cu) [2], which is also considered a fungicide. Cu is also not a fungicide at low physiological levels, at which it is essential for life. The authors previously characterized the taste of Cu in water and wines, and two of them have also studied the taste of pesticides in wines [3]. In France, which is known as a wine producer country, 20% of the pesticides, in tons, sprayed nationwide are used on vineyards, which, however, represent only 3% of all cultivated land [4]. This is probably due to an economic and symbolic belief in the protective value of pesticides in a chemically based quite stable monoculture, since no evidence exists that pest attack is greater on vines than on other plants.

Sulfates or sulfites, as well as Cu, are sometimes considered to be pesticides but are authorized for use in organic agriculture. However, in this work we will differentiate whether or not they are produced by petrochemistry and compare their tastes and toxicities for a clearer understanding.

We underline that S and Cu in different chemical forms for vineyards treatments, as well as synthetic pesticides sprayed on the plants, have the common trait to contain petroleum formulants. They are present with the active declared principles, often as residues of syntheses, but not declared. In different families of pesticides these may constitute between 1.25 and 71.7% of the

preparations [5]. These could be polycyclic aromatic hydrocarbons and heavy metals, including lead or arsenic for glyphosate and non-glyphosate-based herbicides [5,6]. Similarly, S made from petroleum has also been found to contain petroleum residues [7]. This fact obviously modifies the toxicity and secondary effects of the mixture provided to agricultural workers for application on crops, but because these are not declared, workers are denied knowledge of what they may be exposed to. In organic agriculture, adjuvants may be from animal or plant origins, such as oligosaccharides from crustaceans or fruits, to add sticking molecules to the leaves.

Organic vineyards that do not use synthetic petroleum-based pesticides are in development. S and Cu salts are authorized in organic wines, but at lower levels in organic wines which are in fact classified in 3 categories: simply organic, biodynamic, and natural. They may be labelled or not, according to the choice of the wine maker. S and Cu salts are often called organic pesticides because they are the most frequently used in organic production. These minerals may be extracted from mines, in which case they are not petroleum-based. These forms are preferred but not always used in organic agriculture.

In this mini review, we will investigate the roles, taste, and comparative toxicities of S, and how it compares with other substances classified as pesticides, including Cu, because it is a highly debated subject. It has long been known that S is essential for plants and animals or humans but that it is also toxic in excess. Here we thus also characterize its taste, and sensations evoked in volunteer tasters and in the context of the literature.

What is the role of S and sulfites in vineyards and wines?

At low physiological levels in the plants (well below the theoretical regulatory sulfites ADI of 0.7 mg/kg bw), it has long been known that S is an essential mineral element, and, in common with Cu, a necessary catalyst in many enzymes; it can be also coupled with molecules such as sulfates to be excreted, allowing more solubilization. It is essential in nitrogen metabolism and in the synthesis of some amino acids, like methionine and cysteine, serving the protein structure and folding.

There are several simple molecules in the life cycle containing S, such as SO₂ which is used as an antioxidant or antioxidant with antiseptic capacities. It is often used in its free form and can be linked to numerous molecules. It may be considered to belong to the sulfite family (SO₃²⁻). It can be transformed in sulfate (SO₄²⁻) by hydration and can then also be combined to acetaldehydes, ketones, polyphenols, or proteins, for instance. It is then in an inactive or less active form regarding its pesticide properties; some of these associations are reversible. There are other cycles on earth of S transformation into different forms [8], including in anaerobic fermentation forming sulfides (H₂S, HS⁻, S²⁻, for instance with metals).

The molecules of sulfites can be produced and transformed at low levels by plants [9]; they are in fruits like raisins and are produced also at very low natural levels by wild yeasts, present on all fruits in nature that are not treated by fungicides [10]. This process goes on during alcoholic fermentation. This generally leads to a few mg/L of sulfites in natural wines [11], where sulfites may be, or not at all, artificially added in small quantities in comparison to non-natural ones. Natural sulfites may therefore protect some aromas by their antioxidant effects [12]. On the fruits, sulfites perform the role of a bactericide against pathogens [13]. Thus, they constitute also a defence mechanism for the plants or for yeasts. Like many compounds, they have balanced stimulating and inhibiting effects, depending on time, dose, environment, and sensitivity of the target (e.g., bacteria).

Used at high levels, when they can contribute up to 450 mg/L authorized in some countries (see below) in wines, sulfites become then a major fungicide and bactericide. For these reasons they are used for their preservative capacities. In these chemically treated vines and wines, they are generally used with other fungicides [3], killing yeasts and bacteria on raisins and during the fermentation process. Other transformed or laboratory-prepared yeasts and bacterial strains, both in noticeable non-natural quantities, have thus to be added afterwards to allow fermentation of these treated wines. Microorganisms are more sensitive to sulfites than mammals, in which the process of sulfite oxidation is quite rapid in the liver, forming excreted sulfates [14]. Sulfites can be then added to arrest the fermentation process, and to kill the bacteria added, after the possible malolactic fermentation.

It is obvious that when used at environmentally toxic levels or in combination with toxic compounds such as pesticides and their petroleum-based formulants, sulfites will progressively saturate the detoxification system of humans, including that of symbiotic gut microbiota; therefore, they appear to work as a pesticide [15], with all the side effects possible in the environment, wines, and consumers, even in the short term at high levels. The detoxification and sensitivity may begin by indications given by the taste of food

and drinks in healthy humans [3] - a question addressed in the present paper.

Levels of S in wines, and comparisons with other added compounds

Sulfates are less measured and regulated than sulfites. However, the International Organization of Vine and Wine proposes as maximum sulfates acceptable limits 1 or 1.5 g/L (according to the type of wine), and Brazil imposed that for imported wines [16], in particular for taste and toxicity reasons (see below).

As indicated in Table 1, the maximum levels of sulfites authorized in the world (450 mg/L) are reached in wines in Sri Lanka or Malaysia, according to Wine Australia (2020) [17]. In general, higher levels of sulfites are authorized in white wines, because they have fewer natural antioxidants such as polyphenols and flavonoids than in red ones, coming from the skins in general, and sometimes more sugar. Thus, the fermentation process may continue in the bottle. To control this, the sulfites are added at microbiocidal levels. The maximum level decreases to 420 mg/L in Quebec, Canada and 400 in Ontario. China, Hong Kong, Taiwan, and Vietnam also stipulate 400. A slightly reduced limit (350) is authorized in Qatar, Peru, the Philippines, and the USA, and even less (300) in Australia and South Africa.

EU wines reach the lowest maximum authorized levels, starting from 400 mg/L for liquored or late harvest wines, with Botrytis, very sugared, decreasing to a maximum of 300 in sweet wines, 235 in sparkling wines, and then to 200 in white wines and 150 in red ones. Argentine wines are comparable, with up to 210 for the same category. Even renowned wines for export or trading may contain more sulfites than others. In the EU, the organic labelled wines contain maximum levels in general 50 mg/L less, in wines of the same categories. The biodynamic wines have a maximum of 40 (white) to 30 (red) mg/L of sulfites, and the additive-free ones ("natural wines") should have only traces <10, which are due to production by the plant and wild yeasts (Table 1).

In wines in mg/L	Pesticides max	Cu max	Sulfites min	Sulfites max
Not organic	1.1 or +	1-15	150	450
organic	0.0001	0.15	<10	150*
Not org /org	11,000	7-100	30	3
Drinking water	0.0001	2	2.21	90
Taste at max	drying	mineral	irritant	irritant
Not org / water	11,000	7.5	67.87	5
Organic / water	1	0.075	2.5	1.67

Table 1: In non-organic and organic wines, levels of pesticides and Cu found, and sulfites authorized in the world, and ratios of the values.

Legend: Petroleum-derived synthetic pesticides and Cu have no maximum regulatory levels in wines, except that the first are forbidden in organic wine making, and in general are expressed as contaminants in µg/L. Here, all products have been expressed in mg/L to facilitate comparisons. Cu may be considered also to be a fungicide at high levels, as well as sulfites which are considered as pesticides. At low levels, these are natural products by contrast to chemical pesticides. For sulfites, the maximum authorized level (450) is reached in Sri Lanka or Malaysia. Organic wines include different categories and labels (AB and Demeter for instance in France), as well as natural wines (no label, or “wines with natural production method”, or SAINS for instance); the latter have no sulfites added. *except for sugared wines (see text). In drinking water, the maximum regulatory level under European law is indicated for pesticides; the maximum level to comply with a quality standard for Cu follows; no maximum regulatory level is set but the levels found are indicated. The tastes are grossly described for comparisons here but will be detailed below. The two last lines represent the ratio between the levels in wines and drinking water, taken as a reference. A mean of 5 mg/L was taken instead of < 10 for calculations, according to some assessments. References are in the text. max: maximum; min: minimum limit; org: organic.

This comparative Table 1 indicates first that among the chemical inputs present in wines, sulfites are the most added, then Cu, which is more sprayed in the vineyard, and then pesticides, with the latter being around 1000 times less in quantity. However, their comparative toxicities at levels at which they are found, overall, in the long term, can be in the reverse order, based on the levels authorized in tap or drinking water by international safety agencies. This is indicated by the ratios in the last two lines and is detailed below.

While petroleum-based synthetic pesticides are forbidden in organic agriculture, it is admitted that Cu and S are the major chemical components authorized for treatments of organic cultures and corresponding wines. They are also used generally and at higher rates in non-organic agriculture, as evidenced in Table 1. These two compounds may also be included in formulations of synthetic pesticides, increasing the levels in non-organic wines [6,18].

In organic wines, pesticides are not found or are only found in traces [3], even when the cultures are in neighbourhood of non-organic wines. This is probably because the contaminations are hundreds of times less when the chemicals are not sprayed intentionally, also possibly because some detoxifying plants are growing spontaneously, such as dandelion [2]. In chemically treated vineyards these plants are generally removed to facilitate mechanical chemical spraying and harvesting. All this is reflected by the comparative ratio “not organic/organic” (Table 1), indicated

for the different compounds. Non-organic wines always have higher levels of all kinds of pesticides, including Cu and S, and in consequence, in these the level of toxic chemicals is increased.

Cu levels [2] and levels of sulfites advised in drinking water (2 mg/L) or even directly measured [19] are indicated for comparisons (Table 1). Sulfites are more strictly regulated in foods [20] than Cu. For instance, in France [21], the average level of Cu in organic wines was established around 0.15 mg/L; the limit is 1 mg/L. The presence of Cu in non-organic wines is mostly linked to the number and timing of fungicide applications. In addition to the declared active substances, most formulations of synthetic pesticides contain heavy metals and other trace elements [18], including Cu. In treated wines in Croatia, up to 7.6 mg/L of Cu was detected [22] and in Australia, up to 15 mg/L Cu were found in non-organic must and raisin juice before fermentation. It has long been known [23] that from 10 mg/L, Cu inhibits fermentation, as does S and agrochemical residues at higher levels, but often this is compensated for in treated wines by adding significant amounts of modified or selected less diverse yeasts. This is, by the way, a common practice in wines when fungicides including S are applied in the vineyard and detected as major pesticides in non-organic wines [3], since most natural microorganisms are killed by fungicides. In brief, more Cu and cupric residues are found in non-organic wines, either white or red, than in organic ones, due to less chemical applications in the latter, and possibly due to more time (40-50 days) between the last application and the harvest. It is a similar case for sulfites.

Similarly, concerning other microbial or chemical inputs, if we consider laboratory yeast strains authorized for making non-organic wines, these are above 300 in number, while there are a lot less, and normally not genetically modified, for organic cultures. None of these are normally used in natural wines, and there is no need, because organic vineyards are colonized by abundant natural and more diverse yeast strains. Authorized additives are in the order of 50 in non-organic wines, and between 40 and 0 in organic ones, depending on the described subclasses, according to Raisin (2020) and www.dicoduvin.com (2021) [24,25].

Taste of sulfites in water and wines

Many variables can change the tastes of wines - including the variety, maturation, soil, leaf removal during growth or time of harvest, chemical treatments, seeding by aromatic yeasts, or aging and processing by methods chosen by the winemaker. It is also the case for sulfites, we will study that below. Even sulfates are to be considered for their impact on the taste of wines and waters: they participate to dryness [16]. The final composition and taste result from a combination of all these factors. Among those, we have demonstrated that pesticides [3] and Cu [2] play a role in the final taste.

We came to question the taste of sulfites in water and how they can additionally modify the taste of wines. We examined whether they could be detected when present in water, in isolation, at the same levels as in the wines. Then similarly we studied the taste in wines. A total of 30 volunteers accustomed to drinking wines were recruited for the experiment. They included chefs and retailers. This primary detection of sulfites in water was not conducted as a sensory tasting typical for evaluating wine: it was a preliminary trial to know the feasibility of their detection in isolation by humans, at low levels found in some organic wines, i.e., 30 and 90 mg/L. We wanted also to find out if the taste, as well as the smell, was describable. It was surprising to observe the repeatability of the results. Testing was processed silently and independently, with the results being recorded in writing, as already described for pesticides and Cu testing. We first asked the tasters to describe freely the tastes detected in water in a few key words.

A primary test in water and wine

In a first step, dissolved and drinkable sulfite and potash disulfite (E224) used for wine, beer, or cider conservation (Alcofermbrew, Polka) was presented to 30 subjects. It was then dissolved at 30 and 90 mg/L in water that was first without this element, in a blinded manner for the testers. The same water but without the added sulfite and potash disulfite was given as a control, without identification. All glasses were similar and were filled with around 30 mL of liquid. 3 mL was the mean quantity for consumption for this first detection. This was not organized as a classical sensory test because the tastes of these types of products were previously not recognised by the participants in food or drink; this is called for this reason a primary test.

Taste in water

The glasses containing S were detected in all instances, from the first drops in the mouth. In consequence, the number of 30 tasters was considered as enough: this was not a sensory test with a hundred varying results. Similarly, in regulatory toxicology for acute tests, a few mice (4 to 10) are enough to determine a lethal dose or the first irritations of eyes or skin if the results are homogeneous.

Sensations were collected and are described below in order of frequency. These were light or stronger according to the testers, the dose of 30 or 90 mg/L, and less or more persistent. Sensations described were drying, aspirin or lit matches, acidic, slight anaesthesia in mouth, tongue irritation, stripping of tongue, bitter, astringent, mineral, and rotten egg (Table 2). For those who clearly detected S by nose and described the taste at 30 mg/L, the level of S was decreased. One of us (JCH) was able to recognize the glass containing 10 mg/L.

Taste in wine

S was then spiked at 90 mg/L in an organic natural wine where no sulfites were used (<10 mg/L), reaching then the maximal level authorized for biodynamic wines (marketed under the Demeter label). This was performed around 1h before tasting, allowing some combinations of free sulfites in the bottle. The spiked glass was easily differentiated by 28/30 testers from the non-spiked one as control. Of course, the free S could be enhanced in comparison to combined S in this total level of 90 mg/L, because S was not added in the barrel but in the glass, and this will facilitate taste detection of sulfites, even if the chemical combination of SO_3^{2-} can be quite rapid and reversible. Then the effect of sulfites for the testers breaks the complexity of nose and mouth sensations, according to specialists, especially for red wine.

The description was easier for white wine: drying; acidic; decrease or shutdown of the nose and tongue capacities; covers, breaks, flattens, closes, blocks, crushes, hides, or cuts aromas in the wine (according to different tasters); causes the fruits aromas to be lost; disrupts freshness; astringent; less perfume; lacks life (Table 2). Thus, the remarks of the 28 volunteers in this primary test, without exchanging comments with each other during the open writing, are very homogeneous. Burning or irritant effects for sulfites in wines - "reduction of taste, bitter, decrease the sensation of fruits and floral intensity" - in completely different tests, were reported by others [26], citing Leroux in 2014. This on one side validates our primary test approach, and on the other side appears contradictory with the fact that some producers claim that sulfites are there to protect aromas. There is an antioxidant effect on aromas, but this argument could include a misunderstanding. This is because if sulfites block the acetic acid formation or the fermentation, which may to some extent produce aromas, they also obviously block the taste buds, depending on the level and the sensitivity of the person, leading to less appreciation of the wine. It should be underlined that high levels of sulfites could kill the natural yeasts before adding the artificial aromatic yeasts, and then in this process the characterized aromas from lab yeasts would be enhanced.

In confirmation of this work, when eco-labelled and regular wines were tasted, without respecting similar varieties, soils, and years, in large studies using 74,148 bottles from 3,842 Californian vineyards, the organic wines were also significantly preferred [27]. This was confirmed in our results with French wines in a blinded manner, but this time using similar varieties, soils, and years, for two neighbouring vineyards, one sprayed with synthetic pesticides, the other not [3]. The tastes of organic wines in our experiment were judged to be less artificial and to last longer; and we recall that artificial aromatic yeasts are not used in natural wines. Natural yeasts could however be more difficult to control, with a greater year-specific variation.

Body discomforts linked to S detection

In this work, we provide evidence that S concentrations not only influence the taste of wine, but also cause discomfort in the head or rest of the body, from 10-30 mg/L in the water or wine. This could also explain, at least in part, why natural wines with less S may be preferred to wines with synthetic pesticides or containing more S or Cu. In non-organic wines, formulants made with petroleum residues and heavy metals may be added to S, Cu, and pesticides, and some will be absorbed through the leaves [28], and then will be driven to the grapes and may bioaccumulate in wines, also distorting the taste.

One of us (JD) has discovered the taste of pesticides, which was then characterized in detail by 71 specialists in 195 tests [3]. He compares in more detail here the sensations of sulfites in water or wines at levels over 10 mg/L to those of pesticides at levels over

10 µg/L. For both classes of substances these levels are realistic, as they are found in chemically treated wines. For sulfites, there is a general heat in the head, heating of the temples, odour, and taste of a lit match, also resembling aspirin taste, drying acidity at the top and the back of the palate; and in comparison, to pesticides: more volatile sensations with the sulfur, which reaches the postnasal area, also more irritation to the respiratory tract in water. In wine a similar comparison can be made with pesticides: sulfites spoil the taste of wine to a greater degree, by diminishing and blocking perfumes.

By contrast, the pesticides at levels from 10 µg/L in water or wines provoke generally in sensitive or already initiated persons: dryness of the tongue on the sides at the rear, and tingling at the tip of the tongue, burning tongue and blockade of the taste buds, a sticky sensation less volatile than sulfur, and a slight headache which is more concentrated on the forehead at the beginning.

Summary of SO ₂ smell or taste	In water from 30 mg/L and 90 mg/L	In wines when spiked at 90 mg/L
Detected by tasters	30/30 from 30 mg/L 1 professional from 10 mg/L	28/30
Description of tastes by frequency	Drying Like aspirin Smell of lit matches Acidic Slight anaesthesia in mouth Tongue irritation Stripping of tongue Bitter Astringent Mineral Rotten egg	Drying Acidic Decrease or shut down of the nose and tongue capacities Covers, breaks, flattens, closes, blocks, crushes, hides, or cuts aromas (according to different tasters) Causes the fruits aromas to be lost Disrupt freshness Astringent Less perfume Lacks life

Table 2: Summary of sulfites’ smell or taste.

Legend: Tasters were asked to describe the nose or mouth detection in primary and preliminary testing at low levels found in organic wines, which were then spiked with S. The results were clear. Further sensory tests could be organized.

Toxicity of sulfites and comparison with pesticides

In this work, we have not directly considered sulfates since these are not generally measured in wines. These could have laxative effects at high doses in water (over 600 mg/L) [29], and include body discomforts depending on the metals or salts they bind to. Also depending on the sensitivity of the person, and essentially in an acute manner, sulfites may induce headaches, skin redness, hives, stomach pain, diarrhea, allergic intolerance like swellings, and asthma [30]. We found burning of the oesophagus in addition. The vapours of free sulfites can be detected from 1 mg/L, and thus S may enhance alcohol toxicity [31]. This may be amplified by the high levels of sulfites present

and added to some foods for the industrial preservation process.

By contrast at very low levels, a very few mg/L, the balance of sulfur regulates homeostasis. The toxicity of sulfites in mammals and thus humans by overdose is due, among other possible mechanisms, to the inhibition of crucial enzymes and reactions of the immune system, as well as direct toxic effects on the gut microbiota [32], as with pesticides such as Roundup [15]. Any enzymatic or hormonal reaction exhibits a bell curve in the presence of increasing doses of its ligand or substrate. Moreover, sulfites can saturate the detoxification system of any living organism.

If we consider the ADI for humans of 0.7 mg/kg bw/day [33],

an 80 kg person can ingest 56 mg of soluble sulfites (interconvertible forms) per day. For a minimum of <10 (for instance 5) mg/L in a natural wine, 11 L must be consumed per day to reach the acute toxicity level for sulfites; this is without considering the toxicity of alcohol. For a wine containing 150 mg/L (Table 1), 0.37 L a day reaches the toxic levels for sulfites for sensitive persons; but for a wine at the maximum permitted level (450 mg/L), only 0.12 L a day is sufficient to reach a toxic level. Regarding chronic toxicity, we must consider the fact that petroleum residues and heavy metals are included in toxic formulations such as herbicides [6,5] or fungicides [18]. This theoretical difference is interesting, even if the toxicity of alcohol were considered, as well as beneficial effects of some natural wine compounds.

Comparisons of the effects of sulfites with those of Cu and pesticides

We will compare the acute and chronic toxicities of sulfites to that of four other relevant substances: Cu, sprayed on vines, to the two major characteristic fungicides detected in wines in our previous study, boscalid and fenhexamid [3]; and the glyphosate-based herbicide Roundup [34], which is the most frequently used herbicide in vineyards and the most used (and most controversial) herbicide in the world, including on genetically modified organisms (GMO plants). Glyphosate was detected in our study in wines.

Cu

In humans, some medical prescriptions are made for 1 mg Cu/day, particularly to stimulate the defence and immune system. Hepatic and kidney failures may occur when an excess of Cu is consumed, since the liver and kidney are the detoxification organs. If we consider the ADI for humans of 0.15 mg/kg bw/day for Cu, an 80 kg person can ingest 12 mg of soluble Cu (interconvertible forms) per day. For an average of 0.15 mg/L in organic wine, 80 L must be consumed per day to reach the acute toxicity of Cu, which is unrealistic, but for a non-organic wine only 8 L on average would have to be consumed (if its content is 10 times more in general). For chronic toxicity, we must consider the fact that Cu is included in toxic formulations of fungicides [18]. This theoretical difference is interesting, even if for these quantities, alcohol is far more toxic (Table 3). In sum, sulfites are more toxic in wines than Cu due to their higher quantities; if they were present in equal quantities the toxicity would be comparable or more.

Fungicides

The most expensive wine in our previous study (400 euros, 75 cL) was non-organic, produced in 2009, and very well known: it was marked 17/20 by wine critics Bettane and Desseauve, 18/20 by Gault and Millau, 97/100 by Wine Spectator, and was given the supreme ranking in the Parker guide: 100/100, as previously studied [3]. It contained 146 ppb of boscalid, recognizable by taste. It is obvious that these classifications of wines do not consider

the pesticide content. This also represents 146 µg/L (or ppb), two times less than the average found in wines treated with synthetic petroleum-derived pesticides, and 1460 times the level authorized in drinking water for instance in France (0.1 µg/L). The ADI for boscalid is 0.04 mg/kg bw/day, according to the EU Pesticides Database. It is 3.75 times more acutely toxic than Cu, but this is still an admissible level for regulatory authorities. For long-term toxicity, we must consider the petroleum and heavy metal residues in the formulation. In boscalid formulations, there are more than 300 ppb of arsenic, around 300 of cobalt, 1000 of chromium, and 600 pb of nickel [6]. This can be sprayed on vineyards and can very easily enter the human cell membranes; it will increase the toxicity and endocrine disruption by at least a factor of 1000, especially in hepatic cells [6,35,36]. This type of toxicity will be then reached for 0.04 µg/kg bw/day. For a human of 80 kg this will correspond to 3.2 µg of this product consumed per day. This quantity will be reached by consuming only 22 ml of this wine (Table 3). Boscalid has a chlorine or burning taste which is detectable by trained professionals at this level [3].

The calculation is comparable for fenhexamid. In petroleum-derived formulations, in addition to petroleum derivatives shown to be toxic to hepatic cells, as we previously demonstrated [35], heavy metals are present at around 500 ppb for arsenic, 800 for chromium and the same for nickel [6]. Consequently, the toxicity level of this mixture reduces the ADI at least by 1000 times for fenhexamid, i.e., 0.37 µg/kg bw/day (Table 3). This equals just 59 mL of wine, corresponding to approximately half a small glass. Fenhexamid has a surprisingly sweet chemical and artificial strawberry taste, in addition to the drying and papilla blockade effects common to all synthetic pesticides [3]. For these two fungicides sprayed in pesticide formulations and found in wines, the chronic (not acute at these levels) toxicity appears to be greater than that of alcohol consumed at reasonable doses. The chronic toxicity arising from exposure to applications in vineyards has long been demonstrated, for instance regarding bladder cancer [37]. Cu cannot be regarded as equal in any way to synthetic pesticides, for all these reasons, nor S, except if their formulants contain carcinogens.

Herbicides

The last example is a glyphosate-based herbicide; it has been invoked because it is the most used pesticide in the world and the main herbicide used in vineyards. Some Roundup formulations contain glyphosate (around 40% in general) but also petroleum derivatives [38] and arsenic up to 500 ppb, in common with other heavy metals depending on the formulation [6]. Its chronic toxicity has been documented in vivo from 0.1 ppb (or µg/L) [39] and it has been shown to be up to 100,000 times more toxic than glyphosate alone, depending on the type of human cells and the time of exposure [40,41]. Its effects below regulatory limits have been reviewed [42]. Lethal liver and kidney deficiencies caused by ultra-low doses (0.1 ppb) of Roundup over a long-term exposure

period have been confirmed by multi-omics techniques [42,43]. In general, 10-11 ppb (110 times more than the level previously shown to be chronically toxic for the whole formulation) of glyphosate were discovered in several wines [3]. The calculation in Table 3

was performed on this basis. Still, glyphosate in formulation is 12-33 times less toxic than fungicides in formulation. Cu or S are not toxic on the same scale at all.

Compounds	In organic wines µg/ L	In non-organic wines µg /L	Long-term toxicity in formulations µg/kg/day	Quantity of the compound needed (for a 80 kg body) to reach toxicity, in ml of wine hypothetically consumed/day
All sulfites (E220-E228)	<10,000 up to		5,000	80,000**
	150,000	150,000 up to		2,667**
		450,000		889**
Copper (Cu average)	150		150	80,000**
		1500	0.15	8,000**
Boscalid	0	146	0.04	22
Fenhexamid	0	500	0.37	59
Glyphosate	0	11	0.1	727**

Table 3: Quantities of sulfites and copper in wines with their comparative long-term toxicities with some petroleum-derived pesticides.

Legend: The quantities of sulfites and Cu in wines are taken from the text above (Table 1); levels for the other synthetic pesticides, boscalid, fenhexamid, and glyphosate, are the actual maximum levels found in our previous study in some current wines [3]. The long-term toxicity of formulations considers the presence of petroleum-derived residues and heavy metals, together with the combined measured toxicity in human hepatic cells (minimum ADI 1000 times less, see text). Cu and sulfites are preferably not present in petroleum-derived formulations for organic use, but are present in the formulations of petroleum-based synthetic pesticides. The quantities per day needed to reach toxicity for a human body of 80 kg are thus compound-specific. Obviously, the toxicity threshold for alcohol will be reached before that for Cu, and then for sulfites**. For sulfites, the ADI is calculated at 0.7 mg/kg; chronic toxicity could be around 5 mg/kg/day [44]. This is not the case for boscalid and fenhexamid present in non-organic wines, nor is it the case overall if we consider the combined effects of all pesticides present besides these examples, which may be around 5-6 in number or more [3]. Fungicides are more chronically toxic in general..

Finally, as for Cu, natural sulfites cannot be considered as equal to petroleum-derived synthetic pesticides present in non-organic wines from any point of view, especially for long-term toxicity. Sulfites are far less chronically toxic, but with more acute reactions, at the levels reached in wines. We were able to differentiate Cu and S toxicity levels in organic and non-organic treatments due to the presence of petroleum derivatives in chemicals used for non-organic treatments. Moreover, the environmental impact of high sulfur treatments as well as Cu or chemical pesticides in non-organic vineyards and wines appears to affect biodiversity, drinkability, taste, and health.

In conclusion

On the strength of all the results of this trilogy on the taste of chemical pesticides in wines [3] and of Cu [2], and the present results on S, we propose a new concept: taste as a toxicity detector for the chemicals added in wine (Figure 1)

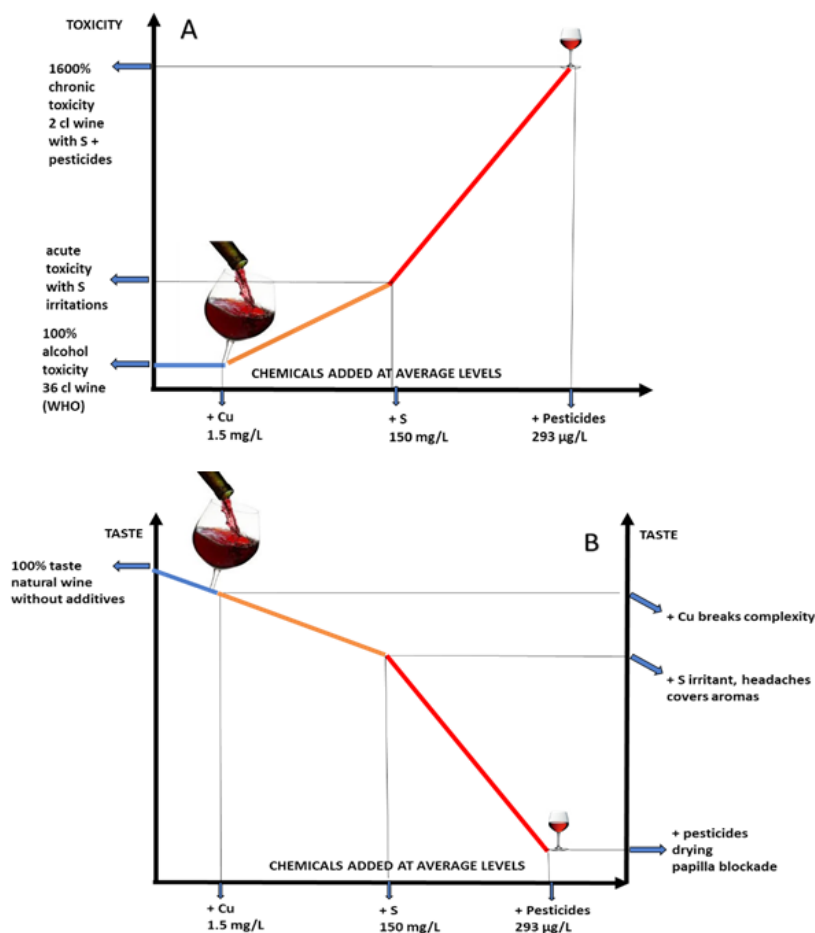


Figure 1: A new concept: Taste as a toxicity detector. (A) Toxicity and (B) taste in wine according to the chemicals added.

Legend: First, Cu is often added in vineyards of organic or non-organic wines, either directly or in the formulations of pesticides for non-organic ones. It is not toxic at 1.5 mg/L but breaks the complexity of the taste, according to our experiments. Then S in different forms may be added to vines or wines at fungicidal levels: at 150 mg/L, or even far below for sensitive persons, an acute immune reaction or headaches are possible; in addition, these compounds are detectable by irritation in the mouth or nose and cover aromas. Synthetic pesticides, at 293 µg/L on average in chemically treated wines, are drying, and provoke a papilla blockade. They have serious chronic toxicity in addition to the toxicity of alcohol. This pesticide level (µg/L range) is a thousand times less than average levels for Cu and S (mg/L). S is by far the most present additive in quantity. Taste could serve as a toxicity detector in wine. The large and small glasses are symbols of what could be chronically absorbed taking the chemicals toxicities into account.

As for some animals due to their more highly developed olfaction, but here in wines, experienced tasters could detect the products applied in vineyards or added to wines. These detected products include the synthetic ones made from petroleum, as well as Cu or S when these two are in excess over physiological levels. In all cases, these additives break the complexity of wines, bringing a drying or slightly irritant or acidic taste that one could have previously attributed to other characteristics of the wines. The skill to detect these products can be acquired in a few days if the products are first given in water at the same level. The chronic toxicity of chemical pesticides is important in comparison to that of alcohol, which is a new finding of this study. Taste as a toxicity detector for humans and wine is also a new original concept.

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