Alternative Pesticide Residue Detection Methods

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Pesticides have been extensively used in agriculture to protect crops and enhance their yields, indicating the need to monitor for their toxic residues in foodstuff. To achieve that, chromatographic methods coupled to mass spectrometry is the common analytical approach, combining low limits of detection, wide linear ranges, and high accuracy. However, these methods are also quite expensive, time-consuming, and require highly skilled personnel, indicating the need to seek for alternatives providing simple, low-cost, rapid, and on-site results. In this study, we critically review the available screening methods for pesticide residues on the basis of optical detection during the period 2016–2020. Optical biosensors are commonly miniaturized analytical platforms introducing the point-of-care (POC) era in the field. Various optical detection principles have been utilized, namely, colorimetry, fluorescence (FL), surface plasmon resonance (SPR), and surface enhanced Raman spectroscopy (SERS). Nanomaterials can significantly enhance optical detection performance and handheld platforms, for example, handheld SERS devices can revolutionize testing. All in all, despite being in an early stage facing several challenges, i.e., long sample preparation protocols, such POC diagnostics pave a new road into the food safety field in which analysis cost will be reduced and a more intensive testing will be achieved.

pesticide residues optical detection screening methods point-of-care diagnostics biosensors

1. Introduction

The ever-increasing demand for food production unfortunately still requires a widespread use of pesticides. According to the European Commission (EC), pesticides “prevent, destroy, or control a harmful organism (“pest”) or disease, or protect plants or plant products during production, storage, and transport”. Pesticides can be clustered on the basis of the target pest (Table 1), for example, compounds combating insects are called insecticides[1]. Another useful classification was proposed by the World Health Organization (WHO) and is based on hazard expressed as lethal dose (LD) in rat specimen (Table 1)[2]. Alternatively, pesticides can be classified focusing on how they enter into the target pest, for instance, systemic pesticides are absorbed by tissues (leaves, roots, etc.) (Table 1)[3].

Table 1. Summary of various classification systems for pesticides.

a. Based on Target Pest

Pesticide Type	Pest
Algicide	Algae
Avicide	Birds
Bactericide	Bacteria
Fungicide	Fungi
Herbicide	Weeds
Insecticide	Insects
Miticide	Mites
Molluscicide	Snails
Nematicide	Nematodes
Piscicide	Fish
Rodenticide	Rodents

b. Based on Toxicity

Type	Toxicity Level	LD_{50} for Rats (mg kg^{-1} Body Weight)	
		Oral	Dermal
Ia	extremely hazardous	<5	<50
Ib	highly hazardous	5 to 50	50–200
II	moderately hazardous	50–2000	200–2000
U	unlikely to present acute hazard	>5000	

c. Based on the Way of Entry into a Pest

Ways of Entry	Details
Systemic	Absorption by tissues such as leaves, stems, and roots
Non-systemic	Physical contact between the pesticides and the target organism
Stomach poisoning	Pesticide digestion
Fumigants	Target organism killing through vapors
Repellents	Inhibit the ability of pests to

acute or chronic. The various pesticide classes can potentially affect their targets in different ways, including humans. In the case of organochlorine (OC) pesticides, which were extensively used during the 20th century, nervous system stimulation has been noticed. For example, lindane inhibits the calcium ion influx and Ca- and Mg-ATPase, causing

a. Based on Target Pest	
Pesticide Type	**Pest**
[4]	[5]

se effects the case

hate (OP) insecticides, their toxicity is related to the inhibition of acetylcholinesterase (AChE), a vital enzyme in the neural system of insects or mammals, including humans. Normally, AChE hydrolyzes the neurotransmitter acetylcholine into choline and acetic acid, an essential reaction that enables the cholinergic neuron to return to its resting state after activation. However, AChE activity is reduced in the presence of CMs and OPs due to carbamylation or phosphorylation of the serine hydroxyl group in the enzyme active cite[6], respectively. This results in acetylcholine accumulation, which can lead to serious health problems, including respiratory and myocardial malfunctions[7]. Another example of pesticide toxicity it is the class of pyrethroid pesticides. Pyrethroids cause neuronal hyperexcitation, resulting in repetitive synaptic firing and persistent depolarization. Their molecular targets are similar in mammals and insects, and include voltage-gated sodium, chloride, and calcium channels; nicotinic acetylcholine receptors; and intercellular gap junctions[8]. Therefore, it is obvious that the presence of pesticide residues in food has to be strictly regulated and monitored to protect consumer health.

2. Optical Screening Methods

2.1. Biochemical Assays

Biochemical assays using antibodies or enzymes as recognition elements have been traditionally used in a microplate format, which provides high-throughput, simplicity, good sensitivity, and ease of operation. The enzyme-linked immunosorbent assay (ELISA) is a striking example of such bioassays. ELISA is based on the specific interaction between an enzyme-labelled analyte-specific antibody and its antigen. Owing to the labelling of the antibody with an enzyme, upon the addition of a substrate, a measurable color change is initiated. A recent review by Wu et al.[36] is recommended for a deeper understanding of the ELISA mechanism, various types (Figure 3a), as well as recent advances. ELISAs have been developed for the screening of various pesticide residues in food matrices, for example, OPs[37][38], CMs[39], neonicotinoids[40], or fungicides[41]. In terms of cholinesterase microplate assays, cholinesterases have been employed as recognition elements (both AChE[42] and butyrylcholinesterase, BChE[43]) to screen for CM and OP. Considering that, in vitro, cholinesterases hydrolase colorless substrates to colored products, the presence of CMs and OPs can be correlated to a color decrease similarly to competitive ELISAs. A great variety of substrates, resulting in different colored products (Figure 3b), have been used including acetylthiocholine and butyrylthiocholine halides for AChE and BChE, respectively; indoxyl acetate; α-naphthyl acetate; 2,6-dichloroindophenol acetate; and others[44]. Importantly, reduced sample and reagent consumption (typically less than 100 μL) as well as low LODs at the μg kg^{-1} level[42][45][46], depending on the matrix, were achieved by cholinesterase microplate assays. However, biochemical assays are still applicable in laboratories as they require certain apparatus and well-trained operators (commonly such assays contain multiple steps).

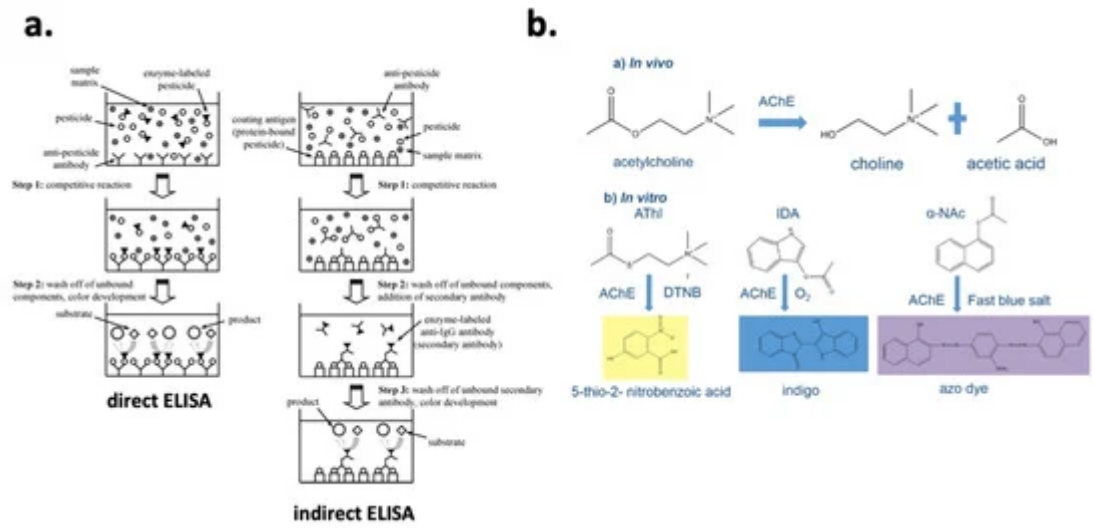

Figure 3. (**a**) Multistep direct and indirect ELISA protocols for pesticide residues screening. Reprinted with permission from[47]. Copyright 2013 American Chemical Society. (**b**) In vivo and in vitro acetylcholinesterase hydrolytic activity producing, in vitro, various colored products depending the catalyzed substrate. Reprinted from [42] under CC BY 4.0.

2.2. Biosensors

Biosensors are analytical platforms that convert a biological response into a quantifiable and processable signal. Besides the described attractive characteristics of biochemical assays, biosensors can be miniaturized and automated, indicating their potential for on-site testing. On the basis of the biorecognition element, we can distinguish three main groups of biosensors, i.e., immunosensors[20], cholinesterase[21] and lipase sensors[48] (enzymatic recognition), and aptasensors[49][50]. It is of note that aptamers emerge as an alternative to counter problems related to antibodies, such as the challenge to trigger an immune response for small molecules or their higher temperature stability, a problem related to biomolecules[51]. Biomolecules can be negatively affected by organic solvents (e.g., denaturation problems resulting in decreased activity), certain pH values (commonly neutral pH values are the optimum for antibodies and enzymes), or hydrostatic and osmotic pressure. Nevertheless, increased stability can be accomplished by immobilizing biomolecules on surfaces as in the case of biosensors[52]. For instance, the immobilization of AChE on cellulose strips resulted in retained enzyme activity over a two-month period[34]. Other less used recognition elements include, but are not limited to, molecularly imprinted polymers (MIPs, synthetic molecules), cells, and DNA probes. In the following paragraphs, further discussion on various biosensors is provided on the basis of the detection principle used, and tables summarizing interesting publications in the field during the period 2016–2020 are presented.

2.2.1. Colorimetric Biosensors

Colorimetry is probably the simplest approach as a biorecognition event is related to a color development. This fact significantly increases colorimetric platforms potential for on-site analysis as colorimetric signals can be monitored even by the naked eye or they can be easily coupled to a smartphone readout (see Section 4.3). On the downside,

colorimetric signals are vulnerable to minor lighting variations while most of the food extracts are colored, which negatively effects method detectability. Of importance is the ever-increased use of analytical platforms commonly based on colorimetric responses such as membrane-based assays (lateral flow (LF) or paper-based assays), microfluidic chips, or lab-on-a-chip (LOC) devices (Table 2). LF assays are membrane tests consisting of various polymeric zones on which various substances can be accommodated and react with an analyte [53]. Liquid samples or extracts containing an analyte move through this lateral device due to capillary forces. Two different formats of LF assays can be distinguished, namely, competitive and sandwich formats. Competitive assays are used for low molecular weight analytes, i.e., pesticide residues, and a positive result is related to the absence of a test line due to the blocking of antibody binding sites to protein conjugates by the analyte. In terms of big molecules, for example, allergens, the sandwich format is used, and the analyte is immobilized between two complementary antibodies. Besides research studies using LF assays for pesticide residue screening[54][55], LF assays are one of the few cases that have reached the commercialization stage[19]. Regarding microfluidics, this is a relatively new field that was established in 2006 following the publication of G.M Whitesides in the prestigious *Nature* journal [56]. In this way, microfluidics are related to the manipulation of fluids in channels with dimensions of tens of micrometers. Fluidic behavior under these micro-level confined regions significantly differs from fluidic behavior in the macroscale. In this context, essential parameters such as viscosity, density, and pressure need to be strictly controlled to reach optimum microfluidic performances[57]. Although no strict criteria have been proposed to define microfluidic systems, the length and internal size of the channels is considered of critical importance. Microfluidic channels are combined to LOC devices to develop fully portable and autonomous analytical platforms. In fact, LOC systems are able to mimic different apparatus such as reactors and pumps to carry out injection, filtration, dilution, and detection in a reduced portion, eliminating handling errors and enhancing robustness while retaining the analysis cost low[58]. Regarding the application of colorimetric microfluidic and LOC platforms, paper-based microfluidics can combat problems related to intolerance towards organic solvents that are used to extract pesticide residues by spontaneous evaporation on the paper-platform before loading an enzyme solution for pesticide recognition[32]. However, overall, such platforms are still in an early stage, with the majority of the studies focusing on proof-of-concept applications[59]. Unfortunately, the majority of colorimetric analytical platforms utilize traditional sample preparation protocols, highlighting the need to automate and simplify sample pretreatment to increase the applicability of such methods in the field.

Table 2. Selected studies on pesticide residue screening using colorimetric biosensors.

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
Methyl-paraoxon and chlorpyrifos-oxon	cabbage and dried mussel	paper-based device coated with nanoceria using an enzyme inhibition assay with	methanol vortex extraction, centrifugation, PSA clean-up, centrifugation, evaporation	0.040 mg kg^{-1}	0.010 mg kg^{-1}	[60]

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
		AChE and ChOX				
Carbofuran and carbofuran-3-hydroxy	water	LF immunoassay	none	7 µg L^{-1} (carbofuran) and 10 µg L^{-1} (carbofuran-3-hydroxy)	0.1 µg L^{-1}	[54]
Malathion	apple	aptasensor employing gold nanoparticles	methanol extraction, filtered and evaporation	5.2 pM (or 0.001 µg kg^{-1})	0.02 mg kg^{-1}	[61]
Paraoxon	vegetable irrigation water	enzyme cascade and iodine starch color reaction	filtration	10 µg L^{-1}	n.a.	[62]
Ethoprophos	tap water	gold nanoparticle aggregation combined to adenosine triphosphate	no	4 µM (or 0.96 mg L^{-1})	0.1 µg L^{-1}	[63]
Paraoxon	rice and cabbage	AChE assay coupled to carbon dots	acetonitrile ultrasonic extraction, centrifugation, filtration through sodium sulfate and evaporation	0.005 mg kg^{-1}	0.01 mg kg^{-1} (cabbage) and 0.02 kg^{-1} (rice)	[64]
Acetamiprid	spinach	aptamer with DNA probe	ethanol ultrasonic extraction, centrifugation, filtration, and 20-times dilution	0.1 nM (or 0.022 µg kg^{-1})	0.6 mg kg^{-1}	[65]

Biosensors with fluorescent detection combine the selectivity provided by the recognition part to the sensitivity of fluorescence (FL), as it is a zero-background method and only specific compounds (based on their structure) are able to fluoresce. Fluorescent biosensors (Table 3) are based on the principle that the interaction of a fluorescent probe (chemical or physical) with an analyte leads to either fluorescence enhancement or quenching[66], which is also known as analyte-induced “on–off” fluorescent behavior[67]. A great variety of fluorescent probes have been used, namely, fluorescent dyes, nanocomposite materials, rare earth elements, or semiconductors[68]. The great advancements in nanomaterial field have further improved fluorescent detection, as they have countered, at a certain extent, bottlenecks related to dyes, e.g., high photobleaching. Quantum dots, which are semiconductor

crystalline nanomaterials with unique optical properties due to quantum confinement effects, are an example of nanocomposite probes that have enhanced fluorescent detection for pesticide residue screening[66]. This was recently demonstrated for the detection of four OP pesticides, namely, paraoxon, dichlorvos, malathion, and triazophos, using CdTe quantum dots as the fluorescent probe coupled to an AChE-choline oxidase enzyme system[69]. In this case, when AChE was active (resulting in choline production), H_2O_2 was produced by choline oxidase, which in turn "turned off" the FL of the CdTe quantum dots. However, in the presence of an OP, the FL induced by CdTe quantum dots was retained and a correlation between OP concentration and FL signal was feasible. Impressively, a LOD of 0.5 ng mL^{-1} was achieved in water, tomato juice, and apple juice, while the fluorescent biosensor could be regenerated using pyridine oximate. In another study, an "off−on−off" strategy was applied by using AChE as the recognition element and lanthanide-doped upconversion nanoparticles (UCNPs) with Cu^{+2} as the fluorescent probe[70]. This analytical platform achieved an LOD of 0.005 mg kg^{-1} for diazinon detection in apple and tea powder and, importantly, the results were cross-confirmed to GC–MS. It should be kept in mind that although it is necessary to benchmark the results attained using screening methods, this practice is commonly omitted in the published literature as it is comprehensively discussed in our previous study[9]. In conclusion, FL biosensors can attain sensitive results, which is extremely important in the food safety field. However, their principles and analytical configuration are commonly more complicated than colorimetric platforms that may influence their applicability within the point-of-care (POC) testing concept.

Table 3. Selected studies on pesticide residue screening using fluorescent biosensors.

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
Acetamiprid	tea	aptasensor	methylene chloride extraction, filtration, and evaporation	0.002 mg kg^{-1}	0.05 mg kg^{-1}	[71]
Dichlorvos	cabbage and fruit juice	carbon dots–Cu(II) system	PBS extraction	0.84 ng mL^{-1}	n.a.	[72]
Paraoxon	water	BChE assay	no	0.25 µg L^{-1}	0.1 µg L^{-1}	[73]
Imidacloprid	Chinese leek, sweet potato, and potato	LF immunoassay	PBS extraction and supernatant dilution with PBS	0.5 ng g^{-1}	0.5 mg kg^{-1}	[74]
Diazinon	cucumber and apple	aptasensor	Dilution with water, water-heated bath, centrifugation	0.13 nM (0.039 µg kg^{-1})	0.01 mg kg^{-1}	[75]
Aldicarb	ginger	AChE-based assay	QuEChERS	100 µg kg^{-1}	0.05 mg	[76]

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
					kg^{-1}	
Eight rodenticides	wheat	LF immunoassay combined with quantum dots	acetonitrile ultrasonic extraction, centrifugation, filtration, and filtrate 10-times dilution in PBS	1–100 µg kg^{-1} depending the analyte [78]	0.01 mg kg^{-1}	[77]

on a thin sensing, which is a great advantage as labeling procedures are omitted, resulting in reduced cost and prevention against false positive signals related to labeling. Moreover, SPR is especially useful to calculate association (or dissociation) kinetics and affinity constants or bounded analyte content in the case of immunorecognition[79]. Interestingly, only a few enzyme-based biosensors have employed SPR detection[80]. Detecting pesticide residues in trace amounts is a challenging task as it is difficult to attain a measurable change in the refractive index due to their low molecular mass. To face this problem, sensor surface modification using nanoparticles is commonly applied since nanomaterials can enhance SPR signals due to their high refractive index. Furthermore, nanomaterials are also preferred because of their facile synthesis, high surface to volume ratio, and high biocompatibility and photostability[81]. The nanomaterials commonly utilized in such analytical platforms include, but are not limited to, metal nanoparticles, i.e., Au or Ag; carbon nanoparticles; and quantum dots. Besides signal enhancement using nanomaterials, SPR phase-measurement instead of amplitude (which is the case in conventional SPR systems) is an alternative approach that is based on the topological nature of the phase of a system. Considering that our study focuses on the analytical developments and applications in pesticide residue analysis, no further discussion on the physics behind phase sensitive SPR measurement is provided, and two studies[82][83] are recommended for a deeper understanding of the phenomenon. In any case, SPR biosensors have found several applications in pesticide residue analysis based mainly on immunorecognition (Table 4). It can be noticed that the problem of laborious sample preparation when analyzing solid food matrices was also the case for SPR-based biosensors. In addition, the low molecular weight of pesticides set a great challenge in terms of detectability and compliance to regulatory limits for SPR-based analytical platforms. More effort is definitely needed to further improve such platforms, considering the miniaturization potential (handheld SPR systems or coupling to smartphones)[84] that can be highly beneficial for the field.

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Aldicarb	ginger	AChE-based assay	QuEChERS	100 μg kg^{-1}	0.05 mg kg^{-1}	[76]
Eight rodenticides	wheat	LF immunoassay combined with quantum dots	acetonitrile ultrasonic extraction, centrifugation, filtration, and filtrate 10-times dilution in PBS	1–100 μg kg^{-1} depending the analyte	0.01 mg kg^{-1}	[77]

lue to its coupling to biorecognition events [20], SERS is in principle a spectroscopic method based on light scattering, specifically to inelastic collisions occurring between a sample and incident photons emitted by a monochromatic light source, such as a laser beam[91]. Combining biorecognition events to SERS can significantly enhance the analytical performance of such methods, but also it increases method complexity and cost. For example, a multiplexed immunochromatographic assay for the simultaneous detection of cypermethrin and esfenvalerate (pyrethroid pesticides) achieved impressive results in milk matrix[92]. Specifically, the acquired LOD was at the parts per trillion level (LOD = 0.005 ng mL^{-1}), a performance that would not be possible without using SERS-based detection considering that immunochromatographic assays mostly provide qualitative results. Regarding direct SERS screening, this is feasible as molecules provide specific Raman spectra due to their unique structure, which is also called “Raman fingerprint”. However, Raman signals are not strong enough, with only 1 out of 10 million of the scattered photons experiencing Raman scattering when incident light interacts with an analyte[93]. Therefore, it is necessary to enhance such signals by employing nanocomposite substrates resulting in electromagnetic and chemical enhancement[94]. Two different types of substrates can be distinguished, namely, colloidal and solid substrates. Although the synthesis of colloidal substrates such as Ag or Au nanoparticles is quite facile and cost-effective, poor reproducibility of signals remains a problem[95]. In terms of solid substrates, these provide more robust signals and counter the risk of nanoparticle aggregation, which is a problem for colloidal substrates. Solid substrates can be immobilized on various surfaces for example paper[96] or hydrogels[97]. In fact, paper-based SERS substrates can further increase the method potential to be applied on-site as such substrates can be used to swab the surface of a sample and then screen using a portable Raman spectrometer. In this way, paper SERS substrate coated with a monolayer of core-shell nanospheres was recently developed and was successfully used for the detection of thiram in orange juice[98]. This simple and non-destructive method achieved a LOD of 0.25 μM

or 0.060 mg L^{-1} by using 4-methylthiobenzoic acid (4-MBA) as the internal standard (IS) to attain quantitative results. Similarly, in another study, 4-MBA was accommodated in Au@Ag nanocubes and exploited as the IS[99]. Moreover, it was noticed that water molecules can be used as a IS since their Raman scattering signal is quite stable [100]. Alternatively, the use of anisotropic nanoparticles, e.g., nanocubes, nanorods, and nanostars, positively affected SERS quantification capabilities by achieving more stable signals[101]. Nevertheless, SERS can mostly detect analytes on the surface of food, which does not correspond to the whole amount of a pesticide in a food matrix. Pesticide residues depending their polarity can be found in the non-polar peel or the polar-aquatic inner part of a fruit. Moreover, LODs have been mostly expressed using the "ng cm^{-2}" unit[102] because pesticide residues were measured on a surface. Nevertheless, such a concentration expression is not in line to the regulated MRL units (mg kg^{-1}). There were also cases in which QuEChERS extraction[103] or other long sample preparation protocols (Table 5) were used prior to SERS screening, an approach that comes in contrast to the non-destructive and direct measurements than can be acquired using SERS. In conclusion, SERS can highly improve the current status of pesticide residue screening at the point of need due to the discussed merits and the ever-decreased price of such portable platforms (approximately EUR 35,000 to 50,000 at the moment).

Table 5. Selected studies on pesticide residue screening using SERS methods.

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
Methyl parathion	apple	portable SERS	none	0.011 μg cm^{-2}	0.010 mg kg^{-1}	[102]
Prometryn and simetryn	wheat and rice	MIP-SERS	QuEChERS	20 μg·kg^{-1}	0.010 mg kg^{-1}	[103]
Thiram	lemon	SERS with nanowire Si paper as a substrate	none	72 ng cm^{-2}	0.100 mg kg^{-1}	[104]
Difenoconazole	pak choi	portable SERS	acetonitrile extraction, centrifugation, dSPE clean-up, evaporation, and reconstitution to ethyl acetate	0.41 mg kg^{-1}	2.0 mg kg^{-1}	[105]
Paraquat	apple and grape juice	portable SERS	none	100 nM (0.025 mg L^{-1})	n.a.	[106]
Dimethoate	olive leaves	portable SERS	none	5 × 10^{-7} M	n.a.	[107]

Analyte	Matrix	Analytical Platform	Sample Preparation	LOD	EU MRL	Reference
Edifenphos	rice	SERS	two times acetone extraction, centrifugation; six times pre-concentration	0.1 mg kg^{-1}	0.01 mg kg^{-1}	[108]
Thiram	apple, pear, and grape	"drop-wipe-test" using portable SERS	none	5 ng cm^{-2}	5 mg kg^{-1} (apple and pear) and 0.1 mg kg^{-1} (grape)	[109]

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