Towards comprehensive identification of pesticide degradation products following thermal processing below and above 120 °C: A review

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Abstract

Characterising pesticide residues from a qualitative and quantitative point of view is key to both risk assessment in the framework of pesticide approval and risk management. In the European Union (EU), these concerns are addressed during the evaluation of active substances at the European level prior to marketing authorisation. In the framework of this review, we will focus on one specific item of the residue section, namely the effect of process (industrial or domestic transformation of the raw commodities) on the nature of the residue in food. A limited number of hydrolysis conditions defined by three parameters (temperature, pH and time) are set to be "representative of the most widely used industrial and domestic food processing technologies". These hydrolysis conditions, however, do not cover processes at temperatures higher than 120 °C, such as cooking with a conventional oven or in a pan, frying or using a microwave oven.

Keywords: evaluation of active substances, thermal processes, pesticides, analytical methods and strategies

Introduction

Plant Protection Products (PPPs) help to keep crops healthy and prevent damage or destruction by disease and infestation. A PPP contains at least one active substance that can be either organic or inorganic, natural or synthetic. Phytopharmaceutical treatment with a PPP may leave residues of the active substance in the form of the parent compound and/or metabolites (breakdown products) in food and/or feed commodities, with possible consumer and/or livestock exposure via ingestion. Consumer exposure to pesticide residues may be of concern depending on the toxicity of the residue

compounds, the amount of residue found in food commodities, and the diet of the considered population *(K. H. Kim et al., 2017)*. Characterising pesticide residues from a qualitative and quantitative point of view is key to both risk assessment in the framework of pesticide approval and risk management. The number of available publications is a clear illustration of increasing concern around consumer exposure to pesticide residues in food: a rapid search in the Scopus database using the terms “pesticide” AND “residue” AND “food” in titles yielded around 10 000 documents since 1951, with a considerable increase in recent decades. Publications have increased by a factor of seven since the year 2000.

In the European Union (EU), these concerns are addressed during the evaluation or re-evaluation of both active substances at the European level and PPPs at the zonal level (administrative zones) prior to marketing authorisation or re-authorisation. To this end, the residue section of the evaluation focuses on the residue definition in food, the amount of residues to be expected in food, and lastly on consumer exposure. In the framework of this review, we will focus on one specific item of this residue section, namely the effect of process (industrial or domestic transformation of the raw commodities) on the nature of the residue in food. Currently, if use of a PPP leads to significant residue levels in a raw agricultural commodity (RAC), a study investigating the degradation pathway of the residue during the process, called a hydrolysis study, is required. These types of studies are carried out on buffer solutions fortified with radiolabelled active substance that undergo different hydrolysis processes *(OCDE, 2007c)*. The use of radiolabelling studies makes it possible to monitor every potential breakdown product that may form during the process. A limited number of hydrolysis conditions defined by three parameters (temperature, pH and time) are set to be “*representative of the most widely used industrial and domestic food processing technologies”*. These hydrolysis conditions, however, do not cover processes at temperatures higher than 120 °C, such as cooking with a conventional oven or in a pan, frying or using a microwave oven. Since further degradation is expected with increasing temperatures, one can assume that certain metabolites may form above 120 °C. As an example, the active substance pyraclostrobin breaks down into several transformation products during deodorisation of olive oil (240 °C), while pyraclostrobin parent compound remains stable at temperatures ranging from 90 to 120 °C *(Germany, 2018)*.

The purpose of the present article is to review academic research literature (referred to as public literature) as well as literature submitted by pesticide manufacturers in the framework of pesticide evaluation. Comparing the two sets of literature aims at discussing the need for future studies to

investigate the pesticide degradation pathway during high temperature processes (> 120 °C) in the regulatory framework of pesticide evaluation as well as conducting hydrolysis studies with high-temperature hydrolysis conditions (> 120 °C). First, we briefly outline the principles followed by European regulations when evaluating the effect of the process on the pesticide residue. We then review the academic research literature following the population (P), intervention (I) or exposure (E), comparator (C) and outcome (O) (PICO/PECO) strategy. Finally, we suggest analytical tools that could be used as alternatives to the radiolabelled studies currently required in the pesticide evaluation in order to conduct such studies.

1. European pesticide regulation requirements

In the EU, a PPP cannot be placed on the market without prior approval of the active substance at the European level, according to Regulation (EC) No 1107/2009. The active substance is evaluated by a Rapporteur Member State (RMS) in the form of a monograph based on data essentially provided by the active substance manufacturer. The evaluation of this monograph is then peer-reviewed by another member state under the supervision of the European Food Safety Authority (EFSA). A peer-review is then published by EFSA with a conclusion on the overall evaluation. Regulation (EC) No 283/2013 reports the data requirements for active substance evaluations and multiple Organisation for economic co-operation and development (OECD) technical guidelines further describe the evaluation criteria. Based on these guidelines, the role of the RMS is to assess the scientific validity of the studies provided by the active substance manufacturer and to decide whether or not sufficient studies are available to characterise the risk.

The residue section of the monograph focuses on consumer risk, i.e., the risk related to ingestion of food contaminated with PPP residues. This section presents the following successive steps: (i) setting of a common (or multiple) residue definition in various food commodities (plant/animal origin, raw/processed commodity), (ii) quantification of residues in raw commodities of plant and animal origin, (iii) study of the effects of industrial and household processes on the degradation of residues in processed commodities, and (iv) estimation of consumer exposure to the residue via food ingestion. In order to set a residue definition, “metabolism studies” are carried out with radiolabelled compound (mostly ^{14}C labelled) in order to follow its degradation pathway. These studies are conducted: (i) on plants treated with radiolabelled active substance according to OECD Guideline 501

(OCDE, 2007a), and (ii) on animals fed with the radiolabelled compound according to OECD Guideline 503 *(OCDE, 2007b)*. For each study, degradation products that account for more than 10% of the initial active substance concentration are chemically identified or at least characterised. Each identified metabolite above that threshold is screened for toxic/genotoxic properties to discuss its inclusion in the residue definition, as well as to define chronic and acute toxicity concentrations.

When residues in a raw commodity of plant origin that can be processed occur at levels > 0.01 mg/kg, the effect of the process on the nature of the residue needs to be investigated. For this purpose, OECD Guideline 507 requires that a buffer solution spiked with radiolabelled compound must undergo three hydrolysis treatments in order to highlight potential further degradation of the residue compounds due to the hydrolysis conditions. Hydrolysis tests are performed in a sealed enclosure to avoid any loss of radiolabelled compound. The buffer solution is considered sufficiently representative of any food matrix since, according to the guideline, "the substrate itself is not likely to have a major effect upon the processing procedure (apart from governing the pH level in some situations)" *(OECD, 2007c)*. The three hydrolysis conditions defined by a combination of three parameters (temperature, processing time and pH) are set to be representative of the majority of processes as follow: (i) pasteurisation (90 °C, 30', pH 4), (ii) baking, brewing, boiling (100 °C, 60', pH 5), and (iii) sterilisation (120 °C, 20', pH 6). Given that most enzymes are inactivated above 90 °C, hydrolysis is expected to be the main degradation mechanism. As hydrolysis is strongly dependent on temperature, the higher the temperature, the more advanced the hydrolysis expected. The criteria to chemically characterise and include or exclude a metabolite in the residue definition for a processed commodity are the same as those stated above for metabolism studies.

Even though the hydrolysis study is a model study, its preset hydrolysis conditions between 90 and 120 °C do not cover typical temperatures of, for example, conventional oven cooking processes reaching up to 240 °C. OECD Guideline 507 addresses this potential issue with the example of the deodorisation process during oil refining and states that "the necessity for these studies should be discussed on a case-by-case basis with regulatory authorities". As a consequence, such studies are rarely provided by manufacturers and rarely required by member state regulatory authorities in the framework of their evaluations.

Because most of the studies provided in the framework of active substance approval lack hydrolysis conditions above 120 °C, we reviewed the public literature targeting studies investigating the effect of process on PPP residues.

2. Review methodology

This review was conducted in accordance with the principles laid down in the EFSA Guidance (EFSA, 2010), which follows the PICO/PECO approach of systematic review (Morgan *et al.,* 2018). The search was performed through the PubMed and Scopus databases for articles published between the year 2000 and up to 20 July 2021. The following search question was formulated: what is the effect of cooking/microwave/high temperature on degradation of pesticides? The key elements of the question (inclusion criteria) were defined as follows (according to the PICO/PECO format, with * as truncation operator):

- population (P): chemical, pesticide, contaminant, substance, molecul*, "plant protection product", food (with food as a required word, i.e., preceded by the Boolean operator AND)
- intervention (I) or exposure (E): "high temperature", cook*, fry*, microwav*, therm* process*, hydroly*, thermal-oxidat*, thermooxidat*, structural-alteration, heat-process*, defrost*, thawing, "thermal degradation", "thermal decomposition", "thermal analys"*, "heat treatment"
- comparator (C): *not applicable*
- outcome (O): by-product*, degradation, "transformation product"*, neoform*, stability, behavio*, metabolit*, residue

Initial queries done on the basic search fields "title-abstract-keywords" retrieved an excessive number of publications (e.g., 12 409 publications on Scopus). The subsequent searches were therefore limited to pesticide or "plant protection product" without using broad meaning words (chemical, contaminant, substance, and molecul*). In total, 642 publications were retrieved from Scopus and 1 155 from PubMed. Various filters were applied as summarised in Table 1, resulting in a total of 73 publications after removal of duplicates (the list of these publications is available as supplementary material (SM)). Of these, we selected the final 31 publications reported in Table 3 (highlighted in the Table S1). This selection was made by retaining papers reporting thermodegradation studies of pesticides and applying an experimental workflow. Most of these papers investigated the removal of active substance from the matrix while cooking it (with in most cases, a processing factor (PF) determination), while a few others investigated the degradation products formed during degradation of the substance of interest. Studies reporting only pesticide analytical methods (without thermal tests), only theoretical degradation (without an experimental

workflow), or only degradation pathways other than thermic (such as biodegradation, storage degradation and soil degradation) were not retained. Other data were also considered, mostly from peer-review regulatory documents. The studies were conducted according to OECD guidelines and met the OECD requirements of good laboratory practice. Some quoted publications were also included by snowballing from those initially selected in the review, such as Amvrazi *(2011)* or Senneca *et al. (2007)*.

3. Thermal degradation of active substances

Many previous reports, summarised in various literature reviews *(Bajwa et al., 2014; Kaushik et al., 2009; Li et al., 2021; Yigit et al., 2020)*, have investigated the impact of certain household and industrial processing steps such as storing, peeling, washing and cooking on several types of food products. A number of parameters can influence pesticide degradation, such as the presence of salts, pH, temperature and the time of the process *(Chauhan et al., 2014)*. Most of these studies aimed to compare the amount of pesticide present in the food product before and after the process, and to calculate percent degradation or processing factor (PF) *(Scholz et al., 2018)*. PFs are calculated for one specific process applied to one specific food product, according to the European guideline *(European Commission, 2007)*. It is important to follow the key parameters indicated in the guideline (pH, temperature, time, and sample weight) to harmonise published results, facilitate comparison between reported PFs and not induce any bias in data interpretation.

Numerous literature reviews have focused on the effects of cooking food on pesticide residues *(Kaushik et al., 2009; Li et al., 2021; Vagi et al., 2020; Yuan et al., 2021)*. They mainly examined two aspects: (i) residue dissipation (cooking considered to be one of the processing treatments) where processing factors are particularly well investigated, and (ii) overall analytical workflow, including extraction of the substances, clean-up and chemical analysis.

Table 3 shows research projects focusing on the impact of temperature on various pesticides. These publications are those from the literature review that mention thermodegradation studies on pesticides. The table shows the name of the studied compound (further information about these compounds is given in the Table S2), the matrix used in each study, the thermal process, the analytical workflow (such as the sample preparation method), and the analytical instrument. Moreover, it is mentioned whether the process was conducted below or above 120 °C, whether processing factors

were calculated, and whether degradation products were investigated. The table is therefore sorted as follows: (i) publications that both reported percent loss of the analysed compounds/processing factors and that investigated degradation products, (ii) publications that reported only percent loss of the analysed compounds/processing factors, and (iii) publications that reported neither percent loss nor degradation products. Even though some reported papers also investigated non-thermal degradation processes (e.g., storing, peeling and washing), only the parts of the studies involving the thermal process were retained. Of the 31 studies published after the year 2000, 27 reported results for experiments < 120 °C and 14 reported results for > 120 °C (ten had results for both < 120 °C and > 120 °C).

3.1. Temperatures below 120 °C

Considering the studies with data on processing below 120 °C *(Bai et al., 2021; Boulaid et al., 2005; Chavarri et al., 2005; Duhan et al., 2010; Göckener et al., 2020; Heshmati et al., 2019; Holden et al., 2001; Huan et al., 2015; Jankowska et al., 2019; S. W. Kim et al., 2015; Kontou et al., 2004b; Lin et al., 2005; Łozowicka & Jankowska, 2016; Medina et al., 2021; Mekonen et al., 2015; Pallavi et al., 2021; Raveendranath et al., 2014; Sakaliene et al., 2009; Shabeer et al., 2015; Shakoori et al., 2018; Shoeibi et al., 2011; Singh et al., 2017; Soliman, 2001; Walia et al., 2010; Watanabe et al., 2018; Yang et al., 2012; F. Zhao et al., 2020)*, even though 23 (85%) reported PFs or degradation percent calculations, only three (11%) *(Göckener et al., 2020; Kontou et al., 2004b; Lin et al., 2005)* investigated the degradation products of the pesticides of interest. Most of the cooking processes were sterilisation, boiling and pasteurisation, even though other processes such as drying, blanching or various cooking processes were also investigated. Most of the investigated thermal processes involved water.

3.2. Temperatures above 120 °C

Considering the studies with data on processing above 120 °C *(Chavarri et al., 2005; Göckener et al., 2020; Göckener et al., 2019; Heshmati et al., 2019; Huan et al., 2015; S. W. Kim et al., 2015; Martin et al., 2020; Mekonen et al., 2015; Planche et al., 2017; Soliman, 2001; Walia et al., 2010; Witczak, 2009; Yang et al., 2012; F. Zhao et al., 2020)*, only three (21%) *(Göckener et al., 2020; Göckener et al., 2019; Martin et al., 2020)* investigated the degradation products of the pesticides of interest.

Other studies also investigated the degradation pathway of various active substances for hydrolytic conditions above 120 °C. The most common processing techniques used were roasting, frying, grilling and microwaving, and all of them were found to impact concentrations of the parent compound in the analysed matrix. The following two mechanisms are expected to affect parent compound concentrations: (i) degradation of the parent compound into its metabolite(s), and (ii) loss of water and organic compounds via volatilisation. It is therefore difficult to predict formed degradation products or to reach general conclusions since this depends for instance on the studied compounds, matrix, process, time of processing, temperature, and water content. Most of these studies calculated PFs taking into account the potential weight loss induced by cooking (mostly water loss).

3.3. Comparison between regulatory documents and studies available in the public literature

As previously described, very few research studies have aimed to elucidate the degradation pathway of pesticide residues in samples under thermic treatment. However, comparing results obtained from the public literature to those from regulatory studies (monographs) would be beneficial to improve knowledge about degradation patterns and formation of degradation products.

Martin *et al. (2020)* investigated the degradation products of non-radiolabelled chlordecone (CLD) in naturally contaminated beef samples above 120 °C to detect two compounds identified as 5b-hydro-CLD and tentatively as mono-hydro-CLD. According to the same publication, these two compounds were already detected in raw liver samples, which does not confirm whether they are degradation products formed only in a biota matrix or both from biota and thermal degradation. The absence of studies investigating the impact of the process in the monograph for CLD does not enable us to confirm the formation of the two detected degradation products following thermal processing.

The two studies by Göckener et al. *(Göckener et al., 2020; Göckener et al., 2019)* investigated the degradation of chlorpropham and prochloraz above 120 °C using radiolabelled compounds. The first study investigated the degradation pathway of chlorpropham in a potato-derived commodity after boiling, frying and baking. Results showed that 3-chloroaniline was produced with increasing storage time, while the three high-temperature processes resulted only in the formation of a small amount of free-chloroaniline. Therefore, the formation of chloroaniline is essentially due to metabolism in the potato tuber during storage and not to degradation during the process. If one relies only on the hydrolysis study performed in the framework of the monograph *(Netherlands, 2016)*, the finding that

the amount of 3-chloroaniline in the buffer solution changes proportionally to temperature (90 °C: 0.36%, 100 °C: 0.6%, 120 °C: 1.3%) suggests that 3-chloroaniline is formed during the thermal process. Although the same degradation product was detected by Göckener *et al.* and in the monograph, assessments about its formation differ since the former highlights formation during the storage step, while the latter highlights formation during thermal processing. A different study was conducted on rapeseed oil spiked with radiolabelled prochloraz in a sealed vial. In the monograph *(Ireland, 2007*, *2011)*, prochloraz was shown to be stable under standard hydrolysis conditions, with a low level of certain degradation products containing 2,4,6-trichlorophenol moiety, such as C449589. However, in the Göckener *et al. (2019)* study, eleven degradation products were identified. Some of these compounds, such as 2,4,6-trichlorophenol itself or BTS 40348, contain this 2,4,6-trichlorophenol moiety. Some other detected degradation products were a combination of prochloraz and other compounds from the oil matrix (fatty acids).

Another study, by Kontou *et al. (2004a)*, investigated the degradation of maneb at different temperatures (50–90 °C) and pH, with the detection of one degradation product called ethylene thiourea (ETU). This product was already identified in the maneb monograph (not published on the EFSA website) as being formed via the effects of pH in maneb hydrolysis. Even though this study was conducted using a non-radiolabelled standard, it enabled the detection of the same degradation product as that from the monograph, obtained with a radiolabelled standard. It also demonstrated that formation of ETU from maneb is pH related.

The last study, already referenced in the cypermethrin monograph *(Belgium, 2017)*, by Lin *et al. (2005)*, investigated the degradation products that are formed when heating cypermethrin to 110 °C. The two detected products were 3-phenoxybenzaldehyde and dichlorovinyl-dimethylcyclopropane carboxylic acid.

The comparison between regulatory and public literature studies demonstrated that the same degradation products can be observed using both radiolabelled and non-radiolabelled compound, as shown for the degradation of maneb to ETU.

Because of the lack of research studies focused on new degradation products formed above 120 °C in buffer solution, clear conclusions cannot be drawn about the possible degradation products that are overlooked by the regulatory studies.

3.4. Overall workflow of degradation studies

To perform thermal analysis of active substances, it is important to be aware of the possible behaviours of the compound during each step of the process (thermal degradation, extraction, chemical analysis) and also the impact of data processing on the search for potential by-products and the fate of the pesticide residue. This would mitigate the risks of bias and misinterpretation when drawing conclusions.

3.4.1. Behaviour of the active substance during the thermal procedure

3.4.1.1. Degradation

The main mechanisms affecting the fate of pesticide residues during storage and food processing are described in various reviews *(Amvrazi, 2011; Yigit et al., 2020)*. The mechanisms involved in high temperature processes are the following:

- In water containing substrate (matrix), hydrolysis is the breaking of chemical bonds in the pesticide compound with the action of water as a nucleophile. At temperatures higher than 120 °C, it is assumed that the hydrolysis mechanism does not involve enzymes, as most of them are inactivated at these temperatures. This mechanism is mainly affected by pH and moisture content in the raw agricultural commodity (RAC), as well as by temperature during the process. Highly soluble pesticides are more susceptible to hydrolysis *(L. Zhao et al., 2018)*.
- In an oxygen-containing atmosphere, oxidation is loss of electrons of the pesticide compound, which results in the formation of oxide pesticide. This mechanism is mainly affected by UV radiation and temperature. Highly water-soluble pesticides are more susceptible to oxidation *(L. Zhao et al., 2018)*. Oxidation also depends on the complexity of the pesticide molecule *(Senneca et al., 2007)*.

3.4.1.2. Volatilisation

The study by Göckener *et al. (2020)* was conducted on potato treated at post-harvest with radiolabelled sprout inhibitor chlorpropham, then stored for up to six months and finally boiled. It showed considerable loss by volatilisation during storage and a significant amount of residue was transferred into boiling water. When the amount of parent compound residue decreases, it leads to uncertainty whether this decrease is due to degradation or volatilisation during heat treatment. It is therefore valuable to analyse in both ways to determine the ratio between degradation and volatilisation. Volatilisation is mainly affected by the equipment used, especially if the system is open

or closed, and air humidity. Pesticides with high vapour pressure are more susceptible to volatilisation *(L. Zhao et al., 2018)*.

3.4.1.3. Reactions with the matrix

The matrix may have significant effects, for example moisture content (*(Göckener et al., 2019)*) or pH *(Kontou et al., 2004a; Lin et al., 2005)*. Göckener *et al. (2019)* demonstrated with radiolabelled prochloraz that metabolites may also form through chemical reactions with matrix components in rapeseed oil. This mechanism, thought to involve triglycerides contained in the oil, is more important than the hydrolysis mechanism during heat treatment of rapeseed oil.

Cooking conditions vary considerably depending on the equipment used (oven, pan, grill, or microwave) and parameters such as temperature, time processing, degree of moisture loss, and whether the system is open or closed affect the different mechanisms mentioned above. This review shows how important it is to determine the possible degradation products for the substances of interest since their toxicity may be higher than that of the substance itself. This was observed in Kontou *et al. (2004a)* with the detection of ETU, a substance that is more toxic than maneb parent compound.

3.4.2. Extraction

There are various types of extraction procedures depending on the analysed matrix. The main extraction methods were developed to extract active substances from complex matrices, such as fruits and vegetables *(Gautam et al., 2017; Wang et al., 2018)*, livestock meat *(Hrynko et al., 2021; Saint-Hilaire et al., 2018)*, soil *(Chatterjee et al., 2013)* and water *(Ahmed, 2001; Farajzadeh et al., 2016)*. The main extraction method to analyse pesticides from food products is called QuEChERS (Quick, Easy, Cheap, Efficient, Rugged and Safe) *(González-Curbelo et al., 2015)* and is widely used to extract compounds within a wide polarity range. Moreover, even though other extraction methods are not labelled as QuEChERS, most of them also use solid liquid extraction with a mix of water and organic solvents, adding certain salts for demixion and thus improving extraction efficiency. Other methods can also be used such as accelerated solvent extraction (ASE) *(Planche et al., 2017)*, Envi-Carb extraction *(Holden et al., 2001)* or Soxhlet extraction *(Witczak, 2009)*. During the extraction steps, it is important to consider intermediary steps such as vortexing, sonication or centrifugation since they can affect the extraction yield and the degradation of the analysed pesticides, mainly during the sonication step *(Yuan et al., 2021)*. Following extraction, clean-up is carried out. This step

aims to remove the matrix with optimum recovery of the compounds of interest. The main clean-up methods for pesticide extraction are dispersive-solid phase extraction (d-SPE, used following QuEChERS extraction) and gel permeation chromatography (GPC) *(Chavarri et al., 2005; Planche et al., 2017; Watanabe et al., 2018)*, but other methods such as solid phase microextraction (SPME) *(Medina et al., 2021)* and matrix solid phase dispersion (MSPD) *(Jankowska et al., 2019; Łozowicka, Jankowska, et al., 2016)* are also used. Following the technical guideline on the evaluation of the extraction efficiency of residue analytical methods (SANTE/2017/10632 Rev.4) *(European Commission, 2022)* for pesticide residues in RACs (or processed commodities derived from RACs), sufficient extraction efficiency should have been demonstrated on the matrix group to which the RAC belongs beforehand, with a radiolabelled study.

Both radiolabelled and non-radiolabelled studies show that the pesticide residue is expected to vary depending on the type of matrix (RAC, homogenised RAC or buffer solution). This also demonstrates whether the residue formed is due to treatment before harvest, post-harvest or during fortification. The extraction in fortified buffer solution is expected to be easier than a fortified RAC due to the complexity of the matrix. Extraction in RAC treated post-harvest is expected to be easier than in RAC treated at the field level due to further metabolism in the plant. Appropriate extraction and clean-up methods need to be applied to prevent bias in the analysis and to provide accurate results.

3.4.3. Chemical analysis

The two main techniques used to separate compounds are liquid chromatography (LC) *(Chawla et al., 2016)* and gas chromatography (GC) *(Karasek et al., 2012)*, both of which are now mainly coupled with mass spectrometry (MS): low-resolution instruments (LR; triple quadrupole (QQQ) or quadrupole ion-trap (QTRAP)) or high-resolution instruments (HR; Q-Exactive or quadrupole time-of-flight (QToF)). Other detectors such as electron capture detectors (ECDs), nitrogen-phosphorous detectors (NPDs), ultraviolet (UV) and flame photometric detectors (FPDs) were also used based on reported publications, all with the aim of detecting compounds based on a specific target compound property or specific atoms (e.g., ECDs are suitable for the detection of halogenated compounds).

3.4.4. Data processing

Various data processing methods can be used depending on the availability of the standard as well as the list of targeted compounds. These methods are called target, suspect screening and non-target analysis and are further described in paragraph 4.

3.4.5. Comparison between radiolabelled and non-radiolabelled analysis

When the aim is to market a new active substance in Europe, thermodegradation studies to identify degradation products are carried out with isotopic labelled standards. These studies are considered to be more accurate since radiolabelled compounds are not present in the environment, and there is as a result no possibility of cross-contamination from the environment. Moreover, radioactivity analysis is specific to the analysed compound and thereby increases the sensitivity of detection of the active substance. Since only one signal should be observed for the analysed compound, the detection of any other signal when increasing the temperature would correspond to a degradation product. In studies using non-radiolabelled compounds, various signals do not correspond to the analysed molecule (such as matrix or noise), and it is therefore more difficult to extract the signal of the active substance as well as unknown degradation products from the overall dataset. Radiolabelled standards have several benefits, but they are expensive to buy or to synthesize and radioactive compounds can only be handled in containment conditions with specific accreditations. For these reasons, ^{14}C standards are rarely used when research laboratories intend to carry out degradation studies.

Overall analytical workflows between radiolabelled and non-radiolabelled studies are relatively similar, but certain differences are observed for each step. In radiolabelled studies carried out on RACs, total residual radioactivity (TRR) can be divided into two main fractions: (i) the extractable radiolabelled residue on which identification or characterisation of the residue is possible, and (ii) non-extractable residue when the pesticide has been extensively degraded into numerous low level metabolites or is associated with biomolecules via incorporation, physiochemical tight-binding or physical encapsulation. Comparing the TRR of the non-extractable fraction and that of the extractable fraction makes it possible to calculate the recovery of the extraction procedure. In non-radiolabelled studies, unextractable fraction and recovery percentages can be determined when the standard is commercialised, which is the case for active substances. This can be done by comparing the intensity of the extracted compound with that of the non-extracted standard and by calculating a recovery percentage. When the standard is not accessible (most often for detected degradation products), it is not possible to accurately calculate the unextractable fraction of these compounds. It is solely possible to approximate it using the standard of the active substance from which the degradation product formed.

The difference in the thermal process is that according to OECD Technical Guideline 507 (radiolabelled study for monographs), the buffer solution fortified with radiolabelled pesticide is contained in a sealed vessel. In this closed system design, loss of buffer solution through volatilisation is very limited. In non-radiolabelled studies, processes most often occur in open systems, and the evaporated fraction should therefore also be analysed.

Lastly, the analytical techniques are almost identical for both radiolabelled and non-radiolabelled studies, except that some radioactivity detectors should be coupled with the analytical instrument to analyse radioactive compounds. Various conclusions can be drawn depending on the observations for the analysed compounds, as summarized in Table 2. When conducting a non-radiolabelled study, data processing tools are important to extract information about the compound of interest and its degradation products while saving time.

4. Towards the identification of pesticide degradation products

In the literature, heating processes led to decreased concentrations of the overall pesticide content in food in most studies. However, as already mentioned, a small percentage of reported publications in this review aimed at elucidating the degradation pathway of the pesticide compound. To serve this purpose, several methods other than the radiolabelled method may be used and are listed below.

For each tool, it is assumed that degradation products and parent structures and formulae are closely related. If the formula and the structure of the detected compound differ too much from the studied pesticide, then it can be concluded that this compound is most likely an artefact and not a degradation product of the target compound *(García-Reyes et al., 2007)*.

4.1. Target analysis

Even though target analysis on the pesticide compound is not meant to elucidate the degradation pathway, one can predict the degradation kinetics of the active substance over time and temperature by monitoring pesticide concentrations at various temperatures and time parameters. A decrease would be indicative of either degradation or volatilisation of the pesticide, while a constant concentration would demonstrate stability of the analysed substance.

4.2. Suspect analysis

Target analysis is often limited to analysis of the pesticide residue in the residue definition(s) set in the thermodegradation framework of the monograph. Another way of exploring new transformation products during the process, without using radiolabelled compounds, could be to expand screening to the metabolites identified in sections other than the residue section of the monograph (e.g., absorption, distribution and excretion in mammals, and fate and behaviour in the environment). Building this type of suspect list could help to focus the analysis on metabolites that are likely to be formed given that they were already identified in other studies, even though not related to thermal processes.

4.3. Non-target analysis

Non-target analysis aims at detecting compounds of interest without having prior knowledge of their formula and structure. It aims at detecting specific mass values in the mass spectra that have a correlation with the studied active substance with a different retention time.

4.3.1. Fold change value

In thermal degradation studies, intensities of the studied compound and its degradation products are monitored over a range of temperatures. When increasing temperature, the intensity of the studied compound is expected to decrease in favour of an increase in the intensity of its degradation products. One mathematical tool, called fold change value, is used to calculate the ratio between a final value and an initial value for a studied feature. This tool can be used for intensity/height of chromatographic peaks to detect the features that have the highest fold change, which means the highest difference between low and high temperature processes. The features that have the highest fold change values should correspond either to the active substance (decreasing value over temperature) or to its degradation products (increasing value over temperature). Following this interpretation step, further identification of degradation products as well as the kinetics study for the formation of degradation products over time/temperature need to be performed. This tool is recommended for use in pure compound analysis to facilitate detection of the degradation products related to the analysed compound, without also considering the degradation of the matrix with the temperature.

4.3.2. Fragmentation of the active substance

Because of the structural similarity between the pesticide and its degradation products, similarities in their mass spectra can also be observed *(Thurman et al., 2005)*. This principle was also demonstrated by García-Reyes *et al. (2007)* who analysed the similarities between the ions detected in the mass spectra of two pesticides (amitraz and malathion) and those of their degradation products formed in food. This "fragmentation-degradation" relationship could also be applied to thermal processing to see whether the degradation products observed after heating the pesticide could be predicted from the fragmentation of the compound in the analytical instrument. Investigation can thereafter be performed following the detection of specific masses to confirm the structure of the detected compound and the possibility of it being a degradation product of the studied pesticide.

Similarities between the mass spectrum of the active substance and its degradation products can also be observed through molecular network analysis. This tool is used in various fields and enables researchers to create connections between variables that are correlated in peak height/peak area/concentration. In analytical chemistry, it is mainly used in metabolomics to study the effect of a variable (e.g., specific disease, drug intake, diet, or smoking) in the up/down regulation of specific biological functions in the body. This tool could also be used to observe the correlation between decreasing concentrations of the pesticide and increasing concentrations of its degradation products following thermal processing. This correlation will be highlighted in the network by forming one group containing both the analysed substance and its degradation products. This correlation would make degradation products easier to detect in further identification steps.

4.3.3. Isotopic pattern recognition

Another way to identify degradation products is to use isotopic pattern recognition. This can be conducted mainly to search for molecules with halogens (chlorine or bromine atoms) since both of them have two natural isomers with high abundance (^{35}Cl 75% / ^{37}Cl 25%, ^{79}Br 50% / ^{81}Br 50%). It is therefore possible to identify the number of chlorine or bromine atoms in a molecule by investigating the isotopic pattern of the molecule *(Wellington Laboratories, 2012)*. The degradation pathway of halogenated compounds may lead to degradation products also containing halogens, easily detectable due to their specific isotopic pattern, which also makes their identification easier.

The detection and identification of certain degradation products formed above 120 °C could therefore be carried out using one tool or a combination of several presented tools.

5. Conclusion

According to Regulation (EC) No 1107/2009 and more specifically article 6.5 Regulation (EU) No 283/2013, hydrolysis and processing studies are required to elucidate the degradation pathway of a pesticide, and to characterise and quantify breakdown products in processed foods (for further risk assessment). Conducted according to OECD Test Guidelines 507 and 508, these regulatory studies may not cover certain common household processes, such as microwave oven cooking, frying or conventional oven heating > 120 °C. By reviewing results published in academic literature and those from regulatory studies, this work aimed to assess to what extent certain processes may be overlooked.

It was determined that very few studies (six) are actually dedicated to the effects of cooking processes on the formation of degradation products from a pesticide, within both the academic and regulatory framework. Most of the academic studies published on the effects of cooking on pesticide residues are in fact limited to measuring the efficacy of food preparation processes through the processing factor (PF), while regulatory studies above 120 °C are very rare as they are not mandatory for placing a new active substance on the market in the European Union.

In most cases, heating the food commodities was found to contribute strongly to a decrease in the overall pesticide content. However, a few studies showed that new degradation products can be formed when increasing the temperature above 120 °C, mostly due to interactions between the active substance and the matrix. Because of the lack of public studies for temperatures above 120 °C in buffer solution, no conclusions could be drawn about potential gaps in regulatory pesticide risk assessment. The very low number of public studies makes it impossible to develop principles helping to define priorities for future studies or regulatory rules. It shows the crucial need to develop knowledge on the fate of pesticides in food subjected to various cooking processes, such as the microwave oven, conventional oven, pan or frying. The main processes and factors driving this fate still need to be clearly described, in connection with the chemical properties of the pesticide. The role of matrices, including buffer solution, should also be explored since they are practically unknown. This clarification is a prerequisite step to understand whether there is a need for the regulatory framework to be updated. In that way, as illustrated by the great diversity of studies reviewed here, two recent significant changes are likely to ease this need for scientific studies in the near future.

The first change is the remarkably fast development of analytical techniques (liquid chromatography, gas chromatography and high-resolution mass spectrometry) that allow for the characterisation and

quantification of breakdown products without using radiolabelled compounds. This would enable a significantly higher number of laboratories to carry out research on this topic. We also found that future studies should develop a comprehensive workflow (sample preparation, chemical analysis and data processing) for the identification of degradation products without using an isotopic labelled standard.

The second change concerns the broad opening of access to scientific data supporting decision-making for the registration of pesticide products, which appears to be a remarkable outcome of the application of EU transparency regulations this past decade *(European Food Safety Authority (EFSA), 2021)*. In this framework, the main key data are becoming more and more accessible, through the publication of EFSA scientific opinions, conclusions of the peer review of the pesticide risk assessment and assessment reports. Still largely underutilised, this corpus of information could be seen as a precious source of experimental data sets produced according to high standards of quality, which could be very useful in developing knowledge on the fate of pesticide residues in food under various cooking processes.

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