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Specific effects on kidneys relevant for performing a dietary cumulative risk assessment of pesticide residues

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

In consistency with the 'EFSA-SANTE Action Plan on Cumulative Risk Assessment for pesticides residues' EFSA initiated a retrospective cumulative risk assessment (CRA) of the effects of pesticide residues on the kidneys. EFSA identified the following specific effects on kidneys of relevance for cumulative risk assessment: glomerular injury, tubular injury, tubular crystals, papillary necrosis, interstitial nephritis, pelvis erosion/ulceration, pelvis calculi/crystals, renal preneoplastic and neoplastic lesions, and pelvis preneoplastic and neoplastic lesions. A list of histological indicators was defined and will be used to collect information on these specific effects, as reported in the assessment reports of the different active substances used as plant protections products. The criteria for inclusion of active substances/metabolites into cumulative assessment groups (CAGs) were also defined, together with the hazard characterisation methodology and the lines of evidence for assessing CAG-membership probabilities. Since no acute CAGs for kidney effects are considered appropriate, the cumulative dietary risk assessment will focus on chronic exposure only. The process of data extraction and establishment of the CAGs is beyond the scope of this report. This part of the CRA process was outsourced and will be the subject of a separate report.

KEYWORDS

cumulative assessment groups, cumulative risk assessment, kidneys, pesticide residues, specific effects

CONTENTS

SUMMARY

EFSA initiated a retrospective dietary cumulative risk assessment (CRA) of the effects of pesticide residues on the kidneys, according to the EFSA-SANTE Action Plan on Cumulative Risk Assessment for pesticides residues. EFSA and the dedicated working group (WG) focused on the identification of the specific effects of relevance for the CRA of pesticide residues in the kidneys. EFSA identified the following specific effects of relevance for cumulative risk assessment on the major anatomical and histological structures of the kidney (tubule, glomerulus, interstitium, papilla and pelvis): glomerular injury, tubular injury, tubular crystals, papillary necrosis, interstitial nephritis, pelvis erosion/ulceration, pelvis calculi/crystals, renal preneoplastic and neoplastic lesions, and pelvis preneoplastic and neoplastic lesions. Histopathology was considered as the most appropriate source of evidence and a list of primary indicators of each specific effect, reflecting the earliest toxic insult on the respective structure, was defined together with their synonyms. Secondary indicators corresponding to a subsequent progression of the initial insult or a regenerative/reparative response intervening at a later stage, were also defined and considered as indirect indicators to be used in the absence of primary indicators. Other renal ancillary endpoints routinely collected in toxicological studies (macroscopic observations, kidney weight, clinical pathology parameters) were considered by the WG as not adequate as primary or secondary indicators, being unspecific and lacking sensitivity in available toxicology regulatory studies.

The WG also defined the criteria for the inclusion of the ASs and their metabolites into cumulative assessment groups (CAGs) and for the hazard characterisation methodology. Finally, the WG established the lines of evidence (LoE) for each specific effect and their weight (high, medium or low) to assess the probability that an AS/metabolite is actually causing the specific effect and has been correctly included into a CAG (CAG-membership probability).

Since it is not possible to characterise the substances allocated to acute CAGs for kidney effects, the dietary cumulative risk assessment will focus on chronic exposure only.

Based upon the output of the WG and the feedback from the public consultation, the specific effects identified and their respective indicators, the criteria for CAG establishment and hazard characterisation methodology and the LoE for CAG-membership probabilities are finalised.

The subsequent step of this work, related to data extraction from regulatory assessment reports of selected prioritised pesticide active substances and metabolites and establishment of the CAGs, has been outsourced to the Benaki Phytopathological Institute (BPI, Greece) under the Framework Partnership Agreement (FPA) GP/EFSA/PREV/2021/01. The data will be collected in the template developed by the WG (annexed to this report). The outcome of this work and the composition of the CAGs (separate report) will be published in the EFSA website.

1 | INTRODUCTION

Cumulative risk assessment (CRA) has been defined as the analysis, characterisation and possible quantification of the combined risks to health or the environment from multiple agents or stressors (U.S. EPA, 2003). It differs from most assessments which consider the effects of one agent or stressors in isolation.

In Europe, Regulation (EC) No 396/2005 on maximum residue levels (MRLs) in or on food and feed of plant and animal origin and Regulation (EC) No 1107/2009 concerning the placing on the market of plant protection products[1] require thorough consideration of the cumulative and the potential synergistic effects of residues of plant protection products (PPPs).

To comply with these provisions, in 2007 EFSA and the Panel on PPPs and their residues (PPR Panel) started the development of the necessary methodologies to carry out CRA of pesticide residues. This methodological development included a tiered approach for the assessment of cumulative risks of pesticides residues (EFSA PPR Panel, 2008) for the consumers of the different commodities, a guidance on the use of probabilistic methodology for modelling dietary exposure to pesticide residues (EFSA PPR Panel, 2012) and a procedure to establish cumulative assessment groups (CAGs) of pesticides on the basis of their toxicological profile (EFSA PPR Panel, 2013). In 2020, EFSA issued the first two reports on retrospective CRA of pesticides that have acute effects on the nervous system (EFSA, 2020a) and chronic effects on the thyroid gland (EFSA, 2020b). These were followed in 2021 by a retrospective CRA regarding chronic acetyl cholinesterase inhibition (EFSA, 2021), in 2022 by a retrospective CRA regarding craniofacial alterations (EFSA, 2022) and more recently the 2024 update on the CRA on thyroid (EFSA, 2024).

In 2021, a long-term EFSA-SANTE Action Plan on CRA for pesticides residues (EFSA-SANTE, 2021) was developed. This plan provides that all toxicological effects of pesticides of relevance for CRA will have been identified by 2030 and CAGs in the respective organs and systems will have been established. This plan relies on two main pillars: prioritisation of ASs/metabolites and organs/systems and cooperation with Member States' competent authorities.

1.1 | Background and Terms of Reference as provided by EFSA

As part of the 2021 long-term EFSA-SANTE action plan, EFSA decided to perform a CRA for the cumulative effects of PPPs residues on the kidneys. This was motivated by the incidence and severity of kidney effects observed in toxicological studies (Technical University of Denmark, DTU, 2012). This was further confirmed by the outcome of the prioritisation exercise of organ/systems requiring CRA performed by EFSA (EFSA, 2024).

The entire CRA process is indicatively planned over 27 months. It consists of a sequence of steps which are described in Figure 1. These 14 steps cover the standard scheme of risk assessment (hazard assessment, exposure assessment and risk characterisation) with periods of consultations (public consultation, consultation with the PPR Panel and/or selected experts).

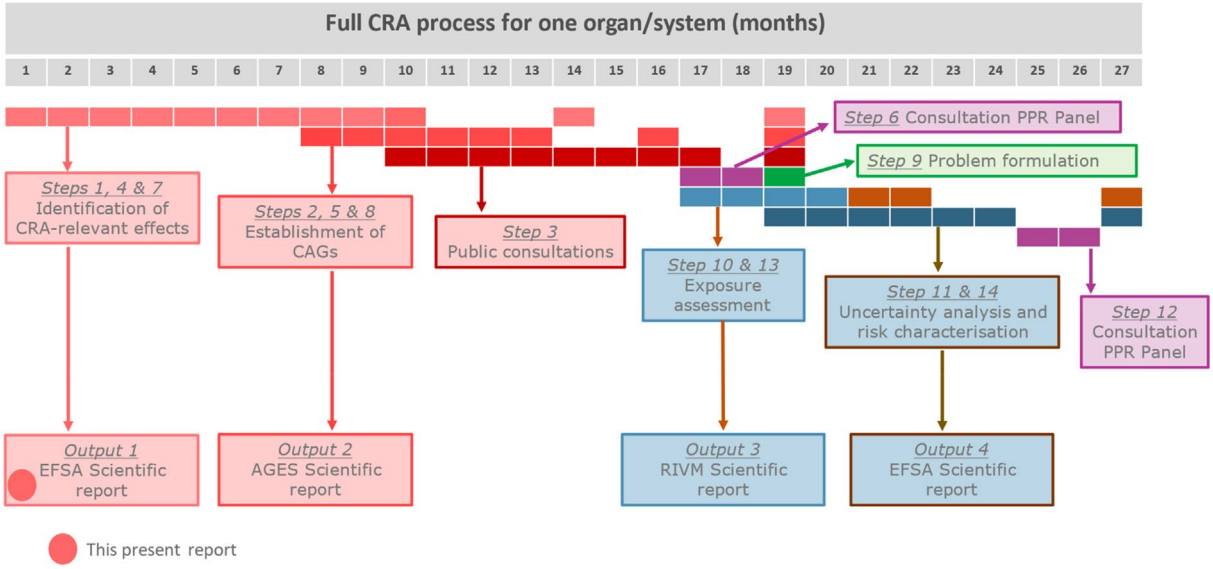

FIGURE 1 The 14 steps of the CRA of pesticide residues. The content of each step is described in detail in Appendix A.

The present CRA process concerning kidneys is performed via a cooperation with the Benaki Phytopathological Institute (BPI), producing the output 2 under the Framework Partnership Agreement (FPA) GP/EFSA/PREV/2021/01.

[1]Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OJ L 309, 24.11.2009, p. 1.

The whole process will be described in the following outputs:

Output 1 (the present report): EFSA scientific report on specific effects on kidneys relevant for performing a dietary CRA of pesticide residues (referred to as 'specific effects' in the rest of the present report). The preparation of this report is supported by the EFSA Working Group (WG) on CRA.

Output 2: Scientific report of BPI on the hazard characterisation (covering the data collection) and establishment of CAGs of pesticides for specific effects on kidneys.

Output 3: EFSA Scientific report on the cumulative dietary exposure assessment of pesticides that have effects on the kidneys using SAS® software.

Output 4: EFSA scientific report on the uncertainty analysis and the cumulative dietary risk characterisation of pesticides that have effects on the kidneys.

Public consultations are foreseen on draft outputs 1 and 2 and the submitted comments will be addressed in an annex of the respective scientific reports.

1.2 | Purpose and content of the present report

The present report (output 1) covers the identification of the specific effects of relevance for the CRA of the pesticide residues for effects on the kidney.

Furthermore, in preparation to the establishment of CAGs, it also defines:

- the indicators relevant to these specific effects;
- the conditions (hazard identification criteria) triggering the inclusion of pesticides (ASs and metabolites) into the respective CAGs;
- the principles for the hazard characterisation of the ASs and metabolites included in the CAGs for the respective specific effects;
- the lines of evidence (LoE) for the assessment of the probability that any pesticide included in a CAG is actually causing the respective specific effect (referred to as CAG-membership probability in the rest of this report);
- the template for the collection of data (see Annex A) for an efficient and consistent establishment of CAGs by BPI (see output 2 under 1.1).

2 | BACKGROUND INFORMATION FOR SETTING THE SPECIFIC EFFECTS ON KIDNEYS RELEVANT FOR PERFORMING A DIETARY CUMULATIVE RISK ASSESSMENT OF PESTICIDE RESIDUES

2.1 | Overview of kidney anatomy and physiology

In mammalian species the kidneys are paired, bean-shaped structures located in the retroperitoneal space. At gross examination, the kidneys are unilobar in all of the most common species used in toxicological studies, while they are multilobar in humans. Different sub gross regions can be identified, including capsule, cortex, medulla (further divided in outer and inner stripe of the outer medulla and inner medulla) and papilla extending into the renal pelvis (Figure 2). Both the renal cortex and the medulla contain different structures of the nephron, the functional unit of the kidney.

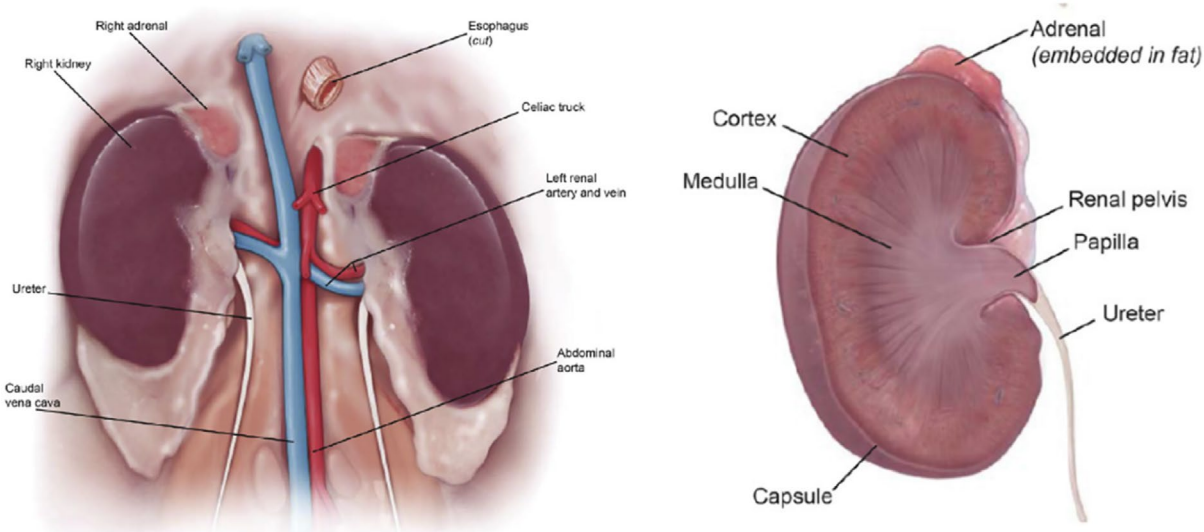

FIGURE 2 Kidneys in rodent (mouse). Regional anatomy (left) and gross structure (right) – (From Treuting et al., 2017).

The kidney performs many physiological processes and is largely responsible for maintaining the normal physiological balance of an individual (Sands & Verlander, 2005).

Kidneys' functions include:

- excretion of the waste products of metabolism (urine formation);
- control of the body's fluid and electrolytes (acid–base regulation, conservation of water and maintenance of normal extracellular potassium ion concentration through passive reabsorption in the proximal tubules and tubular secretion in the distal tubules under the influence of aldosterone);
- endocrine function through renin-angiotensin-aldosterone system, erythropoietin synthesis and metabolic activation of vitamin D;
- Metabolism of chemicals via cytochrome P-450 (CYP450).

Microscopically, the subgross anatomy of kidneys correspond to well-organised combinations of structures (glomeruli, tubular system, interstitium and vasculature) with specific functions (Figure 3).

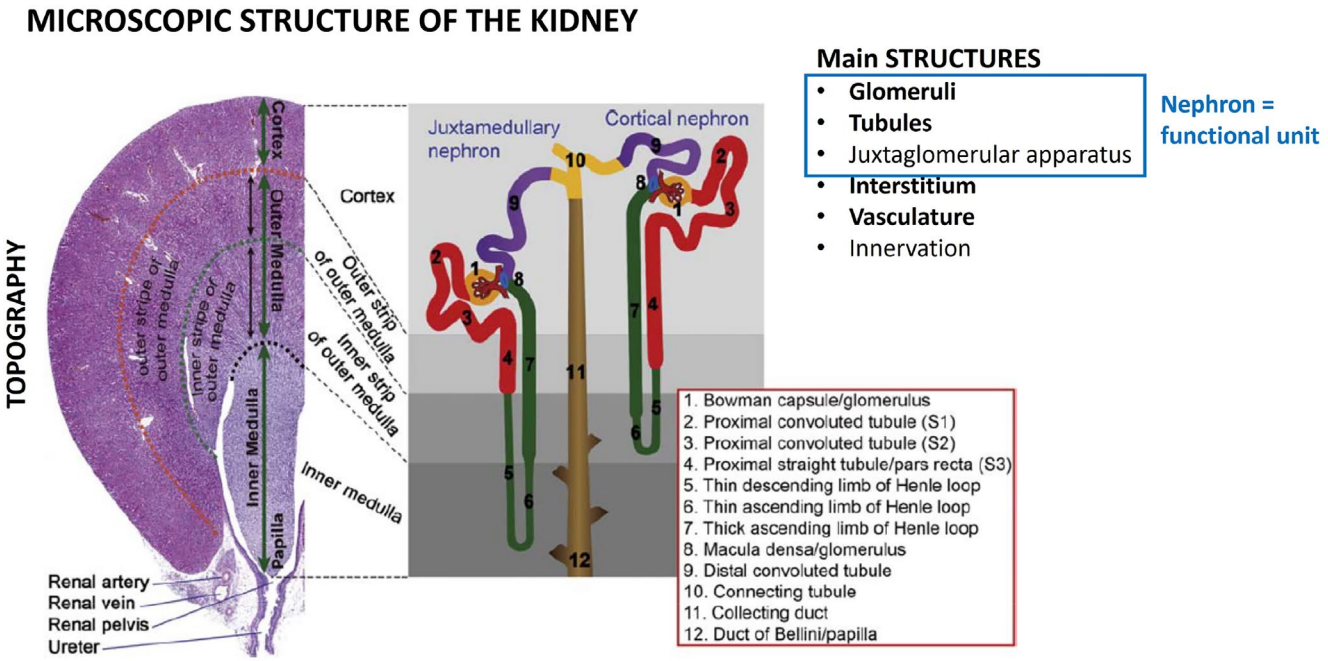

FIGURE 3 Microscopic structure of the kidney, rat (adapted from Khan et al., 2018).

The glomerulus is the key player in plasma ultrafiltration. The glomerular filtration barrier selectively filters molecules based on size (< 70 kDa), electrical charge (the more cationic, the more permeable) and capillary pressure. Thus, the normal glomerulus restricts many proteins and negatively charged molecules from being filtered into the uriniferous (Bowman's) space and proximal tubular lumen whereby allowing permeability to water, proteins (< 70 kDa) and small solutes. This ultrafiltrate of plasma (primary urine), which contains water, salts, ions, glucose and albumin, passes into the uriniferous space and then empties into the proximal convoluted tubule at the urinary pole to traverse through and be acted on by the tubular system (Khan et al., 2018).

The renal tubular system consists of a proximal tubule, loop of Henle, distal tubule and collecting duct (see Figure 3). Proximal tubules are lined by columnar epithelial cells that have a microvillous (brush) border. This arrangement greatly increases their absorptive surface, and their numerous intracellular mitochondria supply energy for the various secretory and absorptive functions. Proximal tubules make up the vast majority (approx 75%) of structural subunits of the cortex. Morphologically and functionally proximal tubules can be divided into a proximal convoluted portion (further subdivided in segments S1 and S2) and a pars recta (representing the segment S3 of the proximal tubule) in most mammalian species, including rat, rabbit, mouse and the rhesus monkey. In the S1 segment the cells are tall cuboidal with a long brush border and elongated vertical mitochondria located adjacent to deep infoldings of the basolateral plasma membrane, and they contain numerous apical endocytic vesicles and a prominent lysosomal compartment. In the S2 segment the cells are typically not as tall as those in the S1 segment, and they have a shorter brush border, fewer mitochondria, and fewer endocytic vesicles and lysosomal elements. In the S3 segment (straight portion) the cells have a long brush border, a less complicated basolateral plasma membrane than in the S1 and S2 segments, and contain numerous peroxisomes, but comparatively few mitochondria, endocytic vesicles and lysosomes (Verlander, 1998). Loop of Henle is divided into a thin descending limb and an ascending limb with a thin and a thick segment. Distal tubules (including thick ascending limb of the loop of

Henle, juxtaglomerular apparatus and distal convoluted tubule) and collecting tubules are lined by flattened/cuboidal epithelial cells that contribute to the concentration of urine by absorptive and secretory activities (Khan et al., 2018). Overall, the tubular functions include reabsorption, secretion via specific transport systems (organic anion transporters and cation transporters) and metabolism (e.g. via CYP450).

The renal interstitium is composed of fibroblasts, connective tissue and extracellular matrix that provide most of the interstitial tissue support. Cells in the interstitium, particularly in the medulla, are responsible for local production of prostaglandins. Blood vessels, nerves and lymphatic vessels are present in the renal interstitium.

The renal pelvis is a dilatation of the ureter, lined by urothelium, serving as a funnel for the urine towards lower urinary system structures (see Figure 3).

2.2 | Elements of chemically-induced renal toxicity

The kidney is a common target organ for toxicity for a variety of reasons (Khan et al., 2018), including:

- the high blood flow relative to the renal mass (25% of the cardiac output) and the consequent high exposure to systemically circulating toxic compounds;
- its role in the excretion of chemicals (through glomerular filtration and tubular secretion mechanisms);
- greater susceptibility of tubular cells to hypoxia;
- the ability to concentrate the ultrafiltrate resulting in high concentration of chemicals (and/or their metabolites) in the tubules;
- the capacity to alter the pH of tubular fluid, with transformation of solutes into reactive forms or into crystals;
- the local metabolism of chemicals via CYP450 with potential formation of active metabolites
- the capacity to dissociate protein-bound toxins.

Renal toxicity can be categorised according to different approaches, including the type of nephrotoxic agent (functional and chemical characteristics of the substance), mechanism of renal damage, lesion morphology and sub-topographical location of the injury (Khan et al., 2018).

Mechanistically, renal toxicity can be triggered by a variety of initiating events, often specifically targeting different structures of the organ (glomerulus, tubular system, interstitium, papilla, pelvis). The specific pattern of renal injury depends on the physico-chemical properties of the nephrotoxic agent, its toxicokinetic and toxicodynamic properties, clearance profile, metabolism, local tissue concentration and duration of exposure.

Glomerular injury can be caused either by direct toxic injury or immune-mediated injury. Direct injury can affect the three main types of cells within the glomerulus: the mesangial, endothelial or visceral epithelial cells (podocytes) and the extracellular matrix. Immune-mediated glomerulopathies can result from various pathways leading to the formation of a number of (auto)antibodies and immune-complexes deposition, which may, ultimately, affect the glomerulus integrity (Hogan et al., 2015; Markowitz et al., 2015).

Tubules are a common target of chemically-induced injury. **Proximal tubular cells** (and the surrounding interstitium) are exposed to nephrotoxic agents via apical contact with the ultrafiltrate present in the tubular lumen, or cellular uptake from tubular lumen or transport from the basolateral circulation with subsequent apical efflux into the urine. The proximal convoluted tubule (S1 and S2 segments) contains a very active endocytosis/lysosomal apparatus, thus representing the site of injury related to lysosomal overload, as well as protein-bound toxic moieties. The S3 segment represents the most susceptible site of injury via metabolic activation, transporter-associated accumulation and hypoxia/reperfusion (Khan & Alden, 2002). Tubular (cell) injury can be caused by several mechanisms (mitochondrial injury, oxidative injury, DNA damage, etc.) that may lead to various types of cellular alterations such as accumulation of material, degeneration and cell death (apoptosis, necrosis). Tubular injury following apical contact, uptake or basolateral transport is a dose-dependent mechanism. In the more **distal part of the tubules** chemicals and their metabolites may precipitate, crystallising or forming casts, and lead to degeneration and necrosis of the adjacent tubular epithelium, or to tubular obstruction (Perazella, 2018, 2019). Tubular crystalluria may also accompany the presence of calculi within the renal pelvis as the etiopathogenesis of the two lesions is similar.

Papillary injury can occur under various conditions that affect medullary blood flow or solute concentration, e.g. diabetic nephropathy, dehydration, amyloidosis and treatment with various chemicals and drugs. Non-steroidal anti-inflammatory drugs (NSAIDs) cause papillary injury in both animals and humans via inhibition of vasodilatory prostaglandins and redistribution of medullary blood flow resulting in ischemia (Khan et al., 2018). Concentration of toxicants in the distal medulla and local metabolic activity (e.g. prostaglandin hydroperoxidase activity) may also play roles in the renal papillary injury (Bach & Nguyen, 1998). The initial targets are the medullary interstitial cells, followed by degenerative changes in the medullary capillaries, loops of Henle and collecting ducts (Schnellman, 1998). Histologic features of papillary necrosis vary with the agent and dose, from mild interstitial oedema or mucoid change in the renal papillary matrix to frank necrosis and haemorrhage till the complete loss of the tip of the papilla. Inflammation can be quite variable from sparse to severe suppurative infiltrates (Frazier et al., 2012).

Interstitial injury can be directly induced by chemicals and their metabolites (primary injury), but often it develops secondarily to glomerular and tubular damage. Pathogenesis of primary interstitial injury involves an immune-mediated

mechanism leading mainly to T cell mediated type IV delayed hypersensitivity reaction, although immune complex deposition or direct activation of T cells by drugs can also occur. Chemicals can act as haptens/prohaptens or through molecular mimicry, damage renal proteins turning them into neo-antigens or induce the formation of immune complexes (Perazella, 2018, 2019). Immune-mediated interstitial injury (nephritis) is considered a dose-independent mechanism in humans (Perazella, 2019). In rats primary interstitial injury is poorly documented (Khan et al., 2018).

Pelvis injury can occur as a result of direct effects by xenobiotics on the renal urothelium or through the formation of crystals and uroliths. Xenobiotics or their metabolites in the urine may directly interact with renal urothelial cell DNA, protein, organelles or cell membranes, or may indirectly alter urine pH or composition. Interaction with DNA is the basis for formation of DNA adducts or mutations potentially leading to carcinogenicity. Interactions with proteins, organelles or cell membranes can lead to renal urothelial cell degeneration, necrosis or renal urothelial hyperplasia. Alterations in urine pH or composition may lead to mineral precipitates, crystals or calculi (Khan et al., 2018).

Spontaneous or induced **renal preneoplastic and neoplastic lesions** in rodent species are generally of epithelial origin involving tubule epithelium (atypical hyperplasia, adenoma, carcinoma), while nonepithelial tubule tumours are infrequent or rarely observed (including connective tissues neoplasms such as lipomas, liposarcomas, fibrosarcomas). Renal carcinogenic mechanisms include: direct DNA reactivity, tumour induction linked to oxidative stress, sustained regenerative cell proliferation either from direct or indirect cytotoxicity, and chemicals aggravating or accelerating the development of spontaneous chronic progressive nephropathy (CPN) (Hard, 1998).

As regards **proliferative changes in the pelvis**, these include urothelial hyperplasia, papillomas and carcinomas. Although normally the urothelium is a quiescent tissue, upon stimulation it can proliferate rapidly. Overall, the incidence of renal pelvic tumours is uncommon to rare in humans (and also in both the rat and mouse).

Nephrotoxic agents tend to target specific kidney structures acting via specific mechanisms, therefore the sub-topographical localisation of the lesion can support identifying the mechanism for xenobiotic-induced lesions. However, it is noted that the structural and functional unit of the kidney (i.e. the nephron) (Figure 3) shows the tendency to respond to injury as a unit rather than only at the sub-topographical site of injury. Thus, whatever the origin, all forms of chronic kidney damage ultimately involve all the structures of the organ, culminating in the so-called end-stage kidney disease, the final stage of chronic kidney disease.

The functional reserve of the kidney is large, and significant damage should occur before any evident functional impairment becomes clinically detectable, (e.g. by observing an increase in serum creatinine).

2.2.1 | Toxicological endpoints of renal toxicity in regulatory settings

The toxicological endpoints presented here are limited to the ones measured/observed in toxicological studies performed in laboratory animal species in agreement with data requirements set for pesticides.

2.2.1.1 | *Clinical pathology parameters*

Blood urea nitrogen (BUN) and creatinine (sCR) are the traditional biomarkers of general renal injury that remain the most common non-invasive markers of kidney toxicity measured in toxicological studies. However, they lack sensitivity since changes very often do not occur before, but are rather the consequence of a histopathological effect in the kidneys, and serum creatinine tends to remain relatively normal even in the presence of kidney damage, until approximately 50% of nephrons are lost. They also lack specificity, since they can change in the absence of renal damage as a consequence of prerenal factors such as severe dehydration or other lesions, e.g. as a consequence of loss of muscle mass leading to increase of sCR. Urinary biomarkers (urinary sediment analysis included) can be considered 'proxies' of renal damage that can increase the specificity for monitoring renal injury; however their analyses are not consistently carried out in toxicological studies. In contrast, the quantitative measurement of urinary biomarkers of proximal tubular function such as urinary albumin, protein or glucose can provide information on both functional status and localisation of injury which may precede changes in BUN and sCR. Nevertheless, also urine biomarkers often detect the manifestations of injury once it is severe; in addition, their variability is often quite large and urinary biomarker data are easily misinterpreted (Ramaiah et al., 2017; Siska et al., 2022).

More sensitive, specific and human-relevant biomarkers such as kidney injury molecule-1 (KIM-1), clusterin (CLU) and neutrophil gelatinase-associated lipocalin (NGAL) are induced in response to renal injury, and differ in the location, onset and duration of upregulation. These biomarkers have demonstrated the ability to detect early damage, localise injury and predict disease progression, severity and associated long-term mortality (Zhang & Parikh, 2019). However, these biomarkers are not routinely measured in urine in the toxicological studies since they are not introduced in the most commonly applied Testing Guidelines of the Organisation for Economic Co-operation and Development (OECD).

The interpretation of kidney biomarker data is based on a weight of evidence approach that includes understanding of the biomarker biology, its context relative to other study parameters and the magnitude of change relative to biological variability and histopathological changes reflecting renal injury (Ramaiah et al., 2017).

2.2.1.2 | *Gross pathology and organ weight*

Gross pathology findings (changes in colour, shape, size) and kidney weights (absolute and relative to body weight) are collected and analysed in the large majority of toxicological studies conducted in line with the OECD Test Guidelines. Changes in these endpoints can corroborate study interpretation when integrated with other findings (clinical pathology[2] and histopathology); however per se they are considered generic and not sufficient to identify specific chemically-induced toxicity. In the absence of a histological lesion, they can be the expression of mild adaptive changes (e.g. a minor and transient homeostatic disturbance or metabolic impairment) of no toxicological relevance; they can be associated with various histological lesions in a non-specific manner (e.g. tubular injury, tubulointerstitial inflammation or obstruction).

Kidney weight changes can provide useful signals indicating test substance-related effects, but should not be necessarily considered adverse if observed in isolation, and they need to be interpreted with caution (Sellers et al., 2007).

It is also noted that organ weights are not usually collected in carcinogenicity studies, where age-related changes and intercurrent disease may contribute to inter-animal variability, which will confound organ weight interpretation (e.g. spontaneous neoplasia, cachexia related to toxicity or neoplasia) (Sellers et al., 2007).

2.2.1.3 | *Histopathology in regulatory toxicological studies and special investigations*

Histopathology examination is the most relevant tool to evaluate renal toxicity since it is sensitive and routinely carried out in most regulatory studies in pesticide assessment datasets. It can inform on the structure affected (glomerulus, tubule, interstitium, papilla, pelvis) and on the type of toxicological insult. Dedicated terminology is available in the species used in toxicological studies (INHAND, International Harmonization of Nomenclature and Diagnostic Criteria; Mann et al., 2012), facilitating the harmonised description and interpretation of findings.

Special investigations can integrate and corroborate histopathology, such as special histochemistry and immunohistochemistry, in situ hybridisation and ultrastructural investigations (transmission and scanning electron microscopy). These can be used to substantiate diagnosis (e.g. immunohistochemistry to identify accumulation of alpha 2 urinary globulin – alpha 2u-globulin – in hyaline droplets) and are generally used in mechanistic studies.

Non-routine investigations might include studies assessing glomerular, tubular or hemodynamic function by using in vivo, in vitro and in silico models.

2.2.2 | The interpretation of renal findings in regulatory toxicological studies and their relevance to humans

The interpretation of renal findings in toxicological studies, and their relevance to humans are not straightforward and should take into account various aspects. Beside obvious anatomical and functional differences, some pathologies (e.g. alpha 2u-globulin nephropathy in male rats) are specific to the animal species and cannot find a counterpart in humans, and vice versa. A perfect correlation of observed effects in human patients and in test animal species cannot be established, based upon different investigational circumstances and methodologies. Renal 'pathology' in humans often manifests as a disease condition, or it is considered in specific cases of occupational medicine; once the medical question is formulated, diagnostic tools are identified, with the main role played by clinical investigations (signs and symptoms) and sensitive and specific biomarkers (in blood and urine). In some cases, functional tests and imaging techniques are used, and biopsies and histopathological investigations can be carried out to confirm the diagnostic question and support the prognosis and therapy. Regulatory toxicological studies aim at studying the 'general' toxicological response following the administration of a test substance, with the kidney being one amongst potential targets. Generally, renal pathology does not manifest as a clear disease condition or syndrome in toxicological species, firstly, because the kidney has a great functional reserve capacity, both at glomerular and tubular level, until an end-stage condition is reached; secondly, because specific clinical signs or sensitive and specific renal pathology biomarkers are not routinely collected (see Section 2.2.1.1), and no specific functional studies are carried out (e.g. studies investigating glomerular, tubular or hemodynamic functions).

Some elements impacting the toxicological response in species used in toxicological studies, and of relevance for extrapolation of renal findings to humans are presented below.

In toxicological animal species, nephrotoxicity can vary according to the species and strain, depending on anatomical, functional and metabolic differences. Also, males and females can respond differently to the same dose of a nephrotoxic agent, and this may be related to toxicokinetic differences. For instance, differences are known to occur between male and female rodents as regards CYP450 enzyme activity; also, transporter activity is different between sexes and may result in different intracellular accumulation of a chemical or reactive metabolite within tubular epithelium (Frazier & Seely, 2018).

Some findings observed in the species used in toxicological studies are not relevant to humans. Alpha 2u-globulin nephropathy is a condition related to the administration of some chemicals occurring exclusively in young male rats. Alpha 2u-globulin is a protein (181 aa, 20kDa) belonging to the lipocalin family. It is predominantly synthesised in the liver of adult male rats under multi-hormonal control, being induced in vivo by androgens, glucocorticoids, growth hormone and insulin, and inhibited by oestrogens. Serving to bind and release pheromones, after glomerular filtration it undergoes

[2]Clinical pathology includes clinical chemistry, haematology, coagulation and urinalysis parameters.

reabsorption and catabolism in proximal tubular cells. A number of chemicals are known to cause alpha 2u-globulin nephropathy in male rats, but not in female rats (or mice) and kidney tumours in the same rat population (Goyak et al., 2022; IARC, 1999). The initiating event of this condition is the reversible binding of some chemicals to alpha 2u-globulin, leading to a decreased rate in its catabolism and accumulation in the renal proximal tubule cells; this results in the formation of tubular epithelium protein (hyaline) droplets (positive to specific antibodies), followed by tubular cell degeneration and necrosis, granular casts and papillary mineralisation. Such nephropathy is associated with a sustained increase in compensatory renal tubule cell proliferation and subsequent formation of kidney tumours in male rats (Goyak et al., 2022). Both the United States Environmental Protection Agency (U.S. EPA, 1991) and International Agency for Research on Cancer (IARC, 1999) have outlined similar specific criteria based on scientific evidence to characterise a male rat kidney carcinogen as operating through the alpha 2u-globulin nephropathy mode of action. Alpha 2u-globulin nephropathy and consequent proliferative changes in male rats are considered as not relevant for humans, not producing alpha 2u-globulins.

The age is also a relevant condition to consider when interpreting nephrotoxicity. Toxicological lesions may be observed in the kidney in studies on juvenile animals, while no renal lesions are noted with the same compound in adult animals at similar doses. Amongst the reasons, different toxicokinetic profiles, differences in fluid dynamics and rapid organ growth or tissue development can contribute to nephrotoxicity (Cappon & Hurtt, 2010; Scheuplein et al., 2002). Renal malformations can follow exposure in uterus (e.g. increased incidence of cysts, dilated pelvis and dysplasias) (Cappon & Hurtt, 2010), however these are not in the scope of this exercise.

Chronic progressive nephropathy (CPN) is a common background condition observed in aging rats, predominantly in males, with Sprague Dawley rats being more affected than Fischer and Wistar rats. It is influenced by various factors such as caloric intake, protein content of diet and male hormones. CPN includes characteristic morphologic features such as tubular basophilia, nuclear crowding and thickened basement membranes; these are typically present in subchronic and chronic studies, when they can ultimately result in end-stage kidney disease. Tubular basophilia can be the only morphologic feature present in very early cases in young animals. CPN is considered a distinctive condition in rats with no clear counterpart in human disease (Hard et al., 2009; WHO, 2015). Exacerbation of incidence and/or severity of CPN can be noted following the administration of chemicals. Because rat CPN is both regenerative and degenerative, with a high rate of cell turnover, advanced disease may be a risk factor for renal tumour development and a strong correlation between high-grade CPN and renal tumour development in rats has been demonstrated (Hard et al., 2012). There are various interpretations as regards the relevance of such condition in regulatory risk assessment. According to WHO (2015), CPN per se, and its exacerbation, should not be acknowledged as an indicator of human toxic hazard considering the species-specificity of the finding. If the mode of action for an increased incidence of renal tumours in a 2-year rat bioassay is determined to be due to exacerbation of CPN, it can be concluded that the rat renal tumours are not relevant to human cancer risk (Hard et al., 2013). However, according to the Environmental Protection Agency (2021), since there is no definitive pathogenesis for this multifactorial disease process, it cannot be fully ruled out that chemicals which exacerbate CPN in rats may also exacerbate disease processes in the human kidney.

3 | METHODOLOGIES FOR THE IDENTIFICATION OF THE KIDNEY SPECIFIC EFFECTS AND PRIORITISATION OF PESTICIDES

3.1 | Methodology

3.1.1 | Criteria for grouping pesticides into cumulative assessment groups (CAGs)

Amongst all possible effects of chemicals on kidneys, those relevant for grouping pesticides into CAGs can be identified by using hazard-driven criteria as advised by the guidance of the EFSA Scientific Committee on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2021).

The framework proposed by the EFSA Scientific Committee is based on the use of a hierarchical approach to evaluate the body of information available for each chemical, as described in Figure 4 below:

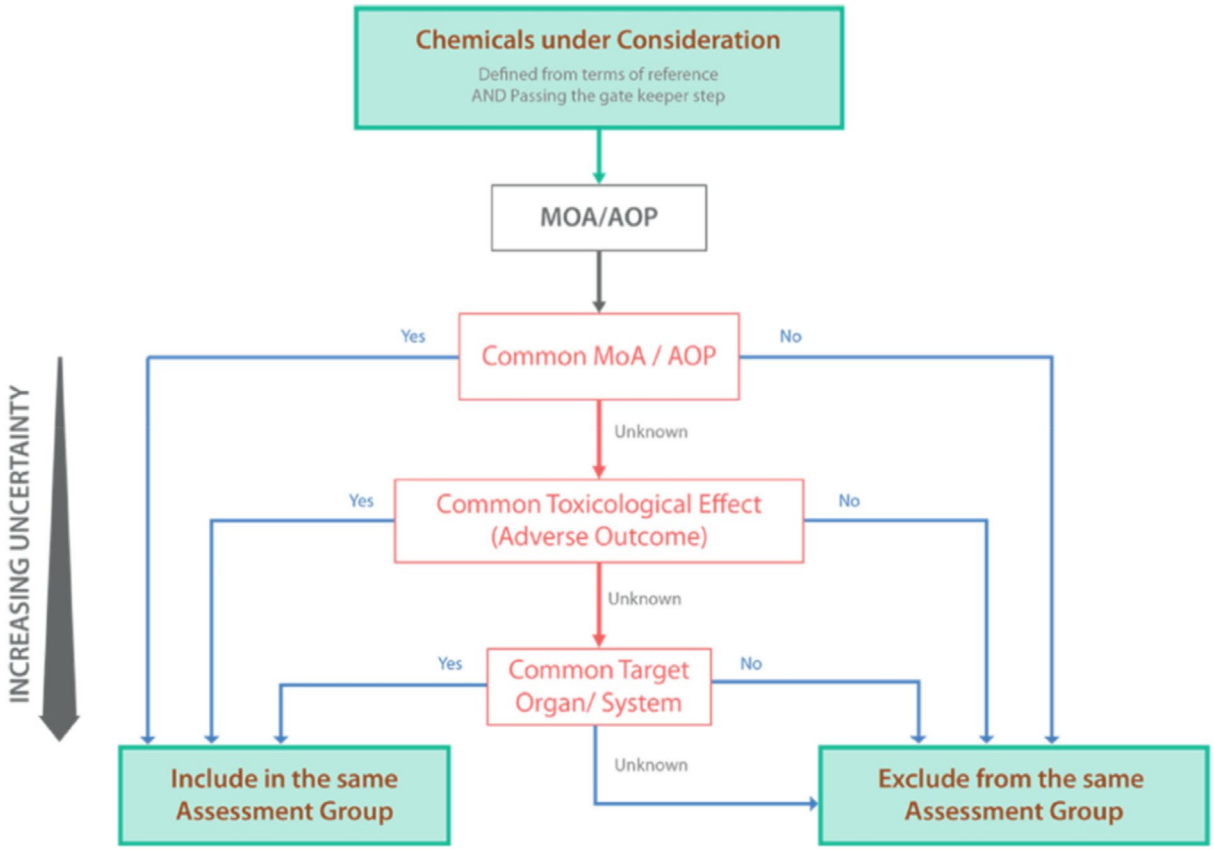

FIGURE 4 Top-down hierarchical process for grouping chemicals into assessment groups using hazard-driven criteria (from EFSA Scientific Committee, 2021).

3.1.1.1 | *Using common MoA/AOP – Relevance of AOPs to identify kidney specific effects*

According to the EFSA Scientific Committee's framework, the golden standard for grouping chemicals is considered to be the common mode of action (MoA) and/or the adverse outcome pathways (AOPs).

In case of incomplete mechanistic information, the grouping may also be based on the commonality of adverse outcomes (AOs). This approach is also of interest in case of AOP networks converging to a same AO even if they have different initiating events and pathways.

AOPs can inform chemical grouping and subsequent data gap filling by read-across or trend analysis. Chemicals that are shown to activate the same AOP based on results of assays or predictions of the molecular initiating events (MIEs) or Key events (KEs) can be grouped together, thereby improving the robustness of the data gap filling approach for the AO. AOPs thus provide an opportunity to group chemicals based on their intrinsic chemical properties, as well as their biological activity at different levels of biological organisation. Whilst a complete knowledge of the AOP from the MIE to the final AO is not considered critical for the purposes of grouping chemicals sharing a common MIE or KE, it is necessary to have the linkages between the MIE or KEs and the AO (or key event relationship/KERs) established in order to justify the data gap filling (such as read-across) performed (OECD, 2017).[3]

The grouping based on common target organ/system toxicity implies a large uncertainty when the dose-addition model is used for quantifying the combined effects of groups of chemicals assembled in this way.

3.1.1.2 | *Using common toxicological effects to identify kidney specific effects*

Considering the possible limitations of currently available AOPs, the ASs and metabolites can be grouped in the respective CAGs based on the common AOs captured as apical toxicological endpoints in regulatory studies, when these can be reasonably assumed to result from a MIE and/or KEs taking place in kidneys.

3.1.2 | Definitions

In the context of this report, the following general definitions are proposed:

[3]Guidance document for the use of adverse outcome pathways in developing integrated approaches to testing and assessment (IATA), Series on Testing & Assessment No. 260, Environment, Health and Safety, Environment Directorate, OECD.

Toxicological effects	**Toxicological effects** are adverse effects induced by xenobiotic substances. They are generally identified and characterised in in vivo toxicological studies performed according to OECD test guidelines.
Toxicological endpoints	**Toxicological endpoints** are endpoints measured/observed in toxicological studies performed in laboratory animal species in agreement with data requirements set for pesticides (i.e. clinical and functional signs, organ weights, clinical pathology parameters, gross pathology or histopathology findings, etc.).
Specific effect	A **specific effect** is a well-characterised and unambiguous toxicological adverse effect relevant for grouping pesticides into cumulative assessment groups (CAGs) in the context of cumulative risk assessment (CRA; EFSA PPR Panel, 2013). When the specific effect is assumed to be associated to one or several AOPs, it can be referred to as 'adverse outcome' (AO; EFSA, 2021) (see Figure 4 under 3.1.1). The specific effect is identified by expert judgement on the basis of its intrinsic relevance for CRA. It should be noted that 'specific effects' do not necessarily consist of a single toxicological effect observed or measured in regulatory toxicology studies, but can also consist of a set of effects representative of a progressive toxicological continuum or a functional/biological dysfunction (e.g. hypothyroidism or functional alterations of the motor division of the nervous system, or renal preneoplastic and neoplastic changes). Criteria for the selection of specific effects relevant for CRA, including their identification and characterisation, were described in the EFSA Scientific Opinion of the PPR Panel published in 2013 (EFSA PPR Panel, 2013). Specific effects are identified based on expert judgement aiming at excluding local effects, non-adverse effects, effects not relevant to humans and non-specific effects (e.g. age-related or occurring at or above the maximum tolerated dose). Specific effects are unambiguous and well-defined in terms of site of occurrence and nature, and it is reasonable to consider that in case of co-exposure to pesticides triggering the same specific effect, the dose-addition (DA) method is valid for the estimation of the risk from the exposure to multiple pesticides. The hypothesis behind the DA method is that all compounds in a mixture act as if they were simple dilutions of one another (Loewe & Muischneck, 1926).
Indicator	An **indicator** describes a change in a toxicological endpoint considered relevant for the identification of a specific effect (EFSA PPR Panel, 2013). A single or a set of indicators can be used to identify a specific effect. In some circumstances, when the specific effect defines a very discrete toxicological insult, specific effects and indicators may be identical. For instance, 'Acetylcholinesterase inhibition' is the single indicator of the specific effect 'Acetylcholinesterase inhibition'. Based on canonical knowledge and expert judgement, indicators are defined as **primary indicators** informing about the direct chemical interaction(s) of the substance with a biological target. Primary indicators are considered the most suitable for the identification of a specific effect (EFSA PPR Panel, 2013). **Secondary indicators** inform about the consequence or progression or a regenerative/reparative response, following the primary toxic insult. They are not necessarily representative of one specific effect only (i.e. they are not unambiguous), nevertheless they can contribute to corroborating the evidence that a substance is actually causing the specific effect.
Ancillary endpoints	**Ancillary endpoints** are toxicological endpoints considered as not sufficiently informative to indicate a specific effect, since they are non-specific and/or not sensitive. However, they could contribute to corroborating the evidence that a substance is actually causing the specific effect.

Further details are provided in Figures 5 and 7, where the role of primary indicators, secondary indicators and ancillary endpoints for the identification of the specific effects in kidney is illustrated.

3.2 | Prioritisation of pesticides

To make best use of resources available for CRA, EFSA developed and implemented a pesticide prioritisation methodology (EFSA, 2024a, 2024b). This methodology was based on short- and long-term exposure assessments for each individual pesticide quantified in at least one food sample of the official monitoring programme of EU Member States over a 3-year monitoring cycle from 2019 to 2021. These assessments were performed by probabilistic modelling in 30 population groups, covering different age groups (adults, children and toddlers) in 17 countries. The threshold to prioritise a pesticide was an acute or chronic hazard quotient (HQ), that is the ratio of the estimated short- or long-term exposure to the acute reference dose (ARfD) or acceptable daily intake (ADI), respectively, equal or exceeding 0.1 (10% of the reference value) at P99.9 of the exposure distribution in at least one of the 30 population groups under consideration. This resulted in a priority list of 67 pesticide residues (ASs and metabolites), of which 21 reached the threshold in short-term exposure assessments only, 15 in long-term exposure assessments only and 31 in both short- and long-term exposure assessments.

4 | ASSESSMENT – IDENTIFICATION OF THE KIDNEY SPECIFIC EFFECTS AND THEIR RESPECTIVE INDICATORS

4.1 | Using MoA/AOP-relevance of AOPs to identify kidney specific effects

In the case of kidney toxicity, no endorsed AOPs are currently available in the OECD Series on Adverse Outcome Pathways, but there is ongoing work which might be considered in the future.

As of 29/09/2022, 16 AOPs were identified in Collaborative Adverse Outcome Pathway Wiki (AOPwiki). The search was repeated on 15/7/2023, applying the same search terms, i.e. 'kidney' and 'renal' in the title or in the text of the AOP, and 2

additional AOPs were identified (18 AOPs in total). None of the retrieved AOPs was currently endorsed by the OECD. Ten (10/16) of the identified AOPs were included in the OECD Workplan for further development.

The most common AOs in the identified kidney-related AOPs were the following:

- Increased, kidney failure;
- Occurrence, kidney toxicity;
- Chronic kidney disease;
- Increased, adenomas/carcinomas (renal tubular);
- Chemical induced Fanconi syndrome (characterised by loss of water and solutes in the urine due to insufficient reabsorption at the proximal tubule);
- Acute kidney injury (AKI) (clinical syndrome) and acute tubular necrosis (ATN).

The following Key Events (KEs) were identified in kidney-related AOPs:

- Mitochondrial dysfunction (e.g. KE 1483-Mitochondrial Dysfunction, KE 40-Mitochondrial Adenosine triphosphate (ATP) production decrease, KE 1968-Mitochondrial Dysfunction Increase, KE 178-Disruption of Mitochondrial electron transport chain).
- Cytotoxicity or necrosis in renal tubular cells (e.g. KE 709-Increased cytotoxicity in renal tubular cells, KE 1097-Occurrence of renal proximal tubular necrosis, KE 710-Increased Regenerative cell proliferation of tubular epithelial cells, KE 1607-Increased Necrosis, KE 768-Increased Cytotoxicity).
- Oxidative stress (e.g. KE 1088-Increased Oxidative Stress, KE 1392-Oxidative Stress, KE 1115-Increased Reactive oxygen species (ROS), KE 926-Oxidation of Glutathione, KE 1538-Decreased protection against oxidative stress). It is however noted that oxidative stress is usually not investigated in toxicological studies and can result from many other causes than exposure to pesticides. In addition, oxidative stress does not fit the unambiguous criterion.
- For AKI and ATN several KEs are mentioned, which have not been encoded. According to the narrative text available, 'the nephrotoxic response following the formation of DNA adducts leads to DNA damage (KE1) and mitochondrial dysfunction (KE2). These events promote the release of ROS (KE3) to induce oxidative stress (KE4), causing cell death (KE5) and inflammation (KE6). As these cells detach from the basement membrane, they are deposited in the tubular lumen. Tubular obstruction and inflammatory responses to proximal tubule insult can cause secondary toxicity and tubular necrosis (KE7), further amplifying kidney injury and a progressive decline of function, finally resulting in kidney failure (AO)' (https://aopwiki.org/aops/472).

It is noted that rotenone represents the chemical stressor involved in several KEs in the AOP related to Fanconi syndrome (KE 887 Inhibition, NADH–ubiquinone oxidoreductase (complex I), KE 1477 Decrease, Oxidative phosphorylation, KE 1562 Decreased Na/K ATPase activity, KE 1563 Decreased proximal tubular vectorial transport).

In addition, some efforts are currently ongoing to develop AOP networks for kidney toxicity being mitochondrial dysfunction, oxidative stress and tubular necrosis the most connected and central KEs (Barnes et al., 2024).

The information provided by the available AOPs at their current status of development is of limited use for the kidney CAG exercise. This information is mainly derived from mechanistic studies, generally not available in regulatory assessment reports and not always linked to apical endpoints. Also, the AOs described relate to functional renal impairment that would need ad hoc functional investigations or would imply severe end-stage renal conditions.

It is recognised that AOPs can provide valuable information for the pesticides CRA of kidneys, and it is recommended to further develop on these (see also recommendations under chapter 9), focusing on AOs available in the pesticide dataset.

4.2 | Using common toxicological effects to identify kidney specific effects

Considering that no OECD-endorsed AOP is currently available for kidney toxicity and that data on mode/mechanism of action are completely lacking or not readily available for most ASs in toxicological studies, the specific effects of relevance for performing CRA of kidney effects have been identified only as common toxicological effects (i.e. AOs) and defined according to the criteria provided in the PPR Scientific Opinion (EFSA PPR Panel, 2013) (i.e. well characterised, unambiguous effects, etc.) by using scientific knowledge and expert judgement (see also the definition of specific effect under 3.1.2).

4.3 | Preparatory work for the identification of specific effects and their indicators

As part of the preparatory work, a template was developed and its applicability tested on three pesticides with effects on kidneys reported in the Draft Assessment Reports (DARs)/Renewal Assessment Reports (RARs). Not necessarily these pesticides were later considered in the data collection, but they were mainly explored for the development and refinement of the template.

Furthermore, to support the identification of kidney specific effects and their respective indicators, data on 17 ASs[4] of PPPs (and their metabolites) showing toxicity in kidneys, according to an earlier data collection performed by DTU (DTU, 2012), were screened in detail for their effects in kidneys in DARs/RARs. This exercise provided a robust insight into the nature of toxicological effects of pesticides on the kidney, their incidence, the type of toxicological endpoints, their eventual concomitancy, as well as the terminology used by the evaluators of Rapporteur Member States (RMSs). This preliminary data collection was performed by collecting information on histopathological findings, and also on kidney weight and clinical pathology changes, when available. This preliminary step was paralleled and followed then by an in-depth analysis of the kidney anatomy and physiology, chemically-induced renal toxicity and revision of the nomenclature used to report kidney histopathological changes as reported by INHAND/GoRENI.[5] An overview of the information on the relevance of kidney weight changes, clinical pathology and urinalysis parameters related to kidney dysfunction/damage was also performed from the open literature.

4.4 | Results

4.4.1 | Approach used for the identification of specific effects

Based on the results of the exercise mentioned under 4.3, histological lesions in the kidney were found to be the most appropriate renal specific effects for CRA, considering the unambiguousness in terms of site of occurrence and nature.

Ancillary endpoints (described by gross pathology findings, changes in kidney weight, clinical pathology including urinalysis parameters) were not considered suitable candidates per se to identify a specific effect in kidneys (see Figure 5) although they are commonly used to interpret kidney findings by using a weight of evidence approach.

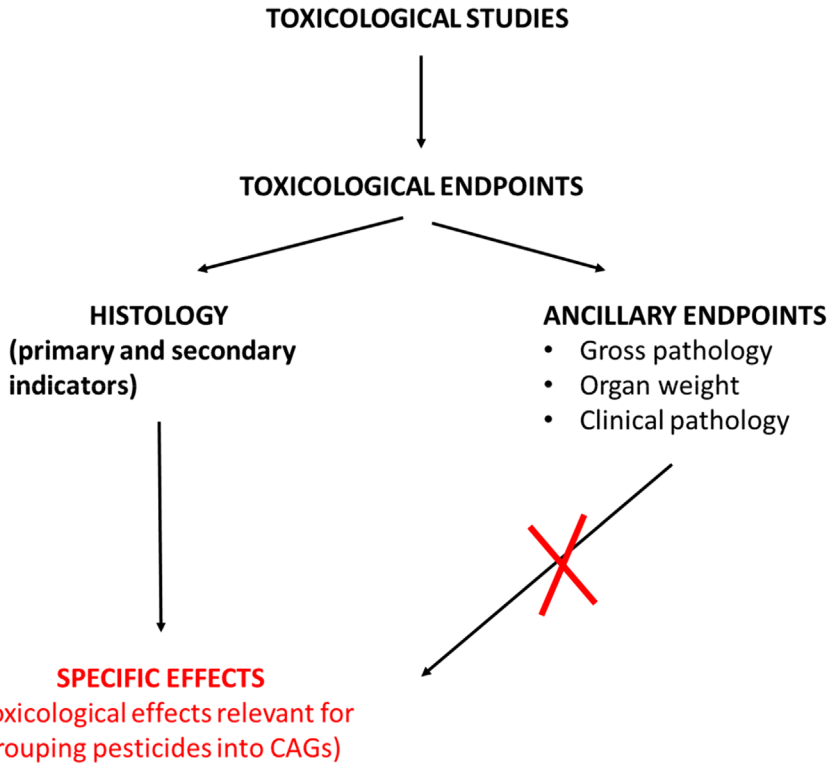

FIGURE 5 Toxicological endpoints considered for the identification of renal specific effects.

The preparatory work described under Section 4.3 was followed by an in-depth analysis of the nomenclature used to report kidney histopathological changes as reported by INHAND/GoRENI.

The histological lesions were further categorised according to the anatomical structure involved (glomerulus, tubules, interstitium, papilla, pelvis). As mentioned earlier, the nephron tends to respond to an insult as a unit, and progression or regenerative/reparative responses generally follow the initial insult, with evolution into complex pictures composed of concomitant primary and secondary lesions.

[4]The selected active substances were 2,4-D, 2-phenylphenol, carbendazim, chlorothalonil, clopyralid, copper compounds, fipronil, iprodione, linuron, MCPB, oxamyl, pirimiphos-methyl, prosulfocarb, pyrimethanil, tetraconazole, thiabendazole and thiophanate-methyl.

[5]International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND).

In detail, to identify and define precisely the kidney specific effects, the following step-by-step approach was followed based on:

1. Exclusion of non-histological kidney lesions (clinical pathology parameter, gross pathology, organ weight alteration) due to the lack of specificity as explained in Section 2.2.1.
2. Revision of the nomenclature of the renal histological lesions according to GoRENI/INHAND to support a precise definition of the specific effects and the lesions they consist of. Synonyms suggested by INHAND or other sources (e.g. National Toxicology Program-NTP, DAR/RAR) were also collected.
3. Exclusion of histological lesions not relevant as specific effects for CAG (see definition of specific effect under Section 3.1.2; see also Figure 6), i.e.:

- Congenital lesions (e.g. congenital pelvic dilation/hydronephrosis, nephroblastoma, renal aplasia/hypoplasia, renal dysplasia).
- Background/spontaneous lesions (e.g. amyloidosis, micro abscesses).
- Species-specific lesions not relevant for humans (e.g. alpha 2u-globulin nephropathy in male rats; see chapter 2.2.2).
- Lesions occurring in the kidney as a consequence to chemically-induced conditions affecting extrarenal compartments (e.g. renal tubular injury by altered hemodynamics consequent to effects on the cardiovascular system or haemorrhages), or in the context of non-specific systemic toxicity (i.e. above the maximum tolerated dose, MTD). These effects are considered not specific to the kidney and therefore they are not relevant for CRA. Since renal histological lesions in these conditions can be similar to those observed in cases of renal specific toxicity, accurate and case-by-case interpretation is necessary to identify such cases.
- Lesions due to genotoxicity (e.g. renal mesenchymal tumours).

4. Definition of the specific effects, based on expert judgement, as previously described (see 4.2), through the categorisation of the remaining renal histological lesions according to the anatomical structure involved (glomerulus, tubule, interstitium, papilla, pelvis).

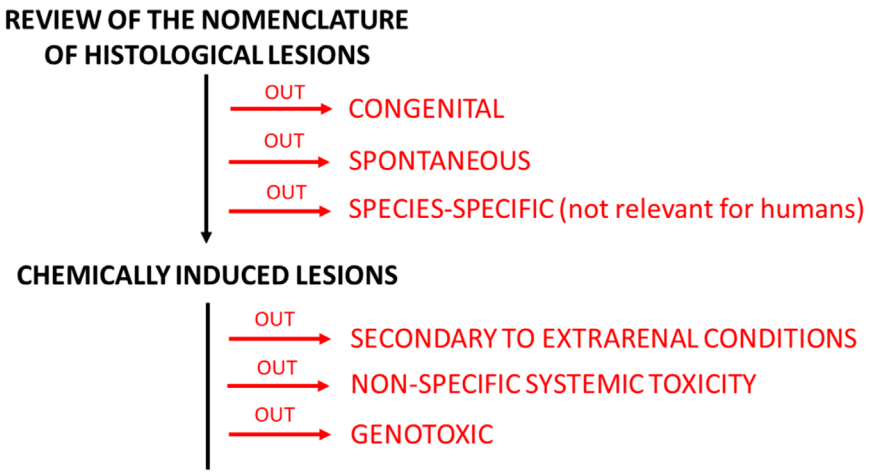

FIGURE 6 Exclusion of lesions not considered relevant for CRA.

The histological lesions were further categorised according to the anatomical structure involved (glomerulus, tubules, interstitium, papilla, pelvis).

4.4.2 | Kidney specific effects

Kidney specific effects are the histological lesions identified as follows (see also Table 1):

- Glomerular injury (acute and chronic in nature)
- Tubular injury (acute and chronic)
- Tubular crystals (chronic)
- Papillary necrosis (acute and chronic)
- Interstitial nephritis (chronic)
- Pelvis erosion/ulceration (acute and chronic)
- Pelvis calculi/crystals (chronic)
- Renal preneoplastic and neoplastic lesions (chronic)
- Pelvis preneoplastic and neoplastic lesions (chronic)

In addition, special considerations have been made on the nature of each specific effect (acute, possibly occurring after one single (over 1 day) exposure; or chronic, i.e. requiring a repeated exposure over several days).

Glomerular injury is a specific effect intended to cover various types of chemically-induced damage (e.g. glomerulonephritis/glomerulopathy, glomerulosclerosis) and/or proliferation of glomerular components (Bowman's capsule hyperplasia) or dilation of Bowman's space (due to glomerular hyperfiltration). This effect can be of **acute and chronic nature**.

Tubular injury is a specific effect intended to include a variety of degenerative changes due to various initiating events and affecting the renal tubular epithelium. These can span from modification in cellular staining properties, to cellular accumulation of various materials and frank necrosis, and, accordingly, they can be described by various indicators. Tubular injury can be of **acute and chronic** nature.

Tubular crystals can occur as a consequence of the precipitation of the administered compound (or metabolite) in the lumina of tubule, generally with a dose–response (Cohen & Arnold, 2011). Crystals are usually located in cortex or outer medulla and should be distinguished from casts and mineralisation due to calcium phosphate (mineralisation). Many crystals are birefringent, the use of polarised light during microscopic examination often aids in visualisation and proper diagnosis. This effect is of **chronic** nature. It is acknowledged that due to differences in pH, solute and protein compositions of the urine filtrate between species, and possible variable renal excretion profiles from one species to another, crystalluria may be confined to one or a limited number of laboratory animal species; for example, calcium phosphate-containing precipitates are considered specific to rat, while melamine-related calculi were seen in species used in toxicological studies and in humans (Cohen et al., 2000). The presence of crystals therefore does not automatically imply that these may occur in human kidney; careful examination of the pharmacokinetic and biochemical profiles of an agent and its metabolites, as well as crystals composition is needed for extrapolation of the potential for crystalluria in humans (Sahota et al., 2018). 'Crystals from any cause may incite degeneration and necrosis in the adjacent tubular epithelium or may be associated with obstructive nephropathy due to blockage of urine flow. Tubular crystalluria may also accompany the presence of calculi within the renal pelvis as the etiopathogenesis of the two lesions is similar' (Frazier et al., 2012).

Papillary necrosis, known to occur with some classes of compounds, such as NSAIDs (see 2.2), is characterised by an initial loss of structural definition at papilla tip involving mainly interstitial cells. This can progress to confluent necrosis extending from tip through full papilla, mineralisation and/or inflammation between necrotic and viable tissue and sloughing of necrotic papilla followed by re-epithelialisation of surface by transitional epithelial cells, which may be followed by transitional cell hyperplasia. Secondary changes include pyelonephritis, cortical tubule dilatation and/or hydronephrosis. This effect can be of **acute and chronic** nature.

Interstitial nephritis consists of generalised inflammatory lesions of the interstitium, regardless of cause. The pathogenesis is not fully understood, but in some cases may be associated with antibody complexes directed at the basement membrane or extracellular matrix elements. Primary interstitial disease in rats, unlike in dogs or humans, is relatively uncommon, however interstitial nephritis is a recognised complication of drug treatment in rodents (goRENI). Interstitial nephritis has to be distinguished from focal interstitial inflammatory infiltrates (common spontaneous finding in rodents) and CPN. This effect is of **chronic** nature.

Pelvis erosion/ulceration are the consequence of the direct injury to the urothelium and can be **acute and chronic**.

Pelvis calculi/crystals are formed as a consequence of the precipitation of administered compounds (or metabolites) as crystals and represents a specific effect of **chronic** nature.

Similar considerations as for tubular crystals can be drawn as regards the relevance to human of crystals/calculi in the pelvis.

Renal and pelvis preneoplastic and neoplastic lesions are AOs in regulatory studies of potential relevance for humans. Such lesions in regulatory rodent studies are often related to non-genotoxic mechanisms, typically triggered by initial damage (tubular injury, crystals, erosion and ulceration) followed by sustained regeneration and (uncontrolled) proliferation in a continuum (damage, simple hyperplasia, atypical hyperplasia, neoplasia). Genotoxic carcinogens are generally identified in dedicated genotoxicity assessment and excluded for subsequent progression to authorisation. This effect is of **chronic** nature. Preneoplastic and neoplastic lesions are grouped together since part of a pathogenetic continuum.

The compatibility of these specific effects for CRA with the dose-addition model, is justified as follows:

Glomerular injury, tubular injury, papillary necrosis, interstitial nephritis: are lesions of well-defined anatomical structures of the kidney. Although it is likely that the ASs/metabolites that will be included in the respective CAG may be acting through a diversity of MIEs/MoAs, it is likely that their contribution to the overall impairment of the function of the respective anatomical structure will result from the convergence of the respective AOPs and that the dose-addition model will be appropriate in such case. Similar considerations could apply to pelvis erosion/ulceration.

Tubular crystals: due to the anatomical and physiological characteristics of the kidney, the precipitation of various compounds (ASs or metabolites) is likely to occur in the same topographical region, contributing to the impairment of the same region in an additive manner. Similar considerations could apply to pelvis calculi/crystals.

Preneoplastic and neoplastic lesions: it is conceivable that the dose-addition model is not applicable to compounds causing such lesions via non-threshold genotoxic mechanisms, i.e. to compounds reacting directly with the DNA (e.g. clastogens); excluding therefore such compounds (based for instance on the outcome of genotoxicity studies), the dose-addition model is applicable to non-genotoxic carcinogens (e.g. with a mode of action involving sustained regeneration and (uncontrolled) proliferation following an initial tissue damage): an additive contribution in triggering proliferative processes is expected.

4.4.3 | Indicators of kidney specific effects

The approach used for the identification of the specific effects was based on toxicopathology knowledge, adapted from Foster et al. (2020). This approach relies on the categorisation of chemically-induced histological lesions into primary and secondary indicators (see definitions in Section 3.1.2). Primary indicators are considered to be the most relevant ones for the identification of specific effects, since it is conceivable that pesticides causing these primary lesions could contribute additively to the specific effect. Secondary indicators can be indicative of a specific effect but are not necessarily peculiar to it (i.e. they are not unambiguous). In the absence of primary indicators, secondary indicators can be used as indirect or surrogate indicators of (one or more) corresponding specific effects (e.g. renal tubular hyperplasia can be the consequence of tubular injury). The uncertainty related to the use of secondary indicators (i.e. they are not unambiguous) will be considered under the CAG-membership probability.

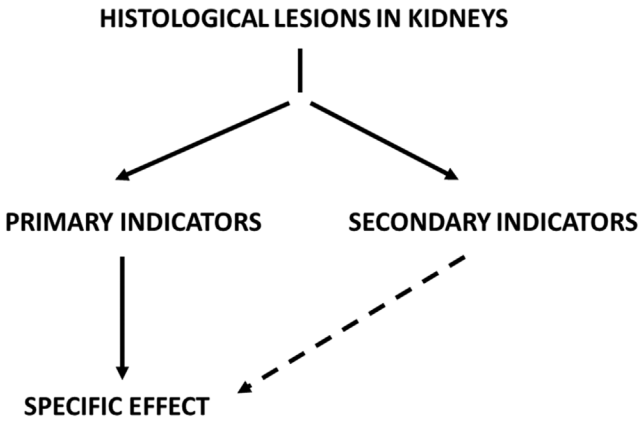

FIGURE 7 In the absence of primary indicators, secondary indicators are considered as the evidence that the pesticide is causing the specific effect.

In the case of renal and pelvis preneoplastic and neoplastic lesions, all the indicators selected are considered as primary even though they are the result of a multistep process arising from distinct MIEs and KEs since they are considered AOs in regulatory studies and thus relevant for the identification of CAGs.

An example of primary and secondary indicators for a few specific effect and their relationship are graphically shown in Figure 8, underlying the complex interaction of different renal compartments and the tendency of the nephron to respond to toxic injury as a unit.

By nature, some histological lesions can be either primary or secondary; for instance tubular degeneration/necrosis can be a primary effect on the renal tubular cells, or secondary to glomerular changes or to crystals; tubular dilation can be primary, occurring following the administration of compounds such as starch or angiotensin converting compounds, or secondary to tubular obstruction by necrosis or crystals. In the dataset for pesticide assessment the classification of the lesions as primary or secondary based on their histopathological characteristics can be challenging: the dataset generally consists of repeated dose toxicological studies, where often primary and secondary lesions coexist, or just secondary lesions can be observed. In the absence of sufficient information, regardless of their primary or secondary nature, these lesions are used as indicators of (one or more) specific effects (e.g. tubular injury, and glomerular injury and crystals).

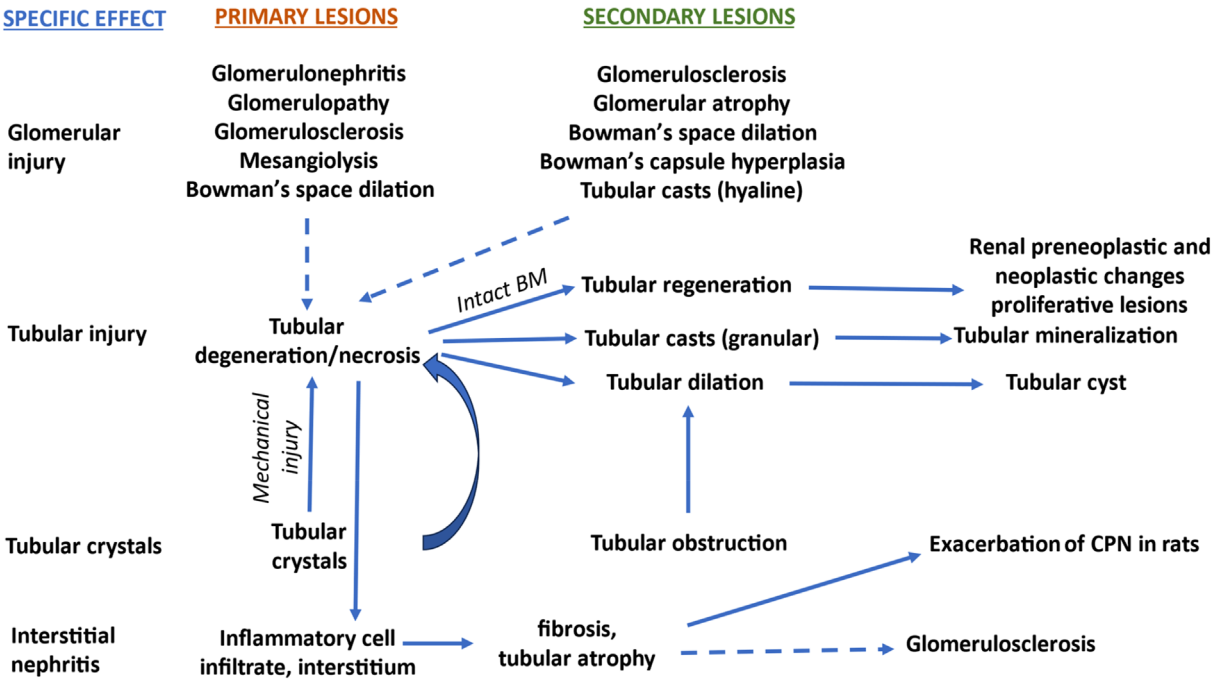

FIGURE 8 Primary and secondary indicators of four specific effects and their relationship, underlying the complex interaction of different renal compartments. (intact BM = intact basal membrane).

Primary indicators describing primary histological lesions and secondary indicators describing secondary histological lesions are listed in the table below.

As explained in Section 4.4.2, some specific effects can be of acute or chronic nature. The indicators of possible acute nature (i.e. susceptible to appear following a single exposure or repeated exposure within a short time interval – 24 h –) have been indicated in italics in Table 1. Such 'possible acute indicators', can be primary or secondary indicators.

TABLE 1 Kidney specific effects and respective indicators (indicators capable of reflecting an injury of chronic or acute nature are reported in italics).

Specific effect	Primary indicator	Secondary indicator
Glomerular injury (acute, chronic)	• Glomerulus, glomerulonephritis/ glomerulopathy • Glomerulus, glomerulosclerosis • Mesangiolysis • *Glomerulus, Bowman's space dilation*	• Glomerulus, glomerulosclerosis • Glomerulus, atrophy • *Glomerulus, Bowman's space dilation* • Glomerulus, Bowman's capsule hyperplasia • Tubule degeneration • Tubule necrosis Secondary histological lesions of tubular injury: • Tubule, mineralisation • Tubule, casts (hyaline, granular) • Interstitium, infiltrate, inflammatory cell[a] • Tubule, regeneration • Tubule, dilation • Tubule – non neoplastic, hyperplasia, tubule, simple • Tubule, cysts • Tubule, atrophy • Exacerbated chronic progressive nephropathy
Tubular injury (acute, chronic)	• *Tubule, degeneration* • Tubule, inclusion bodies (nuclear, cytoplasmic) • *Tubule, necrosis* • *Tubule, vacuolation* • *Tubule, dilation* • *Tubule, basophilia* • *Tubule, karyomegaly* • *Tubule, cytoplasmic alteration*	• Tubule, mineralisation • Tubule, casts (hyaline, granular) • Interstitium, infiltrate, inflammatory cell[a] • Glomerulus, glomerulosclerosis • Tubule, regeneration • Tubule, dilation • Tubule - non neoplastic, hyperplasia, tubule, simple • Tubule, cysts • Tubule, atrophy • Exacerbated chronic progressive nephropathy

(Continues)

TABLE 1 (Continued)

Specific effect	Primary indicator	Secondary indicator
Tubular crystals (chronic)	• Crystals, tubule	• Tubule degeneration • Tubule necrosis Secondary histological lesions of tubular injury: • Tubule, mineralisation • Tubule, casts (hyaline, granular) • Interstitium, infiltrate, inflammatory cell[a] • Glomerulus, glomerulosclerosis • Tubule, regeneration • Tubule, dilation • Tubule – non neoplastic, hyperplasia, tubule, simple • Tubule, cysts • Tubule, atrophy • Exacerbated chronic progressive nephropathy
Papillary necrosis (acute, chronic)	• *Necrosis, papilla*	• Mineralisation, papilla • Interstitium, infiltrate, inflammatory cell[a] • Dilation, tubule • Pyelonephritis • Hydronephrosis
Interstitial nephritis (chronic)	• Interstitium, infiltrate, inflammatory cell (stand-alone)[a]	• Fibrosis, interstitial
Pelvis erosion, ulceration (acute, chronic)	• *Erosion, pelvis* • *Ulcer, pelvis*	• Dilation, pelvis • Pyelonephritis • Pelvis, infiltrate, inflammatory cells • Simple hyperplasia, urothelium
Pelvis calculi, crystals (chronic)	• Calculus, pelvis • Crystals, pelvis	• Dilation, pelvis • Erosion, pelvis • Ulcer, pelvis • Pyelonephritis • Infiltrate inflammatory cells • Simple hyperplasia, urothelium
Renal preneoplastic and neoplastic lesions not caused by genotoxic mechanisms (chronic)	• Tubule, Atypical hyperplasia • Tubule, adenoma • Tubule, carcinoma	
Pelvis preneoplastic and neoplastic lesions not caused by genotoxic mechanisms (chronic)	• Pelvis, urothelium hyperplasia, atypical • Pelvis, transitional cell carcinoma • Pelvis, transitional cell papilloma	

[a]Interstitium, infiltrate, inflammatory cell' if stand-alone is considered a primary indicator of interstitial nephritis; if observed together with other findings, 'infiltrate, inflammatory cell, interstitium' is considered a secondary indicator of 'tubular injury', 'papillary necrosis', 'glomerular injury' and 'tubular crystals'.

A list of synonyms found in DARs/RARs of the indicators listed in the above table is given in the template for data collection in Annex A.

Chronic progressive nephropathy

As anticipated (see Section 2.2.2), the WG further discussed the possible human relevance of chronic progressive nephropathy. This condition is likely multifactorial in origin; however, its aetiology and pathogenesis are not fully elucidated, and it is not clear which renal structure is targeted first (WHO, 2015). When diagnosed, it is usually a morphological complex lesion involving the three main renal structures (glomerulus, tubule and interstitium). Based on the uncertainties on its (lack) of human relevance, the WG agreed to take a conservative approach and considered **exacerbated** chronic progressive nephropathy as a secondary indicator of possible effects occurring in the glomerulus (glomerular injury) and in the tubule (tubular injury).

Because rat CPN is both regenerative and degenerative, with a high rate of cell turnover, advanced disease may be a risk factor for renal tumour development.

Clinical pathology parameters, gross pathology and organ weights

The relevance of non-histological ancillary endpoints (as described under 2.2.1.1 and 2.2.1.2, e.g. changes in organ weight, clinical pathology parameters and macroscopic observations) as potential indicators of the specific effects was considered. However, since they are not sufficiently informative to indicate a specific effect, they were considered insufficient to reflect chemically-induced toxicity. Overall, considering that the above ancillary endpoints can be observed irrespectively of the mechanism or specific type of renal pathology (unspecific) and can be observed in severely compromised kidney conditions (unsensitive), these cannot be used neither as indicators of any specific effect, nor as line of evidence for the assessment of the CAG-membership probability.

5 | INSTRUCTIONS FOR HAZARD IDENTIFICATION CRITERIA TO BE USED FOR THE INCLUSION OF PESTICIDES INTO CAGs

5.1 | Scope of data collection

As indicated under 3.2, a list of 67 pesticides (ASs and metabolites) resulted from the prioritisation methodology (EFSA, 2024). Using a database of toxicological endpoints of pesticides (DTU, 2024), EFSA identified that 35 of the ASs from the priority list and 3 metabolites were associated with endpoints of interest for their possible inclusion in CAGs for the kidneys. The final list of prioritised ASs and metabolites was defined by EFSA according to the methodology described in Section 3.2, communicated to BPI prior to the data collection and will be included in the scientific report on data collection and establishment of CAGs (output 2 in Section 1.1).

5.2 | Instructions for data collection

For the 35 ASs and the 3 metabolites selected, any reported primary and/or secondary indicator(s) for the specific effects defined in Section 4.4.3, Table 1 will be collected from toxicological studies together with any further information needed to implement efficiently the principles of the hazard characterisation (see Section 6) and of the elicitation of CAG-membership probabilities (see Section 7).

The main sources of data are the DARs, RARs generated by RMSs in the context of Regulation (EC) No 1107/2009 and the respective EFSA conclusions. The original study reports might be consulted for further clarification on a case-by-case basis. Additional data sources (e.g. Joint Meeting on Pesticide Residues (JMPR) evaluations) will be considered and potential differences in the evaluation of studies or additional studies from those in DARs and RARs and EFSA conclusions will be captured in the data collection spreadsheet.

The toxicological studies to be considered are all in vivo studies by the oral route that include histopathological examination. Specifically for the reproductive toxicity studies, histopathological examination of the parental animals will only be considered.

The data will be collected in a searchable Excel database. A draft template (see Annex A) is made available to BPI, in charge of the data collection under the FPA GP/EFSA/PREV/2021/01. The outcome of the data collection will be published in a scientific report (see output 2 in Section 1.1).

5.3 | Criteria governing the inclusion of pesticides in a CAG

Only studies by oral route will be considered, with the exception of rodent studies with no more than 5 animals/sex/group, which are not considered robust enough for toxicological characterisation.[6] This rule does not apply to metabolites with the consideration that oral 28-day repeated dose studies, albeit having lower confidence for evaluation than other repeated dose studies (e.g. 90-day), are very often the only toxicity data available and used for setting reference values.

As a general rule, the inclusion of a pesticide in a CAG is determined by the observation of one or more primary indicator(s) or, in the absence of any primary indicator, of one or more secondary indicator(s) of the respective specific effect. Such observations should be treatment-related, statistically significant and/or biologically relevant (i.e. increase in incidence and/or severity of the observation, in alignment with the interpretation given in the DAR/RAR by using HCD when appropriate and available), even if observed in only one sex at a given time point including interim sacrifice, in one in vivo toxicological study conducted by any administration route (e.g. oral, dermal, inhalation), and assessed as « acceptable » and/or « supportive »[7] in the DAR, RAR or equivalent document unless the observation occurred at doses causing decreases in body weight (gain) ≥ 10% associated with feed intake or with clinical behavioural changes or mortality.

Also observations not relevant for human risk assessment (e.g. presence of indicators of preneoplastic/neoplastic changes as consequence of αlpha 2u-globulin nephropathy in male rats) are disregarded.

Due to the possible observation of multiple indicators relevant for more than one specific effect, an AS or a metabolite can be included in one or several CAGs.

With respect to effects which can be of either acute or chronic nature, 2 CAGs need to be established.

A first CAG is established in view of chronic exposure/risk assessments following the above general rule, and without any distinction between the respective indicators of acute and chronic nature. The principles applicable to the characterisation of the substances included in this 'chronic' CAG are described in the following section.

[6]Global Open Registry Nomenclature Information System (goRENI).

[7]The term supportive covers all the possible terminologies used by the authors of the source documents to indicate limitations to the regulatory acceptability of a study. To the extent that the limitations do not impair the evaluation of the specific effect under consideration, the study is considered as equally valid as a study assessed as fully acceptable.

A second tentative CAG is established, in principle in view of acute exposure/risk assessments, since acute effects in kidneys are considered suitable for the setting of an ARfD (Guidance on the setting of acute reference dose, ARfD).[8] In this case, however, indicators of acute nature triggering the inclusion of the substances or metabolite in the CAG could only be extrapolated from repeat dose studies not exceeding 90 days of treatment. Since the design of these toxicological studies is not suitable to identify the possible effects of the amount that can be ingested in a period of 24 h or less without appreciable health risk, it is considered not appropriate to perform hazard characterisation and acute exposure and risk assessment for the substances allocated to the acute CAGs.

6 | INSTRUCTIONS FOR HAZARD CHARACTERISATION OF PESTICIDES INCLUDED IN CAGs IN VIEW OF CRA OF CHRONIC EXPOSURE

This section illustrates the methodology to be followed for characterising the ASs/metabolites included in the CAGs in view of chronic risk assessments.

Each AS/metabolite included in each CAG will be characterised by the identification of a no observed adverse effect level (NOAEL) and a lowest observed adverse effect level (LOAEL) for the specific effect of interest. This may be different from the study NOAEL/LOAEL if the specific effect is not the most critical effect of the study. More specifically, for each substance, a NOAEL/LOAEL will be set for each indicator of the specific effect. Then, amongst all the studies available all different species, the study showing the lowest LOAEL with its respective NOAEL will be used for hazard characterisation as it reflects the most sensitive endpoint (indicator) of the most sensitive species for that specific effect.

In case a study failed to identify a NOAEL and only provided a LOAEL for an indicator(s) of interest, a NOAEL will be derived from this LOAEL by applying an extra uncertainty factor (UF). Although the EFSA guidance on default values to be used in the absence of measured data (EFSA Scientific Committee, 2012) recommends defining the size of such extra UF on a case-by-case basis, the concerned studies will not be reassessed, and, as a systematic approach, a default UF of 10 will be used. This conservative UF is then taken into account in the uncertainty analysis.

All the primary and secondary indicators either of acute or chronic nature are considered equally relevant for the setting of the NOAEL/LOAEL of the respective specific effect, although they may be weighted differently for CAG-membership probability (see CAG-membership probability under 7).

Although the use of sub-acute studies is allowed for CAG-allocation (hazard identification), it is generally not recommended for hazard characterisation when these studies serve as dose range finding (DRF) or mechanistic studies being not suitable to define the NOAEL and LOAEL because of limited power, i.e. low number of animals tested. The use of enhanced sub-acute studies in rodents (e.g. 28-day studies) can be considered for hazard characterisation in the case they are of sufficient power (i.e. 10 animals/sex/group) to enable robust statistical analysis. Any exemption from this will be justified.

6.1 | BMD analysis

Once the risk drivers are identified from the cumulative exposure calculations (step 10 under Appendix A), benchmark dose (BMD) analysis might be applied, if data allow, to support the uncertainty analysis process.

7 | INSTRUCTIONS FOR CAG-MEMBERSHIP PROBABILITY

7.1 | Introduction

The amount, reliability, relevance, specificity and consistency of evidence for causing the specific effects listed in Section 4.4.2 vary between ASs. This makes it uncertain which ASs/metabolites are actually causing the specific effect and therefore should be included in the respective CAG, with some ASs/metabolites being more likely to belong to the CAG than others. This can be quantified by assessing the probability that any AS/metabolite actually causes the specific effect.

In this report, this probability is referred to as CAG-membership probability. This probability can be estimated by a weight of evidence assessment consisting in a stepwise process with the following sequence of tasks:

1. Defining in precise terms the assessment question applicable to selected ASs/metabolites included in the CAG.
2. Identifying the lines of evidence (LoE) that are important for the assessment question: in the assessment of CAG-membership probabilities, LoE typically refer to the indicators of the specific effect under consideration but are not necessarily restricted to these indicators. Depending on the specific effect, additional factors contributing to the evidence can be defined.

[8]Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chemistry Toxicology, 43, 1569–1593. Available online: https://pubmed.ncbi.nlm.nih.gov/16040182/.

3. Rating qualitatively the weight of each line of evidence: the LoE are assessed with respect to their reliability and relevance to the assessment question. This assessment is conducted by expert discussion and results in qualifying each line of evidence for the weight of its contribution to the probability of the AS/metabolite causing the effect.
4. Reviewing the information available and listing the available LoEs for the selected ASs/metabolites included in the CAG.
5. Assessing for the selected ASs/metabolites the probability under consideration using the 'approximate probability scale' from EFSA's uncertainty guidance (EFSA Scientific Committee, 2018).

The present report covers the assessment of the weight of the lines of evidence, while the assessment of the CAG-membership probabilities are addressed in the report on the uncertainty analysis and the risk characterisation (output 4 in Section 1.1). If necessary, additional or refined lines of evidence will be considered under output 4, accordingly.

7.2 | Assessment question

The assessment question applicable to each AS/metabolite included in a CAG was agreed as follows:

'What is the probability that [AS/metabolite X] can cause, as a primary toxicological insult, [specific effect]?'

7.3 | Lines of evidence and assessment of their weight

For each specific effect, the LoEs applicable to the assessment question were defined and agreed (Table 2).

TABLE 2 Strength agreed for each line of evidence.

Specific effect	Lines of evidence	Strength (Agreed score)	Reasoning
Glomerular injury	Observation of at least one primary indicator	High	Observation of at least one primary indicator contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of at least one secondary indicator	Low	Observation of at least one secondary indicator provides limited evidence that an AS/metabolite is actually causing the effect.
	Observation of more than one primary indicator	High	Observation of more than one primary indicator of contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of at least two secondary indicators	Medium	Observation of at least two secondary indicators partly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality link between the exposure to the AS/metabolite and the effect.
	Observation of indicator(s) in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of indicator(s) in two independent studies in the same species	High	Repetition of observations in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS/metabolite has an harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by glomerular injury, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for glomerular injury	High	Whenever available, factual information on the MoA for glomerular injury highly contributes to the evidence that an AS/metabolite is actually causing the effect.
Tubular injury	Observation of at least one primary indicator(s)	High	Observation of at least one primary indicator contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of at least one secondary indicator	Low	Observation of at least one secondary indicator provides limited evidence that an AS/metabolite is actually causing the effect.

(Continues)

TABLE 2 (Continued)

Specific effect	Lines of evidence	Strength (Agreed score)	Reasoning
	Observation of more than one primary indicator	High	Observation of more than one primary indicator contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of at least two secondary indicators	Medium	Observation of at least two secondary indicators contributes less to the evidence that an AS/metabolite is actually causing the effect.
	Observation of degeneration and/or necrosis	High	Whenever degeneration and/or necrosis is/are observed, since definitive and irreversible conditions, this highly contributes to the evidence that an AS/metabolite is actually causing tubular injury.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality link between the exposure to the AS/metabolite and the effect.
	Observation of indicator(s) in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations in more than one species highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of indicator(s) in two independent studies in the same species	High	Repetition of observations in two independent studies in the same species highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by tubular injury effects, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for tubular injury (e.g. oxidative stress)	High	Whenever available, factual information on the MoA for tubular injury highly contributes to the evidence that an AS/metabolite is actually causing the effect.
Tubular crystals	Observation of crystals, tubule	High	Whenever crystals, tubule are observed, being this observation a primary indicator, this contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of degeneration or necrosis, tubule	Low	Observation of secondary histological lesions (degeneration/necrosis, tubule) has limited contribution to the evidence that an AS/metabolite is actually causing the effect.
	Observation of obstructive nephropathy*	Medium	Observation of obstructive nephropathy partly contributes to the evidence that that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality link between the exposure to the AS/metabolite and the effect.
	Observation of crystals, tubule in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations of crystals in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of crystals, tubule in two independent studies in the same species	High	Repetition of observations of crystals in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of degeneration/necrosis (secondary indicator) in more than one species and/or in two independent studies in the same species	Low	Observation of degeneration/necrosis (secondary indicator) in more than one species and/or in two independent studies provides limited contribution to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by crystal formation, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for crystals	High	Whenever available, factual information on the MoA for crystal formation highly contributes to the evidence that an AS/metabolite is actually causing the effect.

TABLE 2 (Continued)

Specific effect	Lines of evidence	Strength (Agreed score)	Reasoning
Papillary necrosis	Observation of papillary necrosis (primary indicator)	High	Whenever papillary necrosis is observed, being this observation a primary indicator, this contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s)	Low	Observation of any of the secondary histological lesions has limited evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality link between the exposure to the AS/metabolite and the effect.
	Observation of papillary necrosis in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations of papillary necrosis in more than species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of papillary necrosis in two independent studies in the same species	High	Repetition of observations of papillary necrosis in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species	Low	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species provides limited contribution to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by papillary necrosis, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for papillary necrosis	High	Whenever available, factual information on the MoA for papillary necrosis highly contributes to the evidence that an AS/metabolite is actually causing the effect.
Interstitial nephritis	Observation of inflammatory cell infiltrate, interstitium as stand-alone lesion (primary indicator)	High	Observation of inflammatory cell infiltrate, interstitium as a stand-alone lesion, being this a primary indicator, contribute to the evidence that an AS/metabolite is actually causing the effect.
	Observation of fibrosis, interstitial (secondary indicator)	Low	Observation of secondary histological lesion (fibrosis, interstitial) provides limited evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes a causal link between the exposure to the AS/metabolite and the effect.
	Observation of inflammatory cell infiltrate, interstitium in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations of inflammatory cell infiltrate, interstitium in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of inflammatory cell infiltrate, interstitium in two independent studies in the same species	High	Repetition of observations of inflammatory cell infiltrate, interstitium in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of fibrosis, interstitial in more than one species and/or in two independent studies in the same species	Low	Observation of fibrosis, interstitial in more than one species and/or in two independent studies in the same species provides limited evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by inflammation in the interstitium, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for inflammation (interstitial nephritis)	High	Whenever available, factual information on the MoA for inflammation (interstitial nephritis) highly contributes to the evidence that an AS/metabolite is actually causing the effect.

(Continues)

TABLE 2 (Continued)

Specific effect	Lines of evidence	Strength (Agreed score)	Reasoning
Pelvis erosion, ulceration	Observation of any of the primary indicator(s)	High	Observation of pelvis erosion and/or pelvis ulceration contributes to the evidence that an AS/metabolite is actually causing the effect.
	Absence of crystals	High	Absence of crystals contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s)	Low	Observation of any of the secondary indicators provides limited evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality between the exposure to the AS/metabolite and the effect.
	Observation of any of the primary indicator(s) in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations of any of the primary indicators in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the primary indicator(s) in two independent studies in the same species	High	Repetition of observations of any of the primary indicators in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species	Low	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species provides limited evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by erosion/ulceration in the pelvis, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for erosion, ulceration in the pelvis	High	Whenever available, factual information on the MoA for erosion, ulceration in the pelvis highly contributes to the evidence that an AS/metabolite is actually causing the effect.
Pelvis calculi, crystals	Observation of any of the primary indicator(s)	High	Observation of any of the primary indicator(s) contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s)	Low	Observation of any of the secondary indicator(s) provides limited evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes causality between the exposure to the AS/metabolite and the effect.
	Observation of primary indicator(s) in more than one species	High	Observation of primary indicator(s) in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of primary indicators(s) in two independent studies in the same species	High	Repetition of observations of any of the primary indicators in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species	Low	Observation of any of the secondary indicator(s) in more than one species and/or in two independent studies in the same species provides limited evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling (STOT RE)	High	Whenever an AS has a harmonised classification and labelling (STOT RE) for kidneys and there is evidence that the classification is driven by the deposition of crystals/calculi in the pelvis, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for calculi/crystals in the pelvis	High	Whenever available, factual information on the MoA for calculi/crystals in the pelvis highly contributes to the evidence that an AS/metabolite is actually causing the effect.

TABLE 2 (Continued)

Specific effect	Lines of evidence	Strength (Agreed score)	Reasoning
Renal preneoplastic and neoplastic changes not caused by genotoxic mechanisms	Evidence of a pathology continuum (sustained regeneration and hyperplasia following tubular injury in short duration studies and/or long duration studies (including the one under investigation) in the same species.	High	Observation of tubular degeneration/necrosis and/or tubular regeneration and/or tubular simple hyperplasia (not atypical) in short and/or long-term studies highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Evidence of exacerbated chronic progressive nephropathy in rodents	High	Observation of exacerbated chronic progressive nephropathy (CPN) in rodents highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes a causal link between the exposure to the AS/metabolite and the effect.
	Observation of any of the primary indicator(s) in more than one species	High	Considering the differences amongst species related to their physiology, repetition of observations in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of any primary indicator(s) in two independent studies in the same species	High	Repetition of observations in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling for carcinogenicity	High	Whenever an AS has a harmonised classification and labelling for carcinogenicity effects in kidneys, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for preneoplastic and neoplastic changes (e.g. cytotoxicity, conjugation with glutathione (GSH) followed by cytotoxicity, exacerbation of CPN)	High	Whenever available, factual information on the MoA for preneoplastic and neoplastic changes (e.g. cytotoxicity, conjugation with glutathione (GSH) followed by cytotoxicity, exacerbation of CPN) highly contributes to the evidence that an AS/metabolite is actually causing the effect.
Pelvis preneoplastic and neoplastic lesions not caused by genotoxic mechanisms	Evidence of a pathology continuum (sustained regeneration and hyperplasia following pelvis erosion/ulceration)	High	Observation of a pathology continuum (sustained regeneration and hyperplasia following pelvis erosion/ulceration) contributes to the evidence that an AS/metabolite is actually causing the effect.
	The AS/metabolite is also included in the CAG for pelvis erosion and/or ulceration	Medium	Whenever an AS/metabolite is also included in the CAG for pelvis calculi, crystals and/or in the CAG for pelvis erosion and/or ulceration, this is considered partly contributing to the evidence that an AS/metabolite is actually causing the effect.
	Evidence of dose–response relationship for any of the indicator(s)	High	When demonstrated, a dose–response relationship establishes a causal link between the exposure to the AS/metabolite and the effect.
	Observation of primary indicator(s) in more than one species	High	Considering the differences amongst species related to their physiology, observation of indicator(s) in more than one species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Observation of primary indicator(s) in two independent studies in the same species	High	Observation of indicator(s) in two independent studies in the same species contributes to the evidence that an AS/metabolite is actually causing the effect.
	Harmonised classification and labelling for carcinogenicity	High	Whenever an AS has a harmonised classification and labelling for carcinogenicity effects in kidneys, this highly contributes to the evidence that an AS/metabolite is actually causing the effect.
	Known MoA: mode of action relevant for preneoplastic and neoplastic changes in the pelvis	High	Whenever available, factual information on the MoA for preneoplastic and neoplastic changes in the pelvis highly contributes to the evidence that an AS/metabolite is actually causing the effect.

*Obstructive nephropathy is not in the list of primary or secondary indicators and will be collected as a finding in any study for risk drivers only.

8 | CONCLUSIONS

The present report defines the specific effects on kidneys eligible for CRA; it is therefore dedicated to hazard identification.

Effects covering different anatomical and histological structures in the kidney were considered and specific effects (in view of the establishment of respective CAGs) on tubule, glomerulus, interstitium and pelvis were defined, together with their respective primary and secondary indicators and their acute and/or chronic nature. Nine specific effects were identified to define the following CAGs: glomerular injury, tubular injury, tubular crystals formation, papillary necrosis, interstitial nephritis, pelvis erosion/ulceration, pelvis calculi/crystals, renal preneoplastic and neoplastic lesions and pelvis preneoplastic and neoplastic lesions.

Criteria for inclusion of ASs/metabolites into CAGs were defined and a hazard characterisation methodology has been elaborated.

Finally, this report illustrates the LoE for assessing the probability that the ASs/metabolites included in the CAGs are actually causing the effect of interest, i.e. CAG-membership probability.

The present report has been finalised after due consideration by the WG of the comments received through the public consultation (https://open.efsa.europa.eu/consultations/a0c0900000HpktxAAB?search=kidneys). The original comments submitted during the public consultation and the way they were addressed by the WG can be found in the Public Consultation Report as Annex of the present output under supporting information.

9 | RECOMMENDATIONS

- In the absence of fully defined and validated AOPs, the kidney specific effects proposed are based exclusively on adverse outcomes (histopathological findings) observed in regulatory studies. However, considering the recent indications provided by the EFSA Scientific Committee, it is recommended to further develop AOPs by focusing on the adverse effects of pesticides.
- For further development of AOPs on kidney effects, the use of in vitro, in silico new approach methodologies (NAMs) should be encouraged to support mechanistic understanding of the observed effects.
- In addition to the standard battery of traditional markers of nephrotoxicity used in the toxicological studies, EFSA recommends to include the analysis of: additional biomarkers in urine such as injury molecule-1 (KIM-1), clusterin (CLU) and neutrophil gelatinase-associated lipocalin NGAL to evaluate acute kidney injury; and the sequential monitoring of serum creatinine or glomerular filtration rate (GFR) (if measured or estimated using appropriate approaches) alongside with urine albumin to evaluate chronic kidney toxicity. Changes in biomarkers/functional assessment could be interpreted together with the histological changes assessed either at specific time-points during sequential animal sacrifice or at the study conclusion. This integrated approach would allow a deeper understanding of the impairment of kidney structure/function and contribute to a more informed establishment of CAGs.
- For future updates of the kidney specific effects relevant for performing CRA of pesticide residues it is recommended to explore the possibility to integrate specific effects with additional indicators (e.g. biomarkers, as above mentioned) to better address renal effects of human clinical relevance, such as 'acute kidney injury' and 'chronic kidney disease' considering their relevance in and clinical setting; this should accompany the development of AOPs with adverse outcomes covering these effects.

ABBREVIATIONS

ADI	acceptable daily intake
AKI	acute kidney injury
AO	adverse Outcome
AOP	adverse outcome pathway
AOP Wiki	Collaborative Adverse Outcome Pathway Wiki
ARfD	acute reference dose
AS(s)	active substances
ATN	acute tubular necrosis
ATP	adenosine triphosphate
BMD	Benchmark dose
BPI	Benaki Phytopathological Institute
BUN	blood urea nitrogen
CAG	cumulative assessment group
CLU	clusterin
CPN	chronic progressive nephropathy
CRA	cumulative risk assessment
CYP450	cytochrome P450
DA	dose addition
DAR	draft assessment report
DRF	dose range finding

DTU	Technical university of Denmark
EKE	expert knowledge elicitation
FPA	Framework Partnership Agreement
GFR	glomerular filtration rate
GSH	gluthathione
HQ	Hazard Quotient
IARC	International Agency for Research on Cancer
INHAND	International Harmonization of Nomenclature and Diagnostic criteria
KEs	Key Events
KERs	key event relationships
KIM-1	kidney injury molecule-1
LOAEL	lowest observed adverse effect level
LoE	line of evidence
MCRA	Monte Carlo Risk Assessment (Software)
MIE	molecular initiating event
MoA	mode of action
MRLs	maximum residue levels
MTD	maximum tolerated dose
NAMs	new approach methodologies
NGAL	neutrophil gelatinase-associated lipocalin
NOAEL	no observed adverse effect level
NSAIDs	non-steroidal anti-inflammatory drugs
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PPP(s)	Plant Protection Products
PPR	Plant Protection Products and its Residues
RAR	renewal assessment report
RMS	Rapporteur Member State
ROS	reactive oxygen species
sCR	serum creatinine
STOT RE	specific target organ toxicity – repeat exposure
UF	uncertainty factor
U.S. EPA	United States Environmental Protection Agency
WG	Working Group
α2u	alpha 2-urinary globulin nephropathy

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REQUESTOR

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX A

The 14 steps of the CRA of pesticide residues

Step	Month	Content description
1	1–10	Hazard identification: Definition of the specific effects of relevance for CRA The task consists in the identification and unambiguous definition of the specific effects of relevance for CRA for the organ/system under consideration, in consistency with the hazard-driven criteria described in the guidance document of the EFSA Scientific Committee on scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals[9]. For each specific effect, respective relevant indicators (i.e. toxicological endpoints identifiable in toxicological studies reflecting the specific effect) are defined. To prepare Step 2, the conditions triggering the inclusion of active substances in each CAG to be established, and the principles of their characterisation for the respective specific effects, are also defined. Where relevant, also the lines of evidence (LoE) to be considered in the assessment of the CAG-membership probability (i.e. the probability that a substance included in a CAG is actually causing the respective specific effect) are defined. A first draft scientific report on the identification of the specific effects for the organ/system under consideration will be prepared by month 10.
2	8–13	Hazard characterisation: Establishment of CAGs For each of the specific effects defined in Step 1, a CAG will be established. The task first consists in collecting all the indicators of the specific effects concerning the active substances prioritised by the prioritisation method and causing effects on the organ/system under consideration, as well as any further information needed to implement efficiently the principles of the hazard characterisation defined in step 1 (e.g. details on the study design needed to allow the combination of studies for the characterisation of substances) or the uncertainty analysis in step 11 (e.g. data allowing the assessment of the dose–response relationship). These data are collected from Draft Assessment Reports, Renewal Assessment Reports and their addenda generated by Rapporteur Member States in the context of Regulation (EC) No 1107/2009, as well as from other sources of similar nature (e.g. JMPR evaluations), in a predefined format/template. A robust quality check procedure of the data collection needs to be put in place. Secondly, the collected data are assessed to decide which active substance is to be included in the CAG(s), using the conditions of inclusion defined in Step 1. Finally, all the substances included in the CAGs are characterised for the respective specific effects, using the characterisation principles defined in Step 1. A first draft scientific report on the establishment of CAGs for the organ/system under consideration is prepared by month 13.
3	10–19	Public consultations Two public consultations are conducted. The first public consultation is launched at month 10 and concerns the draft scientific report on the identification of the specific effects. The second public consultation is launched at month 14 and concerns the draft scientific report on the establishment of the CAGs. Both public consultations are initiated by EFSA and take place on the EFSA website for a 2-month period. Technical reports covering the two public consultations are prepared during month 17. These technical reports describe the results of the public consultations and provide answers to each submitted comment. They are published as annexes to the respective scientific reports.
4	14	Hazard identification (update) A second draft scientific report on the identification of the specific effects for the organ/system under consideration is prepared, based on the outcome of the public consultation, for submission to the PPR panel or two external experts.
5	16	Hazard characterisation (update) A second draft scientific report on the establishment of CAGs for the organ/system under consideration is prepared based on the outcome of the public consultation, for submission to the PPR panel.
6	17–18	PPR panel consultation or consultation with selected experts The PPR panel is consulted for the endorsement of the second draft scientific report on the identification of the specific effects for the organ/system under consideration and the second draft scientific report on the establishment of CAGs for the organ/system under consideration. Alternatively, the draft outputs may be submitted to external review.
7	19	Hazard identification (finalisation) The final scientific report on the identification of the specific effects for the organ/system under consideration (**Output 1**) is prepared, based on the outcome of the consultation of the PPR panel and submitted to the internal EFSA approval during month 19.
8	19	Hazard characterisation (finalisation) The final scientific report on the establishment of CAGs for the organ/system under consideration (**Output 2**) is prepared, based on the outcome of the consultation of the PPR panel submitted to the internal EFSA approval during month 19.

[9]EFSA Scientific Committee, More, S. J., Bampidis, V., Benford, D., Bragard, C., Hernandez-Jerez, A., Bennekou, S. H., Halldorsson, T. I., Koutsoumanis, K. P., Lambré, C., Machera, K., Naegeli, H., Nielsen, S. S., Schlatter, J. R., Schrenk, D., Silano, V., Turck, D., Younes, M., Benfenati, E., Crépet, A., Te Biesebeek, J. D., Testai, E., Dujardin, B., Dorne, J. L. C. M., & Hogstrand, C. (2021). Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals. EFSA Journal, 19(12), 7033. https://doi.org/10.2903/j.efsa.2021.7033.

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Step	Month	Content description
9	19	Problem formulation After establishment of the CAGs for the organ/system under consideration, the assessment questions (i.e. the precise health effects for which a cumulative risk assessment needs to be performed) to be addressed in the following steps of the process (exposure assessment, uncertainty analysis and risk characterisation) are defined. The number of assessment questions per organ/system is not necessarily the same as the number of identified specific effects. If this does not impair the protectiveness of the CRA for the organ/system under consideration, the number of assessment questions can be smaller than the number of CAGs. The scope (e.g. commodities, consumer populations) and type of assessment (e.g. acute, chronic) related to each assessment question are also defined.
10	17–22	Cumulative exposure assessments Cumulative exposure assessments are performed by probabilistic modelling. These assessments meet the following conditions: • They are conducted with the MCRA software[10]. • Indicatively, they are performed for the up to about 30 populations of adults, children and toddlers in different EU Member States. • Indicatively, they use monitoring data from the latest available 3-year cycle of the Member States monitoring programmes on up to about 40 raw primary commodities, some of their processed products and on commodities intended for infants and children. The contribution of residues on water is considered on the basis of critical regulatory scenarios. • Inner loop execution is performed in accordance with the harmonised technical approach on the parameters governing retrospective cumulative exposure assessment of the European commission[11], for the Tier II scenario. • Sampling uncertainties affecting the occurrence and consumption data are quantified by outer loop execution where the inner loop execution is repeated 100 times. Prior to each execution, new consumption and occurrence data sets of the same size are generated from random sampling with replacement from the original data sets. • Sensitivity analyses are performed to explore the impact of sources of uncertainty on the results. At least the following sensitivity analyses are performed: a. Sensitivity with left-censored data imputed at 1/2 LOQ on commodities for which the use of the active substance is authorised. b. Sensitivity analysis with all left-censored data imputed at zero. c. Sensitivity analysis assuming that residues will not be present in any processed food. d. Sensitivity analysis excluding samples obtained through a selective sampling strategy. e. Sensitivity analysis assuming that samples are not subject to unit-to-unit variability (acute effects only). f. Sensitivity analysis assuming that no residues are present in drinking water. g. Sensitivity analysis excluding non-compliant samples (i.e. samples in which residues of at least one pesticide exceeds the MRL multiplied by 2). Additional sensitivity analyses might be performed following the analysis of the detailed results of the calculations and/or according to needs identified during the uncertainty analysis process (step 11 below). The results of cumulative exposure calculations are expressed as total margin of exposure (MOET). 95% confidence intervals (incl. central estimate) are reported for P50, P90, P95, P99 and P99.9 of the exposure distribution at least. Violin plots of the confidence intervals at the threshold for regulatory consideration (i.e. P99.9) are presented for the selected population groups. Detailed information on the input data and output data are formatted and reported in a way similar to the recent EFSA scientific reports on CRA regarding chronic AChE inhibition[12] and craniofacial alterations[13]. A draft scientific report on the cumulative exposure assessment is prepared by month 22. For each CAG, this report includes one Excel workbook containing the input data for the exposure assessment (see https://zenodo.org/record/7143238#.Y1FeXHZBzD4 for guidance on the content) and one excel workbook containing the output data concerning the Tier II exposure assessment (see https://zenodo.org/record/7143238#.Y1FeXHZBzD4 for guidance on the content).

(Continues)

[10]https://mcra.rivm.nl/Select.

[11]https://ec.europa.eu/food/plant/pesticides/max_residue_levels/cumulative_risk/technical-annex_en.

[12]EFSA (European Food Safety Authority), Anastassiadou, M., Choi, J., Coja, T., Dujardin, B., Hart, A., Hernandez-Jerrez, A. F., Jarrah, S., Lostia, A., Machera, K., Mangas, I., Mienne, A., Schepens, M., Widenfalk, A., & Mohimont, L. (2020). Cumulative dietary risk assessment of chronic acetylcholinesterase inhibition by residues of pesticides. EFSA Journal, 19(2), 6392. https://doi.org/10.2903/j.efsa.2021.6392.

[13]EFSA (European Food Safety Authority), Anagnostopoulos, C., Anastassiadou, M., Castoldi, A. F., Cavelier, A., Coja, T., Crivellente, F., Dujardin, B., Hart, A., Hooghe, W., Jarrah, S., Machera, K., Menegola, E., Metruccio, F., Sieke, C., & Mohimont, L. (2022). Scientific Report on retrospective cumulative dietary risk assessment of craniofacial alterations by residues of pesticides. EFSA Journal, 20(10), 7550. https://doi.org/10.2903/j.efsa.2022.7550.

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Step	Month	Content description
11	19 to 24	Uncertainty analysis and risk characterisation An uncertainty analysis is conducted according to the stepwise process described in the recent EFSA scientific reports on CRA regarding chronic AChE inhibition and craniofacial alterations. It includes the following steps: • Step 11.1: Identification of sources of uncertainty and collection of information supporting the assessment of their impact. • Step 11.2: Evaluation of the individual impact of all sources of uncertainty. • Step 11.3: Evaluation of the combined impact of uncertainties relating to exposure on one hand, and relating to toxicology on the other hand. • Step 11.4: 1-D Monte Carlo simulations to combine distributions quantifying uncertainties related to exposure and toxicology with the uncertainty distribution for the MOET at the 99.9th percentile of exposure generated by the exposure model. • Step 11.5: Accounting for dependencies and differences between populations. This uncertainty analysis aims at quantifying the probability of the MOET at 99.9th percentile of the exposure distribution being less than 100 for all populations under consideration, which is the final expression of the cumulative risks. It is supported by expert knowledge elicitation (EKE), following the EFSA guidance[14], or by expert judgement. When the uncertainty analysis is supported by EKE, at least 6 independent experts are involved in each area of scientific expertise necessary for the elicitation process (toxicology and dietary exposure). The process is supported by technical notes which document the impact of uncertainties and by ad hoc sensitivity analyses. These can include recalculation of the cumulative exposure after Benchmark Dose (BMD) modelling for the substances which contribute the most to the cumulative risk. When this is required, the principles described in the guidance of the EFSA Scientific Committee on the use of the BMD approach in risk assessment[15], including its updates, are applied and the corresponding EFSA platform for BMD analysis is used. Step 11.5 is also facilitated by simulations testing the impact of dependencies and the effect of downshift/upshift of the MOET distribution on the probability of the MOET at 99.9th percentile being below 100. Hearings of stakeholders in possession of particular data or knowledge informing the uncertainty analysis can be organised. A draft scientific report on the uncertainty analysis and the risk characterisation is prepared by month 24.
12	25–26	PPR Panel consultation or consultation with selected experts The PPR panel is consulted on the draft scientific report on the cumulative exposure assessment and on the draft scientific report on the uncertainty analysis and the risk characterisation. Alternatively, the draft outputs may be submitted to external review.
13	27	Cumulative exposure assessment (finalisation) The final scientific report on the cumulative exposure assessment (**Output 3**) is prepared based on the outcome of the consultation of the PPR panel.
14	27	Uncertainty analysis and risk characterisation (finalisation) The final scientific report on the uncertainty analysis and the risk characterisation (**Output 4**) is prepared based on the outcome of the consultation of the PPR panel.

As indicated in the above table, for each organ/system, the outcome of the entire process will be reported by the following outputs:

- **Output 1**: Scientific report identifying the specific effects of relevance for CRA and defining the relevant indicators, criteria for inclusion in CAGs, hazard characterisation rules and LoE for eventual assessment of the CAG-membership probability.
- **Output 2**: Scientific report establishing the CAG, covering the data collection, the identification of substances causing the effect and their characterisation by a point of departure.
- **Output 3**: Scientific report on the cumulative exposure assessment for the (critical) specific effects defined in output 1.
- **Output 4**: Scientific report on the uncertainty analysis and the risk characterisation for the specific effects addressed in output 3.

[14]EFSA (European Food Safety Authority). (2014). Guidance on Expert Knowledge Elicitation in Food and Feed Safety Risk Assessment. EFSA Journal, 12(6), 3734. https://doi.org/10.2903/j.efsa.2014.3734.

[15]EFSA Scientific Committee, Hardy, A., Benford, D., Halldorsson, T., Jeger, M. J., Knutsen, K. H., More, S., Mortensen, A., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Silano, V., Solecki, R., Turck, D., Aerts, M., Bodin, L., Davis, A., Edler, L., Gundert-Remy, U. S., & Slob, W., Bottex, B., Abrahantes, J. C., Marques, D. C., Kass, G., & Schlatter, J. R. (2017). Update: Guidance on the use of the benchmark dose approach in risk assessment. EFSA Journal, 15(1), 4658. https://doi.org/10.2903/j.efsa.2017.4658.

ANNEX A

Data collection template

Annex A is available under the Supporting Information section on the online version of the scientific output.

ANNEX B

Public Consultation Report

Annex B is available under the Supporting Information section on the online version of the scientific output.