1 Prioritised substance group: Pesticides

Pyrethroids (group), chlorpyrifos, dimethoate, glyphosate (including the co-formulant POE-tallow amine) and fipronil (D.4.5)

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1.1 Background Information

1.1.1 Hazardous properties

Regulatory hazard classifications of the substances are shown in Table 20. The purpose of this section is to identify knowledge gaps and to pinpoint research areas where epidemiological studies have raised concern for potential adverse health effects in humans at population-level exposures and where more HBM data are needed to further evaluate safe exposure levels (i.e., to to answer the policy-related questions). This is especially important for health outcomes for which animal models might not be sufficiently sensitive, e.g. for developmental neurotoxicity (Fritsche et al. 2018) or for which valid animal models do not exist as for example for childhood leukemia (EFSA Panel on Plant Protection Products and their residues (PPR) et al. 2017).

1.1.1.1 Pyrethroids

Pyrethroids compose one of the major classes of insecticides in the EU and worldwide. They are synthetic analogs of pyrethrins naturally present in the Chrysanthemum flower but, compared to the pyrethrins, they are less susceptible to hydrolysis and photodegradation and therefor more stable in the environment. They are highly toxic to insects, but also fish and cats are particularly sensitive to pyrethroids toxicity. Pyrethroids are chiral compounds and the formulations consist of multiple stereoisomers which often have different toxic potencies and toxicokinetic. Based on structural differences and on signs of acute toxicity in rodents, pyrethroids are divided into type I and type II. Type 1 (e.g. permethrin and allethrin) comprise a wide structural variety of compounds lacking a cyano moiety at the alpha-position and elicit an intoxication syndrome that includes general tremor, convulsive twitching, hypersensitivity and aggression, designated the T (tremor) syndrome. Distinctively, type II pyrethroids (e.g., cypermethrin and deltamethrin) contain the the α-cyano-3- phenoxybenzyl moiety and cause an intoxication syndrome that includes salivation and progressive writhing convulsions (choreoathetosis) designated the CS syndrome. However, some pyrethroids exhibit intermediate signs of intoxication that contain elements of both the T and CS syndromes. (Soderlund 2012). The main mechanism of action for both types of pyrethroids is axonal sodium channel depolarisation causing repetitive nerve impulses both in insects and non-target organisms including mammals. However, each type of pyrethroids exhibit secondary targets, including voltage-gated calcium and gamma-aminobutyric acid (GABA)-gated chloride channels, also involved in their acute neurotoxic actions (Soderlund 2012). Increased sensitivity to acute pyrethroid toxicity during early development (the neonatal period), as seen in animal studies, was suggested to be due to lower metabolic capacity and expression of a more sensitive form of the voltage-gated sodium channel (Meacham et al. 2008).

Compared with other major classes of insecticides, like organophosphates and carbamates, pyrethroids have lower acute toxicity in mammals. However, potential human health effects of pyrethroids at low environmental and dietary exposure levels have only been addressed in few epidemiological studies, despite pyrethroids were introduced for the control of insect pest more than three decades ago. Since pyrethroids are known neurotoxicants and some have endocrine

disrupting properties *in vitro* (Saillenfait et al. 2016a), pyrethroids have the potential to interfere with neurodevelopment (Abreu-Villaca and Levin 2017; Bjorling-Poulsen et al. 2008) and to disturb neuroendocrine axes and reproductive development (Koureas et al. 2012; Saillenfait et al. 2015), especially if exposure occurs in vulnerable developmental periods during childhood and foetal life.

Developmental neurotoxicity (DNT)

In research studies using animal models, developmental exposures to pyrethroids has been related to a wide range of behavioural, neurochemical and molecular effects including altered brain vascular formation, increased blood-brain barrier permeability, decreased monoamine levels and neocortical and hippocampal thickness, alterations in cholinergic muscarinic, dopaminergic and noradrenergic systems, delayed physical and motor development, decreased locomotor activity, impaired motor coordination, and deficient learning and memory, reviewed by (Abreu-Villaca and Levin 2017). In a recent study, offspring of mice orally exposed to the pyrethroid deltamethrin during gestation and lactation showed several ADHD-like features, including hyperactivity, impulse-like behaviours, and deficits in working memory and attention. Elevated dopamine transporter levels, lower synaptic dopamine, and increased D1 dopamine receptor levels accompanied the behavioural effects (Richardson et al. 2015). Although the pathophysiology of ADHD in humans is poorly understood, disruption of dopaminergic, noradrenergic, and serotonergic neurotransmission has been suggested to be central mechanisms (Thapar and Cooper 2016). Thus, these findings indicate that pyrethroids might interfere with neurobehavioral development in humans. Accordingly, some recent epidemiological studies reported associations between exposure to pyrethroids during pregnancy (evaluated by biomonitoring of maternal urinary pyrethroid metabolites) and lower cognitive scores at three months of age (Fluegge et al. 2016), at 12 months of age (Xue et al. 2013), and at 24, but not at 36, months of age (Watkins et al. 2016). One study, found no associations with child cognition at 12 months of age but with lower Social-Emotional scores on the Bayley Scales of Infant Development (Eskenazi et al. 2018). These findings were also supported by a study from New York City in which detectable levels of pyrethroid metabolites in maternal urine were associated with a variety of behavioural functioning deficits among children measured at four, six, and seven to nine years of age by the Behavioural Assessment System for Children and the Behaviour Rating Inventory of Executive Function (Furlong et al. 2017). Likewise, maternal pyrethroid exposure was not associated with cognitive development among the children at age 6 years in the French PELAGIE Cohort (Viel et al. 2015) but with internalising difficulties assessed by the Strengths and Difficulties Questionnaire (Viel et al. 2017), despite the exposure level in this cohort was lower than reported from other cohort studies, i.e., the common pyrethroid metabolite 3-PBA was only detectable in urine samples from 30 % of the women compared to 80-90 % in other studies (McKelvey et al. 2013; Wielgomas et al. 2013).

Since growth and functional development of the human brain continues during childhood, it is assumed that the postnatal period is also vulnerable to neurotoxic exposures (Grandjean and Landrigan 2006).

Accordingly, childhood pyrethroid exposure (child urinary concentrations of pyrethroid metabolites) has been associated with impaired cognitive functions, especially verbal and memory functions (Viel et al. 2015) and increased risk of behavioural problems (Oulhote and Bouchard 2013; Viel et al. 2017) including attention-deficit hyperactive disorder (ADHD) (Wagner-Schuman et al. 2015) even at very low urinary metabolite concentrations as reported from the French PELAGIE-cohort (Viel et al. 2015; Viel et al. 2017). Bifenthrin and alpha-cypermethrin are classified as STOT RE 1 – H372 and SOT RE2 – H373 (Table 3) for effects on the nervous system but they are not classified as developmental neurotoxicants.

Endocrine disrupting properties and carcinogenicity

Besides neurotoxic properties, some pyrethroids, or their metabolites, have been reported to possess endocrine disrupting properties *in vitro* (Brander et al. 2012; Saillenfait et al. 2016a) and several pyrethroids (permethrin, acrinathrin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, etofenprox, lambda-cyhalothrin, tau-fluvalinate and tefluthrin) were categorised as potential endocrine disruptors (EDs) in an impact assessment report of JRC (EC 2016) (see Table 1). Accordingly, most of the pyrethroids approved for use in the EU are included in the TEDX list.

In an EU assessment report from 2014, (Reg (EU) No 528/2012) permethrin was reported to cause histopathological changes in the adrenals and increased liver weight in dogs of both sexes (NOAEL: 5 mg/kg bw/d). In experimental studies, the pyrethroid fenvalerate caused increased gonadotropins and a decline in testosterone in male rats (Mani et al. 2002). Perinatal exposure to cypermethrin, disturbed sexual maturation and later reproductive function in rat male offspring (Singh et al. 2017). Exposure to deltamethrin throughout gestation and lactation caused shorter ano-genital distance (AGD) in male offspring (Kilian et al. 2007) indicating insufficient androgen action, whereas no effects on AGD or on expression of genes involved in testicular steroidogenesis was observed when the exposure period was restricted to the period of sexual differentiation between gestational day 13 and 19 (Saillenfait et al. 2016b).

Several recent epidemiological studies have raised concerns about potentially adverse effects on sperm quality and sperm DNA, reproductive hormones, and pregnancy outcome (Saillenfait et al. 2015). Hence, population representative urinary concentrations of pyrethroid metabolites, have been associated with reduced semen quality (Meeker et al. 2008), higher serum concentrations of FSH and LH, and lower inhibin B, and testosterone (Meeker et al. 2009) and sperm aneuploidy (Radwan et al. 2015). Among Chinese women, urinary concentrations of pyrethroid metabolites were significantly associated with increased risk of primary ovarian function (POI) (Li et al. 2018).

Only very few human studies have addressed other health outcomes and potential associations with e.g., carcinogenicity, immune system function, and metabolic disturbances are unclear (Saillenfait et al. 2015; Xiao et al. 2017). Residential exposure to insecticides after indoor use was associated with increased risk of childhood leukaemia (Chen et al. 2015; Ntzani et al. 2013) as also reported in meta-analyses (Bailey et al. 2015; Chen et al. 2015). In most studies it was not possible to pinpoint specific pesticides but pyrethroids constitute the major group of insecticides used for indoor pest control and a study from Shanghai reported elevated risk of childhood leukaemia associated with urinary levels of pyrethroid metabolites in the children (Ding et al. 2012).

At present, none of the pyrethroids at the EU market is classified as reproductive toxicants (H360-H361d) but etofenprox is classified as a lactational hazard (H362) (Table 3). Bifenthrin is classified as “suspected of causing cancer” (Carc. 2 – H351).

A few pyrethroids (permethrin, fenvalerate and deltamethrin) were reviewed by IARC in 1991 (Volume 53) and assigned to Group 3 (not classifiable as to its carcinogenicity to humans). Permethrin is currently listed as a high priority compound for assessment by IARC and was classified as “likely to be carcinogenic to humans” after oral exposure by the US EPA in 2009 (EPA 2009). Permethrin is also listed on the Annex III inventory as it meets the mutagenicity criteria of Annex III to the REACH regulation. Furthermore, genotoxic properties for different pyrethroids have been indicated in experimental studies (Muranli 2013; Ramos-Chavez et al. 2015; Vardavas et al. 2016).

Also, immunotoxic properties have been indicated in some experimental studies for bifenthrin (Wang et al. 2017) and deltamethrin (Kumar et al. 2015). Permethrin, bifenthrin and esfenvalerate are classified as skin sensitizers (Table 3).

Piperonyl butoxide (PBO) - pyrethroid co-formulant

Pyrethroids are often applied in combination with piperonyl butoxide (PBO), a cytochrome P450 inhibitor causing decreased breakdown of the pyrethroids. In the insects. PBO is also a known inhibitor of human cytochrome P450. Health effects related to use of pyrethroid-containing products may thus be due to combined or synergistic action of the pyrethroid and the synergist PBO. Accordingly, PBO, but not permethrin, measured in maternal hair samples during pregnancy was associated with impaired neurodevelopment at 36 months of age (Horton et al. 2011) and cough in the children at age 5-6 years (Liu et al. 2012). However, whether PBO is a causal factor or rather a proxy for the total pyrethroid exposure cannot be ruled out from the cited study.

PBO is approved as a BP (T18) in the EU (Reg (EU) 2016/2288) (see EU assessment report: http://dissemination.echa.europa.eu/Biocides/ActiveSubstances/1344-18/1344-18_Assessment_Report.pdf) with proposed C&L as Carc.2; H351 (increased incidence of hepatocellular adenomas and carcinomas in mouse), STOT SE 3; H335 (respiratory tract irritation), EUH066. The long and medium term AELs and ADI are equal to 0,2 mg/kg bw/d and the AEL short term is 1,0 mg/kg bw/d. The substance is included in the CoRAP for Substance Evaluation, scheduled to start in 2019 by Sweden. According to the CoRAP justification document, it will be evaluated for ED and PBT properties (https://echa.europa.eu/substance-information/-/substanceinfo/100.000.070)

1.1.1.2 Chlorpyrifos and dimethoate (organophosphates)

Developmental neurotoxicity

Chlorpyrifos and dimethoate are organophosphate (OP) insecticides and both compounds are suspected developmental neurotoxicants and endocrine disruptors. Generally, OPs irreversibly inhibit acetylcholinesterase (AChE), the enzyme that catalyses the breakdown of acetylcholine (ACh) to acetate and choline in synaptic clefts in both insects and off-target organisms' nervous system. In humans and other mammals, when AChE inhibition exceeds 70–75%, acute poisoning results in a severe "cholinergic syndrome", in which accumulation of acetylcholin leads to peripheral signs such as increased sweating and salivation, bronchoconstriction, miosis, increased gastrointestinal motility and tremors; and central nervous system effects such as dizziness, mental confusion, and eventually, convulsions and death (Krieger 2001).

Chlorpyrifos is metabolised to the more toxic intermediate chlorpyrifos-oxon (bioactivation), which is a strong inhibitor of AChE in brain, peripheral tissue, and serum and red blood cells. Besides, AChE inhibition, OPs have been shown in experimental studies to induce a variety of neurotoxic effects, particularly after developmental exposure, even at doses devoid of systemic toxicity.

Hence, developmental OP exposure has been associated with altered function of numerous proteins other than AChE and these additional mechanisms are suggested to be involved in the developmental neurotoxicity of these substances, although the exact mechanisms is not understood (for review see Abreu-Villaca and Levin (2017)). Exposure to chlorpyrifos during developmental has been reported to disrupts neuronal cell replication and differentiation through a variety of cellular mechanisms, culminating in loss of neurons, "mis-wiring" of brain circuits and deficiencies in synaptic function (Slotkin and Seidler 2005, 2009; Slotkin et al. 2012).

Thus, disturbance of brain development is the main health concern related to OP exposure in general and to chlorpyrifos in particular. Several reviews of neurodevelopmental effects of OP in humans have been conducted and most of them conclude that exposure during pregnancy, at levels found among groups of the general population, may have negative effects on children's neurodevelopment (Gonzalez-Alzaga et al. 2014; Munoz-Quezada et al. 2013; Ross et al. 2013).

Most of the human studies have been carried out in the US and have focused on assessing brain functions in children in relation to prenatal organophosphate exposure. In a longitudinal birth cohort study among farmworkers in California (the CHAMACOS cohort), maternal urinary concentrations

of organophosphate metabolites in pregnancy were associated with abnormal reflexes in neonates (Young et al. 2005), adverse mental development at two years of age (Eskenazi et al. 2007), attention problems at three and a half and five years (Marks et al. 2010), and poorer intellectual development at seven years (Bouchard et al. 2011), and higher parent and teacher reported autism spectrum disorder scores at age 7 to 14 years (Sagiv et al. 2018). In accordance with this, a birth cohort study from New York reported impaired cognitive development at the ages 12 and 24 months and six to nine years related to maternal urine concentrations of organophosphates in pregnancy (Engel et al. 2011). However, some recent studies, based on cohorts of pregnant women recruited from the general population and without occupational or extensive residential exposure, did not find indication of impaired neurodevelopment in the children at 1-5 years of age (Donauer et al. 2016) or 6 years of age (Cartier et al. 2016) associated with maternal urinary concentrations of organophosphate in pregnancy. The later study, from the French PELAGIE cohort reported two to six times lower OP metabolite concentrations for pregnant women, than reported from other European studies as well as in studies from the US and Canada (Marks et al. 2010; Spaan et al. 2015; Yolton et al. 2013) (Annex 1, Table 2).

Regarding childhood exposure level, five-year-old children from the CHAMACOS cohort had higher risk scores for development of attention deficit hyperactive disorder (ADHD) if their urine concentration of organophosphate metabolites was elevated (Marks et al. 2010). Based on cross-sectional data from NHANES in the US, the risk of developing ADHD increased by 55 % for a ten-fold increase in urinary concentration of organophosphate metabolites in children between eight and 15 years (Bouchard et al. 2010).

Chlorpyrifos was until 2020 the most used OP in the EU (non-renewal of authorisasation from February 2020) and worldwide and it is also the best studied OP in both animal models and in vitro studies. There is evidence for developmental neurotoxicity of chlorpyrifos both from experimental and epidemiological studies (Abreu-Villaca and Levin 2017).

The strongest evidence for neurodevelopmental effects in humans comes from at study performed at the Columbia Children's Center for Environmental Health (CCCEH) at Columbia University in New York. This inner-city birth cohort study was initiated before chlorpyrifos was banned for residential use in 2000 in the US.

The concentration of chlorpyrifos in umbilical cord blood was significantly associated with delayed psychomotor and mental development in children in the first three years of life (Rauh et al. 2006), poorer working memory and full-scale IQ at seven years of age (Rauh et al. 2011), structural changes, including decreased cortical thickness, in the brain of the children at school age (Rauh et al. 2012), and mild to moderate tremor in the arms at 11 years of age (Rauh et al. 2015). Based on these and other birth cohort studies, chlorpyrifos has been categorised as a human developmental neurotoxicant (Grandjean and Landrigan 2014), but these results were not included when setting the ADI value for chlorpyrifos in the 2014 EU regulatory risk assessment (European Food Safety Authority 2014b). The ADI was reduced from 0.01 to 0.001 mg/kg bw per day based on NOAELs of 0.1 mg/kg bw per day obtained from 2-year rat and dog studies with RBC AChE inhibition as the most sensitive end point (European Food Safety Authority 2014a). However, a risk assessment of chlorpyrifos from the US EPA in 2016 (Britton 2016) concluded that the effects observed in the CCCEH, with supporting results from the other 2 U.S. cohort studies and the seven additional epidemiological studies reviewed in 2015, provides sufficient evidence that there are neurodevelopmental effects occurring at chlorpyrifos exposure levels below those causing a 10% inhibition of AChE activity in red blood cells (RBC), which is currently used as point of departure for regulatory actions. Chlorpyrifos was re-evaluated by EFSA in 2019. The overall conclusion of the evaluation in relation to impacts on human health, was that:

“The information available indicates that the approval criteria as set out in Article 4(1) to (3) of Regulation (EC) No 1107/2009 are not satisfied as concerns were identified with regards to:

- The genotoxic potential of chlorpyrifos, which can not be ruled out based on the information available - positive findings were found in an in vitro chromosome aberration study and two in vitro unscheduled DNA synthesis assays; in vivo positive findings were found in open literature on chromosome aberration and on DNA damage caused through oxidative stress or by topoisomerase II inhibition which is considered a molecular initiating event for infant leukaemia. Consequently, health-based reference values cannot be established for chlorpyrifos and the dietary and non-dietary risk assessments cannot be conducted.
- Developmental neurotoxicity (DNT) - effects were observed in the available study on developmental neurotoxicity in rats (adverse effects were seen at the lowest dose tested in rats and a no observed adverse effects level ‘NOAEL’ could not be established) and epidemiological evidence exists showing an association between exposure to chlorpyrifos and/or chlorpyrifos-methyl13 during development and adverse neurodevelopmental outcomes in children.
- Based on the evidence for DNT, experts during the peer review suggested that classification of chlorpyrifos as toxic for reproduction, category 1B, H360D ‘May damage the unborn child’, in accordance with the criteria set out in Commission Regulation (EC) No 1272/200814 would be appropriate.”

Accordingly, approval of chlorpyrifos was not renewed. A very similar conclusion was drawn for chlorpyrifos-methyl, and the authorisation for both substances in the EU was withdrawn by 16 February 2020. However, both compounds are still used outside the EU and therefor residues in food is still a source of exposure.

Endocrine disrupting properties

Both chlorpyrifos and dimethoate are suspected endocrine disrupting substances (EC 2016) (Table 1) and included in the TEDX list. Chlorpyrifos has been reported to disrupt thyroid function in animal studies. In rat studies, a reduction in brain T_4 levels was seen following prenatal chlorpyrifos exposure whereas postnatal exposure caused a transient elevation in young adulthood (Slotkin et al. 2013). Mice exposed to chlorpyrifos postnatally, at doses that did not cause cholinesterase inhibition, showed a small, but significant reduction in serum concentrations of triiodothyronine and thyroxine (T4). The effect was selective for males and was associated with cellular abnormalities in the thyroid gland (De Angelis et al. 2009). Given the importance of thyroid hormones for brain development (Korevaar et al. 2016) disturbance of brain thyroid hormone levels and function may contribute to neurobehavioral deficits associated with chlorpyrifos exposure. In rats, perinatal low-dose exposure to chlorpyrifos caused disrupted glucose and lipid homeostasis, and excess weight gain in adulthood (Lassiter and Brimijoin 2008; Slotkin 2011). Similar effects have been reported for other OPs and occupational exposure to OPs has been associated with increased risk of obesity and type 2 diabetes (Evangelou et al. 2016; Xiao et al. 2017). Whether exposure levels seen in the general population can disturb glucose and/or lipid metabolism is not known at present.

Both chlorpyrifos and dimethoate decreased the expression of the steroidogenic acute regulatory (StAR) gene and thereby inhibit steroidogenesis in Leydig cell assays (Viswanath et al. 2010; Walsh et al. 2000b). Among male floriculture workers, urinary concentrations of organophosphate metabolites were associated with increased serum concentrations of FSH and prolactin and with decreased serum testosterone and inhibin B (Aguilar-Garduno et al. 2013). In rats, chlorpyrifos at low oral doses (0.01 mg/kg/day) for 100 days increased the number of ducts and alveolar structures in the mammary gland and the incidence of benign proliferative lesions in the mammary

gland of these animals. In addition, circulating steroid hormones and gonadotrophins levels were reduced (Ventura et al. 2016).

Carcinogenicity and immunetoxicity

Only very few human studies have addressed other health outcomes related to chlorpyrifos or to general OP exposure and potential associations with e.g., carcinogenicity, reproductive function, and immune system function are not clear. Neither chlorpyrifos nor dimethoate are classified as reproductive toxicants or carcinogenic but in the latest EFSA risk assessment, no toxicological reference values were established for dimethoate due to genotoxicity concerns because of mutagenic effects in bacterial and mammalian cells (European Food Safety Authority (EFSA) 2018). Some epidemiological studies have associated chlorpyrifos with cancer risk, e.g. lung, rectal, and breast cancer and increased risk of Non-Hodgkin Lymphoma (Alavanja et al. 2004; Engel et al. 2017; Lee et al. 2004; Lee et al. 2007; Waddell et al. 2001).

1.1.1.3 Glyphosate and POEA

The herbicidal action of glyphosate derives from its inhibition of a key plant enzyme, 5-enolpyruvylshikimate-3-phosphate synthase, which is involved in the synthesis of aromatic amino acids. Since this enzyme is not present in vertebrates, it has long been assumed that glyphosate would not affect non-target species, including humans.

In plants and the environment, glyphosate is mainly degraded to aminomethylphosphonic acid (AMPA). In the EFSA risk assessment of glyphosate, it was concluded that AMPA presents a similar toxicological profile to glyphosate and the health guidance values (e.g., ADI) of the latter apply to its metabolite AMPA. No toxicological data were provided on *N*-acetyl-glyphosate (NAG) and *N*-acetyl-AMPA which were identified as relevant compounds in plant/livestock residues where glyphosate tolerant genetically modified (GM) plant varieties are eaten by humans or farm animals. The need for information on this was identified as a data gap (European Food Safety Authority 2015a).

Carcinogenicity and immunetoxicity

In 2015 IARC classified glyphosate as probably carcinogenic to humans (Group 2A) (Guyton et al. 2015), a classification that considerably triggered the debate over health risks of this substance. A 2016 EFSA review of the carcinogenic potential of glyphosate concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential according to Regulation (EC) No 1272/2008 (European Food Safety Authority 2015a). In 2017 ECHA – RAC (Risk Assessment Committee) assessed glyphosate's hazardousness and concluded that the scientific evidence available at the moment warrants the following classifications for glyphosate according to the CLP Regulation: Eye Damage 1; H318 (Causes serious eye damage), Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects). RAC concluded that the available scientific evidence did not meet the criteria in the CLP Regulation to classify glyphosate for specific target organ toxicity, or as a carcinogen, as a mutagen or for reproductive toxicity (ECHA, 2017). The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) concluded in 2016 that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet (JMPR 2017).The US-EPA has also classified glyphosate as "Not likely to be carcinogenic to humans" while the US state of California recently decided to list glyphosate as cancer causing (July 2017). Likewise, the Danish Working Environment Authority (WEA) has listed glyphosate as a carcinogen in 2015.

Potential explanations for the controversy in evaluation of glyphosate has subsequently been discussed (Clausing et al. 2018; Portier et al. 2016; Tarazona et al. 2017; Vandenberg et al. 2017; Williams et al. 2016) and might partly be related to differences in toxicity between glyphosate

(alone) and GBH-formulations. The IARC classification was based on evaluation of both GBHs (including co-formulants as POEA, see below) and glyphosate alone. The epidemiological evidence includes two meta-analyses, both of which found significant increased risk of non-Hodgkin's lymphoma (NHL) associated with occupational exposure to GBH (Chang and Delzell 2016; Schinasi and Leon 2014).

The issue of potential higher toxicity related to GBH-formulations than to "pure" glyphosate is not specific to genotoxicity and/or carcinogenicity and has also been reported for other endpoints in experimental studies, also at doses below regulatory limits for glyphosate (i.e., NOAEL of 50 mg/kg bw/day) (Mesnage et al. 2015). However, at a regulatory level, glyphosate is tested alone for chronic toxicity in animal studies and the data are used for setting ADI and other regulatory norms for glyphosate alone, even though it is never used in this form but only as part of a mixture with adjuvants in the commercial formulations. Accordingly, EFSA has recognised that the genotoxic potential of formulations should be further addressed and other endpoints should be clarified, such as long-term toxicity and carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of formulations (European Food Safety Authority 2015a).

Endocrine disrupting properties

The endocrine disruption potential of glyphosate/GBH has not been fully assessed using the updated test guidelines that include specific endocrine endpoints, but scientific experimental studies indicate ED properties. Thus, glyphosate (alone) was reported to interact with the estrogen receptor and induce estrogenic activity in breast cancer cells (Thongprakaisang et al. 2013), GBH (Roundup) inhibited steroidogenesis by disrupting StAR protein expression in testicular Leydig cells (Walsh et al. 2000a), and glyphosate and GBH reduced the conversion of androgens to oestrogens by inhibiting the enzyme aromatase with formulations causing a stronger effect (Defarge et al. 2016; Richard et al. 2005). In animal studies in rats, gestational glyphosate exposure (50 mg/kg bw/day) caused disrupted gonadotropin expression and disturbed reproductive development and altered mating behaviour in male offspring (Romano et al. 2012) and gestational GBH exposure caused decreased lower sperm production in male offspring during adulthood (Dallegrave et al. 2007).

Postnatal GBH exposure changed the progression of puberty and caused reduced testosterone production in males (Romano et al. 2010). In females, postnatal GBH exposure caused morphological changes and alterations in expression proteins involved in uterine development (Guerrero Schimpf et al. 2017), enhanced sensitivity of the uterus to estradiol by modulating the expression of estrogen-sensitive genes (Guerrero Schimpf et al. 2018; Varayoud et al. 2017), and higher post-implantation embryo loss (Ingaramo et al. 2016). Glyphosate is included as a potential ED cat. 2 on the TEDX-list.

Neurotoxicity

The neurotoxic potential of glyphosate has also not been assessed in regulatory studies despite some evidence of neurotoxic effects from the academic literature. These studies show that glyphosate affected the axonal differentiation and growth of cultured neurons (Coullery et al. 2016) and induced behavioural changes (hypoactivity) and alterations in dopaminergic markers in adult rats (Hernandez-Plata et al. 2015). Some epidemiological studies have reported associations between maternal peri-conceptional residential proximity to GBH sprayed crops and increased odds of neural tube defects (Rull et al. 2006), paternal occupational GBH exposure and higher risk of abortion (Arbuckle et al. 2001), and higher risk of ADHD in children of male pesticide applicators who had applied GBH (Garry et al. 2002) while other studies did not find associations with adverse pregnancy outcomes (de Araujo et al. 2016).

Gut microbiota

Besides, glyphosate has known antibacterial properties and has been reported to affect the gut microbiota of farm animals, i.e., laboratory studies where pathogenic bacteria were less inhibited by glyphosate than non-pathogenic bacteria (Ackermann et al. 2015; Kruger et al. 2013; Shehata et al. 2013). Glyphosate is also known to bind essential metals such as manganese, zink, and cobalt which may affect mineral status as suggested by a study where glyphosate in the urine of Danish cows occurred concurrent with low levels of cobalt and manganese in the blood (Krüger et al. 2013). Both these properties might have secondary effects on health.

Co-formulants

Generally, GBHs (Roundup) are mixtures of 36-48% glyphosate, water, salts, and 10-20% adjuvants such as polyethoxylated alkyl amines (POEA) (Defarge et al. 2018) but the composition vary between different brands. Glyphosate is never used without its adjuvants, which allow and enhance its herbicidal activity by promoting its uptake and toxicity. However, adjuvants are declared as inert ingredients and classified as confidential. However, there is convincing data available that the toxicity of GBH-formulants is higher than that of glyphosate alone either because the adjuvants enhance the toxicity of glyphosate or because of their own toxic properties as demonstrated for POEA (Defarge et al. 2016; Defarge et al. 2018; Mesnage et al. 2013). The variability in adjuvants between formulations hamper the possibilities to compare results between studies unless exactly the same GBH-formulations have been used.

1.1.1.4 Polyethoxylated tallow amine (POEA)

POEA belongs to a group of petroleum-based oxidised substances used as surfactants, which are present in many GBHs and there is strong evidence that POEA surfactants decisive increase the toxicity of these formulations (Defarge et al. 2018; European Food Safety Authority 2015b). Thus, POEA-containing formulations had higher toxicity for all investigated outcomes than glyphosate alone and the conclusion from a statement from EFSA in 2015 was: “Concerns were highlighted for its genotoxic potential regarding DNA damage at concentrations not causing cytotoxicity; potentially severe adverse effects were reported with regard to the reproductive and developmental toxicity which identify the need to investigate the potential for endocrine disruption of POE-tallowamine.

No data are available regarding long-term toxicity and carcinogenicity, and developmental toxicity was not investigated in a second species (rabbits)” and “ The genotoxicity, long-term toxicity and carcinogenicity, reproductive/developmental toxicity and endocrine disrupting potential of POE-tallowamine should be further clarified. There is no information regarding the residues in plants and livestock. Therefore, the available data are insufficient to perform a risk assessment in the area of human and animal health for the co-formulant POE-tallowamine” (European Food Safety Authority 2015b). According to the Rapporteur Member State for glyphosate, Germany, POEA should be

classified and labelled for acute oral toxicity Tox. 4, H302, 'Harmful if swallowed', for skin and severe eye irritation, as Skin Irrit. 2, H315, 'Causes skin irritation', and Eye Dam. 1, H318, 'Causes serious eye damage' and skin sensitisation Skin Sens. 1, H302, 'May cause an allergic skin reaction' according to CLP criteria. Most likely, classification for inhalative toxicity would be also needed (European Food Safety Authority 2015b). A recent study found higher toxicity of POEA than of glyphosate in both plant and mammalian cells and stronger inhibition of the enzyme aromatase, which converts androgens to estrogens, and is a marker of cellular ED properties (Defarge et al. 2018). POEA is only one out of many adjuvants used in pesticide formulations as solvents, surfactants, antifoaming agents etc. Many of these substances have toxic properties (Mesnage and Antoniou 2017; Székács 2017) and may add to or enhance the toxicity of the "active" ingredient.

However, they are generally not included in the risk assessment of long-term health effects or included in surveys of dietary exposure to pesticide residues or HBM studies. This data gap represents an important source of error and may result in underestimation of health risk related to pesticide exposure.

1.1.1.5 Fipronil

Fipronil (IUPAC: (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-para-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile) is a phenylpyrazole insecticide. In insects, fipronil or its major metabolite (fipronil sulfone) noncompetitively binds to $GABA_A$-gated chloride channels, thereby blocking the inhibitory action of $GABA_A$ in the central nervous system (CNS). This leads to hyperexcitation at low doses, and paralysis and death at higher doses. Fipronil exhibits a >500-fold selective toxicity to insects over mammals, primarily because of affinity differences in receptor binding between insect and mammalian receptors. However, this selectivity is less pronounced for fipronil metabolites (sulfone and desulfinyl) and especially fipronil-sulfone is reported to be twenty times more active at mammalian chloride channels than at insect chloride channels (Zhao et al. 2005). It should also be emphasised that fipronil-sulfone is rapidly formed in humans and experimental animals and persist much longer in the body than fipronil. The toxicity of another metabolite, fipronil desulfinyl, is qualitatively similar to that of fipronil, but the dose-effect curve for neurotoxic effects appears to be steeper for fipronil desulfinyl. Also, fipronil desulfinyl appears to have a much greater affinity to bind to sites in the chloride ion channel of the rat brain GABA receptor. This finding appears to be consistent with the greater toxicity of fipronil desulfinyl in the CNS of mammals. Therefore, toxic effects in mammals are likely due to the sulfone metabolite and to the primary environmental metabolite (photoproduct) fipronil-desulfinyl. Fipronil elicits neurotoxicity in mammals by inhibition of GABA-gated chloride channels, producing hyperexcitability of the central nervous system (Gupta and Milatovic 2014). Accordingly, fipronil is classified as STOT-RE 1 (H372) "Causes damages to organs through prolonged or repeated exposure" for the nervous system (table 1). Fipronil has also been reported to be a developmental neurotoxicant and to induce thyroid disruption in rats (Gupta and Milatovic 2014) and fipronil has been included in the TEDX list since 2011 and in the EU impact report as potential endocrine disruptor Cat. 2 (JRC) (EC 2016).

Besides, the US EPA has classified fipronil as "Group C - Possible Human Carcinogen" based on increases in thyroid follicular cell tumours in both sexes of the rat (Jackson et al. 2009), but fipronil did not show genotoxicity/mutagenicity potential in a battery of in vitro and in vivo tests (EU Standing Committee on Biocidal Products 2011).

1.1.1.6 Possibility of mixture effects

The general population is exposed to a mixture of many different pesticides from the diet and occupational exposure settings will also often include mixtures of pesticides. Many currently used pesticides possess neurotoxic and/or endocrine disrupting properties and although the exposure

level to the individual pesticides is low, exposure to several pesticides with similar mode of action (e.g., different pyrethroids) or same target organ (e.g., the nervous system for pyrethroids and OPs) are likely to be additive. Monitoring pesticides through HBM4EU will help describe the aggregated exposure of the general EU population. Such data can contribute to the EuroMix project (https://www.euromixproject.eu) which aims at developing a strategy for refining future risk assessment of mixtures relevant to national food safety authorities, public health institutes, the European Food Safety Authority (EFSA), the European Chemical Agency (ECHA), industry, regulatory bodies and other stakeholders.

Many epidemiological studies provide evidence of adverse health effects related to mixtures of pesticides although individual pesticides or pesticide groups could not be pinpointed. One example is a study among women undergoing infertility treatment, and for whom intake of fruit and vegetables with high content of pesticide residues was found to be associated with lower probabilities of pregnancy and live birth (Chiu et al. 2018a) whereas in men, intake of fruit and vegetables with low pesticide content was associated with higher total sperm count and sperm concentration (Chiu et al. 2016). Assessment of pesticide exposure in these studies was based on data obtained from food frequency questionnaires combined with surveillance data on pesticide residues in commodities. This approach was previously validated by comparing the results with biomonitoring data showing that higher intake of high-pesticide residue fruit and vegetables was associated with higher urinary concentrations of metabolites of organophosphate and pyrethroid insecticides and the phenoxy acetic acid herbicide 2,4-D (Chiu et al. 2018b), all of which are frequently detected in fruit and vegetables at the European marked (European Food Safety Authority 2017). The risk is especially high if exposure occurs during vulnerable time periods in foetal life or childhood. Thus, maternal occupational exposure to mixtures of pesticides, in the first trimester before the pregnancy was recognised, was found associated with impaired reproductive development in the boys (Andersen et al. 2008; Wohlfahrt-Veje et al. 2012a), earlier puberty and impaired neurobehavioral function in the girls (Andersen et al. 2015; Wohlfahrt-Veje et al. 2012b), and lower birth weight followed by increased body fat accumulation during childhood (Wohlfahrt-Veje et al. 2011).

1.1.2 Exposure characteristics

1.1.2.1 Trends in production volume and environmental/food concentrations

In 2015, the countries with the highest pesticide sale per hectare of agricultural land were Malta, the Netherlands, Cyprus, Belgium, Ireland, Italy and Portugal. These countries were above 5 kg of pesticide active ingredient/ha, with Malta at 15 kg active ingredient/ha. The EU average was 3.8 kg of pesticide active ingredient/ha. (calculated by EEA based on Eurostat data for pesticide sales (see: https://www.eea.europa.eu/airs/2017/environment-and-health/pesticides-sales).

Data for the sale of specific groups of pesticides in the EU is available only for 2016 (Eurostat) and therefor time trends in sale cannot be evaluated. For pyrethroids, 965 tons (active substance) were sold in 17 of the member states (no available data for 3 and confidential data from 8 countries. For organophosphate insecticides, 2736 tons (active substance) were sold in 12 of the member states (no available data for 5 and confidential data for 11 countries) and almost half (1159 tons) of the OPs were sold in Poland (Eurostat). No specific data on the sale of glyphosate or fipronil is available.

For the general population, pesticide residues in food constitute the main source of exposure. This has been illustrated in intervention studies where the urinary excretion of pesticides reduced markedly after one week of limiting consumption to organic food (Bradman et al. 2015; C Lu et al. 2006; Liza Oates et al. 2014). Similar conclusions have emerged from studies investigating associations between urinary concentrations of pesticides and questionnaire information on food intake and organic food choices. Thus a high intake of fruit and vegetables is positively correlated

with pesticide excretion (Berman et al. 2016; Ye et al. 2015) and frequent consumption of organic produce is associated with lower urinary pesticide concentration (Berman et al. 2016; Curl et al. 2015).

Children have higher food intake per kg body weight leading to higher exposure levels as also confirmed in most HBM-studies. Besides, non-dietary sources (e.g., residential use or living in the vicinity of pesticide treated crops) can also be important determinants of exposure (Babina et al. 2012; Curl et al. 2015; Curwin et al. 2007; Dereumeaux et al. 2018; Fortes et al. 2013; Glorennec et al. 2017; CS Lu et al. 2006; L. Oates et al. 2014; Roca et al. 2014; Ye et al. 2015).

The EFSA reports on pesticide residues in food samples collected in 2015 (published in 2017) and in 2016 (published 2018) shows the combined results from the coordinated control programme (EUCP) and the national control programmes (NP) from the member states, Iceland and Norway (European Food Safety Authority 2017, 2018). Several pyrethroids, chlorpyrifos, dimethoate, and glyphosate were quantified in more than 1% of the plant products analysed.

Baby food products are included in the control programs and the Commission has defined specific rules for foods specially manufactured for infants (below 12 months of age) and young children (between 1 and 3 years of age) in Directive 2006/141/EC. It requires that infant formula and follow-on formula contain no detectable levels of pesticide residues, meaning not more than 0.01 milligrams of pesticide residues per kilogram. The Directive also prohibits the use of certain very toxic pesticides (including omethoate and a few other organophosphates) in the production of infant and follow-on formulae and establishes levels lower than the general maximum level of 0.01 milligrams per kilogram for a few other very toxic pesticides (including fipronil and some organophosphates). However, the pesticide content in human breast milk is not covered by the control programs, and while persistent organochlorine pesticides are commonly detected in breast milk, only few studies have included lipophilic pesticides in current use. In studies from the US, the detection frequency for chlorpyrifos in human milk samples collected between 2007 and 2011 was 100% with median concentrations of 0.06 ng/ml milk (Chen et al. 2014) and 0.03 ng/g milk (Weldon et al. 2011). Pyrethroids were not detected in human milk samples (n=10) in one of these studies (Chen et al. 2014) while permethrin was detected in all samples from the other study in concentrations of approximately 0.10 ng/g milk (Weldon et al. 2011). The pyrethroids, cypermethrin, lambda-cyhalothrin, permethrin and deltamethrin were detected in all human milk samples collected between 2009 and 2010 in Brazil (n= 20), Columbia (n=27) and Spain (n=6) from areas without pyrethroid use for malaria control (Corcellas et al. 2015). In samples (n=127) from Punjab in India, a median concentration of cyfluthrin of 189 ng/g milk and a max concentration of 4.1 mg/g milk was reported (Sharma et al. 2014).

Since breastfeeding is known to confer numerous long-lasting benefits to infants (Victora et al. 2016) and exclusively breastfeeding for the first 6 months therefor is recommended by WHO, it is important to limit contaminants in breast milk that might compromise the health benefits. Thus, knowledge on pyrethroid and chlorpyrifos concentrations in human breast milk collected in the EU would provide information on the risk of lactational transfer to infants for these substances.

Due to neurotoxic and endocrine disrupting properties of most of the included pesticides, pregnant women and children are considered the most vulnerable population groups. So far only studies based on the PELAGIE cohort in France mentioned above have addressed associations between urinary levels of pyrethroid and OP metabolites and child neurodevelopment at exposure levels occurring in the general population. The exposure levels measured for both OPs and pyrethroids in the PELAGIE cohort were considerably lower than reported from other cohorts and more representative exposure levels for EU citizens are needed to characterise the risk of adverse effects on neurodevelopment in European populations.

Population groups with higher exposure levels than the general population will also have enhanced risk of adverse health effects. Among these are agricultural workers who mix and/or apply pesticides onto crops and or handle the crops/plants after treatment, and workers employed in companies applying biocides in residents and institutions. These groups might have high dermal and inhalation exposure.

1.1.2.2 Pyrethroids

Pyrethroids compose a large class of insecticides used to control a wide range of insects both as components of plant protection products (PPP) as insecticides and of biocidal products (BP) for wood preservation (T8) and to combat insects in animal facilities, indoors in public and commercial buildings (e.g., warehouses and hotels) as well as dwellings (T18). Some pyrethroids are also used in veterinary medicinal products and applied on animals (livestock, pets) and for treatment of scabies and head lice in humans. Currently, 16 different pyrethroids are approved as either PPP (n=13) or BP (n=13) or both (n=7), but authorisation status differs between member states. Besides, some additional pyrethroids (n=5) are under review for use as BP. Thus, the potential for human exposure is high both from intake of residues in food items and by dermal and inhalation exposures via direct contact and from dust.

Residues in food

Pyrethroid are lipophilic substances and several of the pyrethroids meet some of the criteria of the REACH Annex XIII regulation for persistency and/or bioaccumulation and are potential candidates for substitution under the Pesticides Regulation (EC) No 1107/2009 (List of candidates for substitution (Draft, January 2015)). Among these are bifenthrin, esfenvalerate, etofenprox, and lambda-cyhalothrin. Also, the co-formulant PBO meets the criteria for being very persistent (vP) according to Annex XIII to REACH (Reg 2016/2288 (EU). Accordingly, pyrethroids have been detected in both fishes and marine mammals (Alonso et al. 2012). A recent study from Spain found pyrethroids in 100% of tissue samples collected from riverine fish (Corcellas et al. 2015). Currently, MRLs for pyrethroids in fish products have not been established in the EU.

In food items of plant origin, residues of bifenthrin, cypermethrin and lambda-cyhalothrin were the most frequently detected in 2015 (European Food Safety Authority 2017) and cypermethrin, deltamethrin, etofenprox, and lambda-cyhalothrin were the most frequently detected in 2016 (European Food Safety Authority 2018). In addition, cypermethrin and permethrin were quantified in few samples of food products of animal origin covered by the EUCP (butter and eggs in 2015 and milk and swine fat in 2016). As mentioned above, pyrethroids have been detected in human breast milk from the US, India, and South America (including six samples from Spain) but the European level is unknown at present.

Dermal and inhalation exposure

Besides dietary exposure, studies from the US have demonstrated that residential use of pyrethroids can contribute markedly to the internal exposure. Hence, floor wipe concentrations for pyrethroid insecticides were found to be significant predictors of child creatinine-adjusted urinary metabolite concentrations (Trunnelle et al. 2014b).

A review of 15 studies in the US that examined children’s exposure to pyrethroids concluded that children were exposed to pyrethroids from several sources including food, dust, and/or on surfaces at residences and for children living in homes with frequent pesticide applications dermal and inhalation exposure routes might exceed the exposure from dietary ingestion (Morgan 2012).

Most pyrethroids are rather stable in the indoor environment and increased content of pyrethroids in dust has been found more than one year after application (Leng et al. 2005).

HBM data

The exposure level to pyrethroids is likely increasing because they have replaced organophosphate and carbamate insecticides in biocides and also, to some degree, as insecticides in agriculture. HBM data are available from studies in many countries including USA, Canada, China, Japan and also from a few EU countries (France, Poland, Denmark, UK, Germany, and Spain) (Dalsager et al. 2018; Dereumeaux et al. 2018; Roca et al. 2014; Schulz et al. 2009; Viel et al. 2015; Wielgomas et al. 2013) but EU-wide data are not available. The studies from EU indicate widespread exposure to pyrethroids within the general population, including pregnant women and children but also some difference in exposure levels between countries and population groups (see Table 1 in Annex 1). Including urinary concentrations of the common pyrethroid metabolite 3-PBA in HBM4EU will provide an estimate of the aggregated exposure to pyrethroids and allow comparison with other studies and with levels associated with adverse health outcomes. That is important from a risk assessment point of view, since so many different pyrethroids are used, they replace each other, and their effects are likely additive. Besides, it will be valuable to include specific metabolites of the most used pyrethroids to get information on exposure levels while for pyrethroids that are used only to a lesser extent, detection frequency will be low, and measurements will not provide useful information.

Pyrethroids are lipophilic substances, and their concentration in human breast milk samples was inversely associated with the number of pregnancies (Corcellas et al. 2015; Sharma et al. 2014). This might indicate some accumulation in fat tissue in humans at continuous exposures as also predicted from toxicokinetic modelling (Cote et al. 2014) and mentioned in the EFSA risk assessment for e.g., bifenthrin: “Potential for accumulation in fat, terminal half-life of up to 51 days” and “Elimination complete within 48 hours, urine (13-25%) and faeces (63-88%), 3% remained in tissues and organs”. Excretion via breast milk would be a potential risk for breast-feeding infants and therefor analysis of human breast milk samples would be relevant.

Thus, urinary concentrations of pyrethroid metabolites will reflect the current body burden which might depend on number of pregnancies/breast feeding periods and BMI/body fat content but such associations have not yet been explored in humans, except for one recent study reporting higher urinary 3-PBA concentrations among primiparous women compared to women with previous pregnancies and a positive association between 3-PBA and pre-pregnancy BMI (Dalsager et al. 2018).

No HBM studies including PBO were identified. After dermal application to the arms of human volunteers, about 2% of the dose was absorbed (Selim et al. 1999). The percutaneous absorption when applied to the scalp was found to be 8.3% (DrugBank). The fraction absorbed after oral exposure is less clear but was reported to be low with 64-80% excreted in faeces. After absorption, PBO is partly metabolised (the fraction is unclear) and excreted unchanged and as different metabolites in urine. PBO was not detected in any urine samples analysed at Environmental Medicine at SDU (DK) although one or more pyrethroid metabolites were detectable in all samples (unpublished results). PBO was detected in one child urine sample (3.8 μg/L) out of 14 from an agricultural population in Spain (Cazorla-Reyes et al. 2011).

1.1.2.3 Organophosphates – chlorpyrifos and dimethoate

Chlorpyrifos, chlorpyrifos-methyl, and dimethoate are authorised as insecticide and acaricide according to Reg. (EC) No. 1107/2009 in 20, 17 and 23 of the member states, respectively. Chlorpyrifos is one of the most commonly used pesticides in the EU and worldwide. None OPs are approved as biocides in the EU.

Residues in food

Chlorpyrifos, and also dimethoate, are commonly detected in commodities produced in Europe as well as in commodities imported from third countries. Exceeding of MRLs are frequently reported for both substances, and also exceeding of acute reference values (ARfd) has been reported for chlorpyrifos based on exposure levels calculated from dietary intake estimates (European Food Safety Authority 2017). In 2014 the ADI for chlorpyrifos was reduced by a factor 10 to 0.001 mg/kg bw/day and accordingly MRLs for chlorpyrifos were lowered for many crops during 2016 leading to a higher number of exceedances in 2016 (59 exceedances out of the 10,212 samples analysed for this pesticide). In addition, a number of MRL exceedances were reported by France for dimethoate in tomatoes produced in the Mayotte oversea territory (32 exceedances in 9,618 samples reported) (European Food Safety Authority 2018). MRLs for chlorpyrifos for more commodities have been lowered during 2018. For dimethoate, the long-term dietary exposure assessment was calculated to be 101 and 6.1 % of the ADI for upper-bound and lower bound scenarios, respectively. The corresponding values for chlorpyrifos was 45.8 and 12.6 % (European Food Safety Authority 2018)

HBM data

There are some EU HBM studies including OPs but few of these were performed after 2010. (Annex 1, Table 2). Since restrictions have been imposed on the use of OPs both at EU and national level the exposure levels might be lower today, especially in countries with most restrictions on their usage. Most studies have used unspecific urinary organophosphate metabolites, i.e., dialkyl phosphates (DAPs) as a marker for the total OP exposure level. DAPs are divided into group-specific metabolites: diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate. The studies indicate wide variation in exposure level across countries and population groups. Relatively few EU HBM studies have included the metabolite 3,5,6-Trichlor-2-pyridinol (TCPY), which is specific for chlorpyrifos and chlorpyrifos-methyl (Annex 1 Table 3). No EU studies have included urine concentrations of dimethoate or its specific metabolite omethoate. Omethoate is rapidly metabolised to unspecific DMPs and only a minor fraction (approx. 1 %) is excreted in urine as dimethoate and omethoate. Accordingly, very low detection frequencies (< 1%) for dimethoate and omethoate was reported in NHANES from the US. Including DAPs in HBM4EU will allow comparison with previous studies and analyses of time-trends. Besides, it will provide an estimate of the total exposure to OPs which is likely more relevant for potential health risks than the exposure level to individual OPs, since OPs are assumed to act additively because of similar mode of actions.

1.1.2.4 Glyphosate and POEA

Glyphosate is the ISO common name for N-(phosphonomethyl)glycine (IUPAC) and a range of different salt derivatives of glyphosate are used in GBH-formulations.

Since the late 1970s, the volume of GBHs applied world-wide has increased approximately 100-fold, especially after the introduction of genetically modified plants tolerant to glyphosate, and GBHs are the most used pesticide formulations in the EU and worldwide. The estimated global use of glyphosate (as active ingredient (a.i.)) was 825.804 tons in 2014 (Benbrook 2016). The current sale of glyphosate in the EU cannot be extracted from Eurostat but it likely contributes the major part of “other herbicides” of approx. 65.210 tons (a.i.) in 2016 (Eurostat).

Residues in food

Application of GBHs on crops result in residues of glyphosate and its primary degradation product, aminomethyl phosphonic acid (AMPA) in food items and especially the use for pre-harvest treatment (desiccation) has probably led to higher content in food items. In the EU survey of pesticide residues in food for 2016, 3.6% of the samples analysed for glyphosate contained quantified residues. The highest quantification rate was observed for dry lentils (38%), linseeds

(20%), soya beans (16%), dry peas (12%) and tea (10%). In cereals, glyphosate was mainly found in buckwheat and other pseudo-cereals (24%), followed by barley (19%), millet (18%), wheat (13%) and rye (4%). Among the 6,761 samples analysed, 19 samples (0.28%) exceeded the MRL for glyphosate (European Food Safety Authority 2018). Although AMPA has been assesses to presents a similar toxicological profile to glyphosate and to apply to the same health guidance value (e.g, ADI) as glyphosate, neither AMPA nor N-acetyl-glyphosate (NAG) and N-acetyl-AMPA are included in the food surveys. NAG and N-acetyl-AMPA were identified as relevant compounds in plant/livestock residues where glyphosate tolerant genetically modified (GM) plant varieties are eaten by humans or farm animals. Accordingly, EFSA has proposed a residue definition for glyphosate for risk assessment as: - 'sum glyphosate, N-acetyl glyphosate, AMPA and N-acetyl-AMPA expressed as glyphosate' (European Food Safety Authority 2018). Besides, adjuvants in pesticide formulations are not included in the food surveys and therefor no data on POEA in food items are available.

Other exposure sources

Besides exposure from residues in food, the population can be exposed to GBHs from contamination of water supply (mainly AMPA), use for home gardening and from drifting from agricultural areas for residents close to treated fields. Additionally, field workers (sprayers and re-entry workers) and bystanders (including farm families) are expected to be exposed as well via the dermal route and via inhalation.

Because of concerns about the health and environment effects of glyphosate, numerous measures have been taken at the national and municipality level in order to restrict the use of GBHs. Besides, POEA has been banned from glyphosate-containing products since 2016 (https://ec.europa.eu/food/plant/pesticides/glyphosate_en) and will be put on the 'negative list' (chemicals not to be used in formulations of plant protection products) that is being set up in the EU. These measures are expected to affect the population's exposure to glyphosate and POEA, but since POEA has been reported to be rather persistent in agricultural soils (Tush and Meyer 2016) and is still approved in countries outside the EU, the substance may still be present in food items although there is no available information regarding residues in plants and livestock.

After the ban of POEA in GBHs at the EU market, it will likely be substituted by replacement surfactants. Thus, it might be important to monitor both POEA and future substitute substances in both food items and human samples.

HBM4EU research may be focused on the development of suspect screening approaches of POEA and eventually other relevant surfactants, permitting to generate a first level of exposure data enabling documentation of human exposure to better justify further investment in a full quantitative and validated method development.

HBM data

Glyphosate is rapidly but incompletely absorbed after oral administration (around 20 % of the administered dose based on urinary excretion after 48 hours and comparison of kinetic behaviour after oral and iv administrations), being mostly eliminated unchanged via faeces. Absorbed glyphosate is poorly metabolised, widely distributed in the body, does not undergo enterohepatic circulation and is rapidly excreted unchanged in urine; showing no potential for bioaccumulation (European Food Safety Authority 2015a). Humans are also exposed to AMPA and both glyphosate and AMPA have been measured in human urine samples and seem to be ubiquitous in human urine. However, only limited HBM data are available from the US (Curwin et al. 2007; Mills et al. 2017; Niemann et al. 2015) and Europe (Connolly et al. 2018; Conrad et al. 2017; Knudsen et al. 2017) (see Annex 1 Table 4) although GBHs has been widely used for many years. US levels seem higher than those seen in Europe. In a study from California, the urinary concentrations of both glyphosate and AMPA among adults had increased considerably between 1993 and 2016 (Mills et al. 2017). For further elucidation of the variation in the population's exposure and time trends, the German Environment Agency is analysing morning urine samples acquired in the cross-sectionally designed population-representative German Environmental Survey for Children and Adolescents (GerES 2014–2017) for glyphosate and AMPA. A recent study from Ireland, reported higher urinary glyphosate concentrations among horticulturalists using GBHs with peak levels up to 3 h after completing the application (Connolly et al. 2018). No HBM data for POEA are available.

So far, no epidemiological studies on GBH-related health effects using HBM exposure data have been published. The scientific community has raised concerns on the safety of glyphosate and glyphosate-based products, and there is a need for HBM data for glyphosate and its metabolites to characterise the exposure situation in the population, HBM-based epidemiological studies on potential related health effects, especially among occupationally exposed agricultural workers, pregnant women and their children and more evaluations of GBH-formulations, recognising that these mixtures likely have effects that are not predicted by studying glyphosate alone.

1.1.2.5 Fipronil

Fipronil is approved in the EU as an active biocidal agent (BP T18) used for ant and cockroach control. Only professional use indoors by application in locations normally inaccessible after application to man and domestic animals has been addressed in the EU risk assessment (Dir 2011/79/EU). It is also authorised in the EU as veterinary medicine in two products (EMA) and in more products at Member State level. As example, fipronil is the active ingredient of one of the popular ecto-parasiticide veterinary products, Frontline, which is commonly used on pets to kill fleas, and all stages of ticks. Until 2017 fipronil was also approved as insecticidal pesticide in plant protection products. A recent (2017) case of illegal use of non-approved veterinary medicinal products in poultry farms caused a large-scale contamination of eggs in several EU-countries and fipronil was detected in quantities between 0.0031 and 1.2 mg/kg (ppm) in eggs in several EU countries.

Fipronil is rapidly and extensively absorbed after oral intake and an uptake of approximately 90% has been estimated. Uptake after dermal exposure was 0.1-10% dependent on the concentration and duration of exposure (Jackson et al. 2009). After absorption, fipronil is metabolised to fipronil-sulfone and fipronil and especially the sulfone metabolite persists in in the body (especially in fatty tissues, but also in brain, liver, kidney, and adrenals) for weeks. Thus, the half-life of fipronil-sulfone in blood is long (6-10 days) reflecting a slow release of the metabolite from fat tissue (Gupta and Milatovic 2014; Jackson et al. 2009).

Since fipronil is used as insecticide in agriculture outside the EU, residues may occur in imported commodities. In the 2016 EU survey of residues in food, fipronil (sum of fipronil and sulfone

metabolite) was quantified in 57 out of 51430 analysed samples (0.11 %), 44 samples exceed the MRL and 40 of these samples were from third countries outside the EU (European Food Safety Authority 2018). Besides, pet owners and especially their children, professional biocide applicators, residents in buildings after treatment, and veterinary personal using fipronil-containing products can be exposed. However, no HBM data are available from the EU to evaluate exposure level and how widespread the exposure is after authorised uses. In a US study fipronil sulfone was present in the serum of approximately 25% of the samples (at concentrations ranged from 0.1 to 4 ng/mL) collected from volunteers (n=96) with no known pesticide/biocide exposure. In contrast no fipronil metabolites were detected in the urine samples (McMahen et al. 2015).

To investigate the transfer of fipronil from dogs treated with a spot-on product (Frontline containing 9.8% fipronil), Frontline (1.34 ml) was applied topically on adult household dogs and gloves worn for 5 min during petting were collected 24 hr and 1-, 2-, 3-, 4- and 5-weeks post-Frontline application for fipronil residue determinations using GC/MS. The highest concentration of fipronil (589 ± 206) was detected 24 h after Frontline application and decreased steadily over time to 448 ± 118 ppm after 8 days, and were undetectable after 36 days (Jennings et al. 2002). A recent study estimated the acute post-application absorbed doses to be as high as 0.56 µg/kg/day for toddlers in households with treated pets based on current US EPA standard operating procedures (SOPs) (Cochran et al. 2015). Only one study investigating fipronil exposure among pet owners have included HBM the authors could not exclude contamination of some urine samples and therefor the HBM results were not presented (Dyk et al. 2012). Thus, especially small children with close contact to treated pets might be relatively high exposed. Further, repeated exposure among veterinary personnel who handle many dogs/cats daily, require proper protection to avoid cumulative exposure. More HBM studies are needed to characterise the exposure level for these groups and for the general population.

The following AELs has been proposed by French Rapporteur Member State for placing fipronil as a biocidal product on the market:

- AEL acute-term (secondary exposure) = 0.025 mg/kg bw.
- AEL medium-term (operator exposure) = 0.0035 mg/kg bw/d.
- AEL long-term = 0.0002 mg/kg bw/d.

1.1.2.6 Health based guidance values available for HBM data

No health-based guidance values (HBM-I or HBM-II) have been established for the pesticides but some national reference values RV95 have been stablished.

The German Human Biomonitoring Commission has established reference values (RV95) for organophosphate and pyrethroid metabolites in urine of both children 3-14 years of age and adults from the German population (Schulz et al. 2011) and the Institute of Environment and Health (IEH) from the Cranfield University has established RV95 for pyrethroid metabolites in urine of the general adult (>18 years) UK population (Bevan et al. 2013). The ongoing National Biomonitoring Programme (NHANES) in the US, are routinely measuring pyrethroid and organophosphate biomarkers (US Centres for Disease Control and Prevention 2017). For comparison RV95 values from the NHANES study are included (children 6-11 years and adults 20-59 years). No RV95 data on glyphosate and fipronil are available from the German Human Biomonitoring Commission and these substances are not included in NHANES.

Metabolite	Reference values (RV95) Urine concentration (µg/L)				
	Germany children	Germany adults	UK adults	NHANES children	NHANES adults
Sampling year	2003-06	1998	?	2007-08	2007-08
3-PBA	2	2	6.1	9.9	6.7
Trans-DCCA	2	2	1.6	4.0	5.4
Cis-DCCA	1	1	0.8	-	-
Cis-DBCA	-	-	1.6	<LOD	<LOD
DMP	75	135	-	43.3	30.3
DMTP	100	160	-	52.5	30.6
DMDTP	10	-	-	6.7	4.3
DEP	30	16	-	20.2	14.0
DETP	10	-	-	6.4	4.2
TCPY	-	-	-	6.0	5.9

Biomonitoring guidance values (BGVs) derived for chlorpyrifos based on biomonitoring data and PBPK/PD modelling of AChE inhibition was recently suggested (Arnold et al. 2015) to be 2100 µg/L and 520 µg/L urine for TCPy in adults and infants, respectively. These limits were based on 10% AChE inhibition in red blood cells (RBC) claimed to be precursor for adverse neurological symptoms and therefor used as point of departure. However, epidemiological studies have raised concern that this limit is not protective for neurodevelopmental effects e.g., by the US EPA (Britton 2016; Drew et al. 2016). Recently a new approach for Benchmark Dose estimation using PBPK/PD modelling and and a novel pharmacodynamic (PD) dose–response model was suggested.

Simulated peak brain chlorpyrifos concentrations, were used to develop a dose–response model to predict chlorpyrifos-induced spatial memory deficits and a 15% cognitive deficit was used as point of point of departure leading to lower benchmark dose (reference dose) than when 10% AChE inhibition was used (Zurlinden and Reisfeld 2018). Corresponding urinary TCPy concentrations were not calculated.

Recently, a Human Biomonitoring Equivalent (BE) value for interpretation of urinary levels for 3-PBA was proposed (Aylward et al. 2018). Using the lowest (most stringent) BE value (Tier 1) or a weighted average based on information regarding relative exposure potential (Tier 2) combined with information on molar urinary excretion fraction of the metabolites led to 3-PBA BE values of 1.7 µg/L (Tier 1) and 87 µg/L (Tier 2).

Urinary pyrethroid and organophosphate (alkyl diphosphate) metabolites are included in the German External Quality Assessment Scheme (G-EQUAS).

1.1.3 Policy relevance

Plant protection products (all substances except permethrin (and other pyrethroids not approved for PPP as indicated in Table 1) and fipronil) are regulated under Regulation (EC) 1107/2009. Under this regulation, the pyrethroids bifenthrin, esfenvalerate, etofenprox, lambda-cyhalothrin as well as dimethoate and fipronil are included in the draft list of candidates for substitution (January 2015).

Fipronil and pyrethroids approved as biocides are regulated under Regulation (EC) 528/2012.

Permethrin and fipronil are also used in medicinal products for human and veterinary use and regulated under Regulation (EC) 726/2004.

Residues of all the substances in food and feed is regulated under Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

Specific rules on the presence of pesticides residues in infant and follow-on formulae are regulated by Directive 2006/141/EC (Annex VIII) which also encompasses the rules, previously set out in Commission Directive 1999/50/EC. It requires that infant formula and follow-on formula contain no detectable levels of pesticide residues, meaning not more than 0.01 milligrams of pesticide residues per kilogram. The Directive also prohibits the use of certain very toxic pesticides in the production of infant and follow-on formulae and establishes levels lower than the general maximum level of 0.01 milligrams per kilogram for a few other very toxic pesticides. Omethoate, an OPs but also a metabolite of dimethoate, is one of the pesticides prohibited for use in the production. For fipronil the MRL is set to 0.004 mg/kg food produced for infants/young children.

Classifications of the substances related to human health outcomes according to Regulation EC 1272/2008 are shown in Table 1.

1.1.4 Technical aspects

- Biomarkers available for parent compounds or metabolites in human matrices and main characteristics of analytical methods (quantitative, semi-quantitative…).

Since the pesticides included in HBM4EU are generally metabolised and excreted within few days, urine is a better matrix than blood/serum for biomonitoring studies (Barr et al. 2005; Needham and Sexton 2000; Yusa et al. 2015). Methods for measuring multiple pesticides at the same time in hair samples (including chlorpyrifos and other organophosphates and pyrethroids) have been published (Hardy et al. 2015; Lehmann et al. 2018).

An advantage is that hair samples will reflect exposure during a longer time period than urine samples, but further development and validation of the methods is needed. However, hair samples are not available in the HBM4EU alignment studies and therefor this matrix is not considered relevant. Chlorpyriphos and pyrethroids (parent compounds) can also be analysed in human breast milk samples (Chen et al. 2014; Corcellas et al. 2012; Weldon et al. 2011). This matrix is considered relevant for a pilot study if bio-banked breast milk samples are available from EU studies.

There are established, validated sensitive methods for analysing metabolites of pyrethroids, chlorpyrifos/chlorpyrifos-methyl, and organophosphates (group-specific) and glyphosate in urine samples as described below and shown in Annex 2. Harmonisation of the methods within partner countries might be necessary.

The following urinary metabolites of pyrethroids have been used as biomarkers in most previous studies:

- 3-phenoxybenzoic acid (3-PBA) is a common metabolite of most pyrethroids and estimate for the total exposure:
- cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DCCA) and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA) are metabolites of the respective isomers of permethrin, cypermethrin and cyfluthrin;
- cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DBCA) is a specific metabolite of deltamethrin,
- 4-fluoro-3-phenoxybenzoic acid (F-PBA) is a metabolite of cyfluthrin.

The urinary pyrethroid metabolites (+ the specific metabolite (TCPy) of chlorpyrifos/chlorpyrifos, see below) can be measured in a single run using high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) (Dalsager et al. 2018; Davis et al. 2013). However, LODs for the specific metabolites are lower if analysed by gas chromatograph/mass spectrometry (GC-MS) (Viel et al. 2015). The detection frequency (percentage of population with concentrations above LOD) for the specific metabolites is lower than for 3-PBA in most studies, and for some metabolites none, or only a few percent, of the samples are above LOD. Pyrethroid formulations generally consist of multiple stereoisomers with different toxicokinetic properties. Most studies report a higher concentration of *trans*-DCCA than *cis*-DCCA in urine reflecting the major exposure route. The urinary excretion pattern is affected by the exposure route with higher urinary trans-DCCA concentrations relative to cis-DCCA after oral exposure, while dermal (and probably also inhalation) exposure results in a more equal ratio (Cote et al. 2014). Besides, methods (GS-MS) for analysing specific urinary metabolites for bifenthrin, esfenvalerate, and lambda-cyhalothrin (Tao et al. 2013) and for bifenthrin combined with cyhalothrin (Bevan et al. 2013) have been described.

Day-to-day variability in individual urinary concentrations of 3-PBA has been reported to be low and much more stable than for organophosphate metabolites (Wielgomas 2013), probably because excretion from storage in fat tissue prolong the excretion time after continuous exposures (Cote et al. 2014). Inter-individual variability in urinary concentrations of the metabolites of specific pyrethroids is unknown but is likely larger.

Unspecific OP metabolites, dialkyl phosphates (DAPs), as a marker for the total OP exposure level, can be quantified in human urine using capillary gas chromatography/tandem-mass spectrometry (GC/MS/MS)(Bravo et al. 2004) (Barr, D. et al., 2010). DAPs are divided into group-specific diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate.

The specific main metabolite, 3,5,6-trichlor-2-pyridinol (TCPy) of chlorpyrifos and chlorpyrifos-methyl can be quantified in human urine using capillary gas chromatography/mass spectrometric detection (GC-EI/MS) (Koch et al. 2001) or LC-MS/MS (Dalsager et al. 2018; Davis et al. 2013).

No sensitive specific urinary biomarker is available for dimethoate, but dimethoate is metabolised to DMPs and thus included in that biomarker.

Glyphosate and the environmental metabolite AMPA can be analysed in urine by GC-MS-MS analysis (Conrad et al. 2017). Glyphosate has also been analysed in urine by ELISA and seem to be comparable with results obtained by GC-MS (high correlation) but the maximum concentrations found in human urine by the two methods differed (Krüger et al. 2014) and more validation of the ELISA method would be needed before applying this approach in a large HBM study. Glyphosate has also been determined in serum using HPLC with fluorescence detection (Kongtip et al. 2017)

but urine is the preferred matrix for non-persistent compounds at low exposure levels as explained above.

No HBM methods for POEA, or related surfactants, are described in the literature.

Fipronil sulfone in serum seem to be the best exposure biomarker for fipronil exposure because the metabolite is rather stable and probably also the main responsible for toxic effects. A time-of-flight mass spectrometry (LC/TOF-MS) method to measure fipronil sulfone in serum and milk samples is available (McMahen et al. 2015) and an ELISA developed for the detection of total fipronil (parent compound and metabolites) in serum has also been used (Mohamed et al. 2004). Recently, a LC-MS/MS method to measure hydroxyl-fipronil in urine was developed using rat urine (Vasylieva et al. 2017) but this method has not yet been applied on human samples.

1.1.5 Societal concern

The general population is exposed to pesticide residues in the food and according to a Eurobarometer survey from 2014, 43% were worried about the impact on their health of chemicals used in everyday products and 29% were worried about agricultural pollution (use of pesticides, fertilisers etc). In a Danish survey from Beredskabsstyrelsen (2016), 30% were concerned about toxic pollutants in food and drinking water. There is a lot of media attention both in the EU and globally, in particular related to concerns about impact on health, especially related to endocrine and developmental neurotoxicity of pesticides as well as the carcinogenic potential of especially glyphosate. Glyphosate assessment by EFSA and by ECHA has generated a wide media coverage, with a wide alliance of European NGO campaigning against its reauthorisation and many municipal and regional governments taking measures to reduce its use. Also, the European Citizens' Initiative calling on the European Commission to propose to member states a ban on glyphosate, to reform the pesticide approval procedure, and to set EU-wide mandatory reduction targets for pesticide use, has collected over 1,320,517 signatures.

Also, the recent discovery of fipronil in eggs on the EU market, as a result of misuse of the active substance in chicken stable areas that were directly accessible to the chickens, gained wide attention and societal concern.

Although the regulatory risk assessment of pesticides currently practiced in the EU is comprehensive there are some concerns in the scientific community, that this risk assessment is inadequate at addressing mixed exposures, specifically for carcinogenic effects (Goodson et al. 2015), endocrine-disrupting effects (Jacobsen et al. 2012; Kortenkamp 2014), and developmental neurotoxicity (Bjorling-Poulsen et al. 2008). Furthermore, there are concerns that test protocols lag behind independent science (Beronius et al. 2013) and that studies from independent science, including epidemiological studies, are not fully considered (Tweedale et al. 2014). In 2015 a Steering Committee of scientists adapted the Intergovernmental Panel on Climate Change weight-of-evidence characterisation for probability of causation based upon levels of available epidemiological and toxicological evidence for one or more chemicals contributing to disease by an endocrine disruptor mechanism. A mean cost of €157 billion annually in EU was estimated by Monte Carlo simulations (Trasande et al. 2015). Effects on brain development are likely to be lasting and one main outcome is cognitive deficits, often expressed in terms of losses of IQ points. When US data on adverse effects on children's IQ levels was utilised to calculate the approximate costs of organophosphate exposure in the EU, the total number of IQ points lost due to these pesticides was estimated to be 13 million per year, representing a value of about € 125 billion (Bellanger et al. 2015). Although this estimate is somewhat uncertain (most likely underestimated as it focused only on one group of pesticides and on one outcome), this calculation emphasises the need to generate better and stricter safety information on pesticides, limit human pesticide exposure further through regulation and public information, obtain better exposure assessments for

population-wide pesticide exposures, and acquire better documentation on the adverse health effects associated with current pesticide exposure.

Finally, the controversy related to glyphosate versus GBHs has emphasised the need to include the whole pesticide formulations including adjuvants in the risk assessment procedure.

1.2 Categorisation of Substances

Table 1-1: Substances included in the substance group, listed according to availability of toxicology and human biomarker data, in category A, B, C, D, E substances (see general introduction)

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B	PYR	Pyrethroids (group)*			
B		Permethrin (proposed lead substance)	52645-53-1	Acute Tox. 4 - H302; Acute Tox. 4 - H332; STOT SE 3 - H335; Skin Sens. 1 B H317; AELlong-term: 0.05 mg/kg bw/d ADI: 0.05 mg/kg bw/d (WHO/FAO JMPR) Potential ED cat.2 (JRC) (EC 2016)	Not approved as plant protection product (PPP) in EU Approved as biocidal product (BP) T8 and T18 Reg. (EU) No 1090/2014
B		Acrinathrin	101007-06-1	No classification ADI: 0.01 mg/kg bw/d; ARfD: 0.01 mg/kg, AOEL: 0.007 mg/kg bw/dg (Reg (EU) 2017/358); Identified as Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP Reg. (EU) no 2017/358, No 540/2011, No 974/2011 (2008/934) Not approved as BP
B		Allethrin	584-79-2		Not approved as PPP or BP in EU
B		Alpha-cypermethrin (alphamethrin)	67375-30-8	Acute Tox 3 – H301, STOT SE 3 – H335, STOT RE 2 – H373 (nervous system) ADI: 0.015 mg/kg bw/d, ARfD: =.04 mg/kg bw, AOEL: 0.01 mg/kg bw/d (Dir 4/58)	Approved as PPP 04/58/EC, Reg. (EU) 018/917, Reg. (EU) No 540/201 Approved as BP T18 Reg. (EU) 2015/405
B		Bifenthrin	82657-04-3	Acute Tox 2 – H300, Acute tox 3 – H331, STOT RE 1 – H372 (nervous system), Skin Sens 1B – H317, Carc 2 – H351, ADI: 0.015 mg/kg bw/dg, ARfd: 0.03 m/kg b, AOEL: 0.0075 mg/kg bw/d (Reg (EU) 2018/291 Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP Reg. (EU) 2017/195, Reg. (EU) 2018/291, Reg. (EU) No 582/2012 Approved as BP T8: directive 2011/10/EU

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Cyfluthrin	68359-37-5	Acute tox 2 – H300 ADI: 0.003 mg/kg bw/d, ARfD: 0.02 mg/kg bw, AOEL: 0.02 mg/kg bw/d (Dir 03/31) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP (as beta-cyfluthrin) 03/31/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011Reg. (EU) No 823/2012 (Reg. (EU) 2016/950) Approved as BP T18 reg (EU) 2016/1937
B		Cypermethrin	52315-07-8	Acute tox 4 – H302, Acute tox 4 – H332, STOT SE 3 – H335 ADI: 0.05 mg/kg bw/d, ARfD: 0.2 mg/kg bw, AOEL: 0.06 mg/kg bw/d (Dir 05/53), ADI: 0.02 mg/kg bw/d, ARfD: 0.04 mg/kg bw (JMPR 2006) Potential ED cat.1 (JRC) (EC 2016)	Approved as PPP 05/53/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011 (Reg. (EU) No 533/2013) Approved as BP T8 Reg(EU) 945/2013 and T18 Reg (EU) 2018/1130
B		Zeta-cypermethrin	52315-07-8 (same as cypermethrin)	No classification ADI: 0.04 mg (kg bw/d, ARfD: 0.125 mg/kg bw, AOEL: 0.02 mg/kg bw/d (EFSA 08)	Approved as PPP, 2009/37 Reg. (EU) No 540/2011
B		Zyphenothrin (or cyphenothrin)	39515-40-7		Approved as BP PT18
B		d-Allethrin	231937-89-6; 584-79-2	Acute tox 4	Under review as BP T18
B		Deltamethrin	52918-63-5	Acute tox 3 – H301, Acute tox 3 – H331 ADI: 0.01 mg/kg bw/d, ARfD: 0.01 mg/kg bw, AOEL: 0.0075 mg/kg bw/d (Dir 03/5) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 03/5/ECReg. (EU) 2017/1511Reg. (EU) No 540/2011Reg. (EU) No 823/2012. Full dossier is currently under review for renewal (AIR-3 programme). Approved as BP T18, directive 2011/81/EU
B		d-Tetramethrin	1166-46-7	Acute tox 4, Carc 2, STOT SE 2 (nervous system, inhalation)	Under review as BP T18
B		Empenthrin	54406-48-3		Under review as BP T18
B		Epsilon-momfluorothrin	1065124-65-3	Acute tox 4, STOT SE 2 (nervous system)	Approved as BP T18 Reg (EU) 2016/2289

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Esbiothrin	260359-57-7		Under review as BP T18
B		Esfenvalerate	66230-04-4	Acute tox 3 – H301, Acute tox 3 – H331, Skin Sens1 – H317 ADI: 0.0175 mg/kg bw/d, ARfD: 0.0175 mg/kg bw, AOEL: 0.011 mg/kg bw/d (Reg (EU) 2015/2047) Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 00/67/ECReg. (EU) 2015/2047Reg. (EU) No 540/2011 (2010/77/EU, Reg. (EU) 2015/1885)
B		Etofenprox	80844-07-1	Lact.- H362 ADI: 0.03 mg/kg bw/d, ARfD: 1 mg/kg bw, AOEL: 0.06 mg/kg bw/d (EFSA 08) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP 2009/77/ECReg. (EU) No 540/2011 Approved as BP T8 (Dir 2008/16/EC) and T18 (Reg (EU) 1036/2013
B		Fenpropathrin	39515-41-8	Acute tox – H301, Acute tox 4 – H312, Acute tox 2 – H330, ADI: 0.03 mg/kg bw/d, ARfD 0.03 mg/kg bw (JMPR 2012)	Not approved as PPP (Reg (EC) No 1107/2009
B		Fenvalerate	51630-58-1	No classification ADI: 0.0125 mg/kg bw/d (EMEA)	Not Approved as PPP (98/270/EC)
B		Imiprothrin	72963-72-5	Acute tox 4	Approved as BP T18 Reg (EU) 2017/2326
B		Lambda-cyhalothrin	91465-08-6	Acute tox 3 – H301, Acute tox 4 – H312, Acute tox 2 – H330 ADI: 0.0025 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.00063 mg/kg bw/d (Reg (EU) 2016/146 Potential ED cat.2 (JRC) (EC 2016)	Approved as PPP 00/80/EC Reg. (EU), 016/146 Reg. (EU) No 540/2011 Approved as BP T18 (Dir 2011/10/EU)
B		Gamma-cyhalothrin	76703-62-3	No classification ADI: 0.0012 mg/kg bw/d, ARfD: 0.0025 mg/kg bw, AOEL: mg/kg bw/d (Reg (EU) No 1334/2014.	Approved as PPP Reg. (EU) No 1334/2014
B		Metofluthrin	240494-71-7	Acute tox 3 and 4, STOT SE 1 (nervous system), STOT RE 2	Approved as BP T18 (Dir. 2010/71/EU)

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Prallethrin	23031-36-9	Acute tox 3 and 4	Under review as PB T18.
B		Tau-fluvalinate	102851-06-9	Acute tox 4 – H302, Skin Irrit 2 – H315. ADI: 0.005 mg/kg bw/d, ARfD: 0.05 mg/kg bw, AOEL: 0.0044 mg/kg bw/d Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP Reg (EU) 2011/19/ No 540/2011
B		Tefluthrin	79538-32-2	Acute tox 2 – H300, Acute tox 2 – H310, Acute tox 1 – H330 ADI: 0.005 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.0015 mg/kg bw/dg (EFSA 10) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP Reg. (EU) No 800/2011
B		Tetramethrin	7696-12-0	Acute Tox. 4, Carc. 2, STOT SE 2 (nervous system, inhalation)	Not approved as PPP (2002/2076) Under review as BP T18
B		Transfluthrin	118712-89-3	Skin Irrit 2	Approved as BP T18, Reg (EU) 407/2014
B		1R-trans-phenothrin (or D-phenothrin)	26046-85-5		Approved as BP T18, Dir. 2013/41/EU
B	PBO	Piperonyl butoxide (co-formulant, synergist)	51-03-6	Carc.2; H351 (increased incidence of hepatocellular adenomas and carcinomas in mouse), STOT SE 3; H335 (respiratory tract irritation), EUH066. The long and medium term AELs and ADI are equal to 0.2 mg/kg bw/d and the AEL short term is 1.0 mg/kg bw/d. Classified as Group C Possible Human Carcinogen by US-EPA	Approved as BP T18 Reg. (EU) No 528/2012, Reg (EU) 2016/2288
B	OP	Organophosphates			

Cat.	Abbrev. / Acronym	Systematic name	CAS No.	Classification (EC1272/2008) and thresholds	Regulation
B		Chlorpyrifos (OP)	2921-88-2	Acute tox 3 – H301, ADI: 0.001 mg/kg bw/d, ARfD: 0.005 mg/kg bw, AOEL: 0.001 mg/kg bw/d (EFSA 2014) Potential ED cat.3 (JRC) (EC 2016)	Approved as PPP, 05/72/EC, Reg. (EU) No 540/2011, Reg. (EU) No 762/2013, Reg. (EU) No 84/2018. Full dossier is currently under review for renewal (AIR-3 programme). Chlorpyrifos, and chlorpyrifos-methyl, did not get renewal and their authorisations were withdrawn by 16 February 2020. Max. period of grace: 16 April 2020.
C		Dimethoate (OP)	60-51-5	Acute tox 4 – H302, Acute tox 4 – H312 ADI: 0.001 mg/kg bw/d, ARfD: 0.01 mg/kg bw, AOEL: 0.001 mg/kg bw/d (EFSA 2013). In an EFSA risk assessment published in 2018 no toxicological reference values were established due to genotoxicity concerns (European Food Safety Authority (EFSA) 2018). Potential ED cat.2 (JRC) (EC 2016).	Approved as PPP, 07/25/EC, Reg. (EU) 2018/917, Reg. (EU) No 540/2011. Full dossier is currently under review for renewal (AIR-3 programme).
C		Fipronil	120068-37-3	Acute tox 3 – H301, Acute tox 3- H311, Acute tox 3 – H331, STOT RE – H372 (nervous system), ADI: 0.0002 mg/kg bw/d, ARfD: 0.009 mg/kg bw, AOEL: 0.0035 mg/kg bw/d (Dir 07/52) Potential ED cat.2 (JRC) (EC 2016) Classified as group C "possible human carcinogen" by US-EPA.	Not approved as PPP Reg. (EU) 2016/2035Reg. (EU) No 540/2011Reg. (EU) No 781/2013 Approved as BP T18 (Dir 2011/79/EU)
B		Glyphosate	1071-83-6	Eye Dam 1 – H318 ADI: 0.5 mg/kg bw/d, ARfD: 0.5 mg/kg bw, AOEL: 0.1 mg/kg bw/d (Reg (EU) 2017/2324) Potential ED cat.2 (JRC) (EC 2016) Classified as a "probable human carcinogen" group 2A by IARC.	Appoved as PPP Reg. (EU) 2017/2324, Reg. (EU) No 540/2011 2016/1056, Reg. (EU) 2016/1313) Latest approval 17/12 2017 for 5 years, expiring 15712 2022
C	POE-Tallowamine	Polyethoxylated tallow amine (co-formulant for glyphosate)	61791-26-2		No registration (but many pre-registrations) under REACH (According to information from ECHA January 2019).

PPP: plant protection product, BP: Biocidal product, T8: wood preservative; T18: Insecticides, acaricides and products to control other arthropods

1.3 Policy-related questions

1. Which are the most suitable methods and biomarkers of exposure?
2. What are the current exposure levels of the EU population to the prioritised pesticides: pyrethroids, chlorpyrifos and dimethoate, glyphosate (in combination with polyethoxylated tallow amine (POEA)), and fipronil and do the exposure levels differ between countries?
3. What are the main dietary sources of exposure across the member states?
4. What are other sources and pathways of exposure?
5. What are exposure levels among occupationally exposed workers?
6. Are the exposure levels of health-relevance/concern for vulnerable groups (infants, children and pregnant women) or high exposure population groups (e.g., occupational exposure)?
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?
8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?

1.4 Research Activities to be undertaken

While completing this table please think of data and gaps concerning toxicology (and exposure [in three dimensions: **location** (differences between the countries), **time** (trends) and **age** (data available for which age group)]. If no HBM method is available or the method has to be harmonised within partner countries, please indicate this too.

Table 1-2: Listing of research activities to be carried out to answer the policy questions summed up in 1.3

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
1. Which are the most suitable methods and exposure biomarkers?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	There are established and validated methods for analysing urinary metabolites as marker for the total pyrethroid exposure (3-PBA), the combined exposure to cypermethrin, permethrin and cyfluthrin (cis- and trans-DCCA), and for some specific pyrethroids (deltamethrin, cyfluthrin, bifenthrin). The detection frequency is low for most specific pyrethroid metabolites but depends on the limit of detection (LOD) which vary between different analytical approaches and labs. Furthermore, pyrethroids are often metabolised to several different metabolites with low fractions of each specific metabolite. There are available methods for analysing the metabolite, TCPy, which is specific for chlorpyrifos and chlorpyrifos-methyl and for group-specific urinary organophosphate metabolites, i.e., dialkyl phosphates (DAPs) as a marker for the total OP exposure level. DAPs are divided into diethyl phosphates (DEPs) and dimethyl phosphates (DMPs). DEPs include chlorpyrifos while DMPs include chlorpyrifos-methyl and dimethoate. A method exist to measure some pyrethroids (total and some specific) and chlorpyrifos simultaneously. Glyphosate is primarily excreted in urine as unchanged parent molecule. Humans are also exposed to AMPA which is the main metabolite found in water. Both glyphosate and AMPA can be measured in urine with established methods.	Activities: 1. Evaluation and selection of best suited biomarkers of exposure (Y3-Y4) (WP9) 2. The methods for analysing urinary metabolites of pyrethroids, chlorpyrifos, organophosphates (DMPs and DEPs), and glyphosate, need to be harmonised within partner countries to obtain comparable values and LODs (WP9) 3. Development/validation of methods to include more specific pyrethroid metabolites could be considered based on expected prevalent exposure and whether major specific metabolites are formed (WP9).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POEA)	Because dimethoate and the specific metabolite are rapidly metabolised to DMPs, establishment of a sensitive specific urinary biomarker for dimethoate is not possible. Dimethoate will be included in the DMPs (see above for Cat B) After fipronil exposure, the major metabolite, fipronil sulfone, is rapidly formed. This metabolite is rather persistent and toxic in mammals. A method to measure fipronil sulfone in serum is available and seem to be the best biomarker for fipronil exposure. Recently, a method to measure hydroxyl-fipronil in urine was developed using rat urine but this method has not yet been applied on human samples.	Activities: 1. If prioritised to include fipronil (Q2), urine will be the preferred matric, allowing analyses of all the pesticides in the same samples. Thus, a method to measure its metabolite in human urine should be further developed and validated (WP9) 2. If prioritised to include POEA (Q2), the first step will be to collect available information on toxicokinetic i.e., absorption after different exposure routes, metabolism, and major urinary metabolites to evaluate if it is possible to establish a sensitive and reliable biomarker method (WP9).
2. What are the current exposure levels of the EU general population to the prioritised pesticides?	Cat B (pyrethroids, chlorpyrifos, and glyphosate)	HBM studies including these substances have been performed in some EU countries but not EU-wide. The studies indicate widespread exposure in the general population. The exposure to pyrethroids is expected to be increasing as they replace organophosphates (OPs) in biocidal products and to some degree also as insecticides in agriculture. Children have higher food intake per kg body weight leading to higher exposure levels from pesticide residues in food as also confirmed in previous HBM-studies	Gaps: Few studies have been performed after 2010 and data are lacking for many EU countries. More data are needed to evaluate differences between countries and population groups, time trends, and age-related differences in exposure. Activities: 1. Collecting, comparison, and evaluation of existing biomonitoring data in the EU and integration into IPCHEM (Y4) (WP8/WP10) 2. Further identify and prioritise knowledge and data gaps and related research needs (Y4) (WP4) 3. Planning and analysing supplementary urine samples from the alignment studies preferentially from children and from studies with available information on dietary habits and/or residential use of pesticides (Y4-Y5) (WP8) 3. Data-analyses of time-trends and differences between countries and population groups, including identification of subpopulations with highest exposure levels (Y4-Y5) (WP10). 4. Data analysis to identify differences between population groups related to e.g., age, dietary habits, residence near agricultural pesticide applications, and indoor residential use, occupational exposure (Y4-Y5) (WP10)

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
	Cat C (dimethoate, fipronil, and POE-tallow amine (POEA))	Studies from the US reported very low detection frequencies (< 1%) for dimethoate and omethoate, because they are rapidly metabolised to unspecific dimethyl phosphates (DMPs). Urinary DMPs and diethyl phosphates (DEPs) have been included in many studies as biomarker for the total OP exposure. Thus, including DMPs and DEPs will allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies. A recent case of fipronil misuse caused large scale contamination of chicken eggs but otherwise fipronil is seldom detected in commodities at the EU market. Fipronil is approved as biocide and for veterinary use but no longer for agricultural use in EU. There is reliable evidence that POEA increase the toxicity of some glyphosate formulations. Although POEA was recently banned in the EU, exposure from residues in food items (imported or due to contaminated soils) is very likely but there is no monitoring data from commodities or other potential human exposure sources to underpin the relevance of HBM.	Gaps: No EU HBM studies have included urine concentrations of dimethoate or its specific metabolite omethoate. There is no HBM data from EU on fipronil or POEA. Activities: 1. Consider to include DMPs and DEPs in the analyses of supplementary urine samples from the alignment studies, as suggested above for the cat. B substances, to allow assessment of the total OP exposure (including dimethoate and chlorpyrifos) and comparison with previous studies (WP8). 2. Prior to method development for POEA and fipronil (see Q1) it should be considered wheter to prioritise to monitor these substances in human matrices (preferentially urine) at present (WP4). 3. If prioritised, methods for analysing POEA and fipronil in urine has to be developed (WP9) – see Q2 for Cat C substances below, and samples from the alignment studies or from targeted studies will be analysed for fipronil and/or POEA (WP8)
3. What are the main dietary sources of exposure across the member states?	Cat B and C (all substances)	Residues in the diet is the main continuous exposure source for pesticides in the general population. Pesticide residues in food is measured under coordinated control programmes (EUCP) and the national control programmes (NP). The coordinated multiannual control programme for 2018, 2019, and 2020 (Regulation (EU) 2017/660) includes many of the HBM4EU selected pesticides (i.e., 12 different pyrethroids, chlorpyrifos/chlorpyrifos methyl, dimethoate, glyphosate and fipronil). These data are collected and stored by EFSA (European Food Safety Authority 2017, 2018). Human breast milk samples are not included in the control programmes. Chlorpyrifos and pyrethroids have been found in breast milk samples from other countries (e.g., USA, India, Brazil and Colombia) sometimes in concentrations exceeding the MRL of 0.01 mg/kg for food for infants and young children (Directive 2006/141/EC). Only six samples from EU (Spain) have been analysed. Methods to analyse pyrethroids and chlorpyrifos in human milk samples are available.	Activities: 1. Continue to analyse/model HBM data in relation to monitoring data on residues in food samples (EUCP, EFSA) to 1) compare and complement exposure assessment performed by EFSA and 2) identify the major dietary exposure sources across member states (Y4-Y5) (WP12) 2. Consider if possible, to perform a pilot study analysing selected pyrethroids and chlorpyrifos (parent compounds) in existing bio-banked milks samples (WP9, WP8).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
4. What are other sources and pathways of exposure?	Cat B and C (all substances)	Living near agricultural areas where pesticides are applied may enhance the exposure level to pesticides due to drifting, as demonstrated in studies from the US. No HBM data from EU are currently available. A targeted survey including families (children and adults) living close to pesticide treated agricultural areas (3-5 countries) using a new developed multi-target screening of multiple pesticides in urine samples is planned in WP15 and WP16 (Survey on Pesticide Mixtures in Europe, SPECIMEn) Indoor use of pyrethroids and/or fipronil as biocides has been shown in studies from the US to contribute markedly to the exposure level – especially among children.	Activities: 1. Analysing the urine samples from the WP15/16 survey using the above-mentioned methods, will allow quantification of these pesticide metabolites and subsequent data analyses to compare the levels with those obtained from the alignment studies (WP10) and comparison with the result obtained by the multi-target screening method in WP15/16 ((Y4-Y5) (WP8/WP9/WP10) Data gap: Biocidal use of pyrethroids might be increasing in the EU but there is no HBM studies investigating this exposure situation. Activities: 1. A targeted study, focusing on children living in homes with repeated residential use of biocides would be highly relevant, e.g., with urine sampling before and fixed time points after treatment (WP8). 2. If a targeted study is not prioritised it may be possible to get some information by analysing HBM data from the alignment studies (including additional analyses of urine samples from children) in relation to questionnaire information on residential use, if such data are available (preferentially with information on time interval between sampling and treatment). Data on authorisation and sale of biocidal products might also be included in data analysis of HBM data from the alignment studies to investigate exposure differences between member states (WP10).

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
5. What are exposure levels among occupationally exposed workers?	Cat B and C (all substances)	Occupational exposure to agricultural workers who mix and/or apply pesticides onto crops can be substantial, with dermal exposure considered the most important pathway, although inhalation may also be important. Also, workers handling crops/plant after treatment have enhanced exposure and, since many young women in fertile age groups, are employed in agriculture/horticulture/floriculture they constitute a special risk group. Further, workers employed in companies applying biocides (pyrethroids and/or fipronil) in dwellings and institutions might have high dermal and inhalation exposure.	Data gap: There is no HBM data from EU covering occupational exposure of the selected pesticides. Investigating occupational exposure levels is important to identify high exposure groups. Activities: A targeted study addressing occupational exposure levels is highly relevant (WP8) as this Q cannot be answered based on the alignment study. The WP15/WP16 mixture survey will provide data on exposure profile/level among residents (children and mothers) close to agricultural fields (orchards) in five EU-countries. Including urine sampling also from agricultural workers who mix and/or apply the pesticides in this survey would allow additional information on occupational exposure (WP15, WP16, WP8)
6. Are the exposure levels of health-relevance/concern for vulnerable groups or high exposure population groups?		Most of the prioritised pesticides are neurotoxicants (OPs, pyrethroids, fipronil) and some also have ED or genotoxic/carcinogenic properties. The main health concerns are adverse effects on neurodevelopment and/or endocrine disturbances affecting reproduction, metabolism etc.	Activities: 1. Combining and analysing HBM data (, e.g, from birth cohort studies) with relevant health outcomes – if possible using meta-analysis (Y4-Y5) (WP13) 2. Identify/suggest adverse outcome pathways (AOPs) for and effect biomarkers for relevant health outcomes (including neurodevelopment and endocrine disrupting effects) (Y4-Y5) (WP13/WP14). 3. Identify/suggest relevant effect biomarkers (WP14) 4. Comparison of exposure levels (based on HBM data) with toxicologically derived guidance values (ADI values) and findings on associations with health outcomes in epidemiological studies (Y4-Y5) (WP5, WP13)
7. How can cumulative risks of pesticide mixtures on health outcomes be assessed and integrated in regulation?		Assumed additivity within the pesticide groups (similar mode of action; (e.g. pyrethroids) but also across groups (similar adverse effects; e.g., neurotoxicity of pyrethroids and OPs)	Input from WP15 and activities mentioned for Q6
8. Is it possible to establish EU wide accepted health-based guidance values for the pesticides, preferably taking potential mixture effects and evidence from epidemiological studies into account?			See activities for Q6 Input from WP5

Policy question	Substance	Available knowledge	Knowledge gaps and activities needed
9. How can HBM data from HBM4EU feed into prioritisation of the pesticides for risk assessments and regulatory decision-making?			Input from WP5

Annex 1: HBM data on pyrethroids (3-PBA), OPs (DAPs), chlorpyrifos/chlorpyrifos-methyl (TCPY) and glyphosate

Table 1-3: HBM data on pyrethroid exposure based on urinary concentrations of the generic pyrethroid metabolite 3-PBA. The values represent volume-based concentrations (μg/L) in spot urine samples unless otherwise stated.

Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95th pct	Remark	Ref
Europe										
PELAGIE, France	Pregnant Women Children, 6 yrs	02-06	205 284	0.008	30.2 63.7	- -	<LOD 0.018	- -	first trim, first-morning-voids	Viel et al. (2015)
Elfe, France	Pregnant women, at delivery	2011	1077	0.004	99.7	0.36	0.36	1.89	Analysed in Canada	Dereumeaux et al. (2016)
GerES, Germany,	Children 3-14 yrs	03-06	598	0.1	98	-	0.43	3.80		Schulz et al. (2009)
Poland (North)	All <18 yrs >18 yrs	12	374 184 190	0.1	82.4 - -	0.26 0.29 0.23	0.25 - -	1.24 - -		Wielgomas and Piskunowicz (2013)
Poland (Lodz)	Adult men, age < 45 y	2008-11	195	0.1		0.17	0.16	0.50		Radwan et al. (2015)
Poland, Gdansk)	Genral pop, 5-77 y	2010-11	132	0.1	80	0.26	0.25	1.15	First morning voids	Wielgomas et al. (2013)
Spain	Children 6-11 yrs	10		0.8	23	-	<LOD	12.33*	*μg/g creatinine	Roca et al. (2014)
OCC, Denmark	Pregnant Women	10-12	858	0.03	94.3	0.22	0.20	2.18	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children, Denmark	Children 10-16 yrs Children 10-16 yrs	11-13	143 128	0.03	100 100	0.66 0.51	0.56 0.49	8.90 8.98	Non-fasting Fasting	unpublished
Greenhouse Cohort Children, Denmark	6-11 yrs	07-08	173	0.8	41.0	0.66	<LOD	4.11	first-morning-voids	Andersen et al. (2012)

Study	Population	Sampling year	N	LOD	%>LOD	GM	50th pct (median)	95th pct	Remark	Ref
America										
NHANES, USA	Children 6-11 yrs 20-59 yrs	09-10	383 1296	0.1		0.55 0.42	0.48 0.39	8.51 6.95		CDC (2015)
NYC HANES, USA	>20 yrs	04	1452	0.64	58.5	-	0.76	5.23		McKelvey et al. (2013)
SUPERB, USA	Children 2-8 yrs 18-57 yrs	07-09	83 64	0.75	60 90		1.56 1.58	4.69 9.44	Residential use	Trunnelle et al. (2014b)
MICASA, USA	Children 2-8 yrs Mothers 23-52 yrs	09	103 105	0.1	78 82	1.11 1.17	1.93 1.63	7.36 13.29	Farm worker families	Trunnelle et al. (2014a)
CHAMOCOS, USA	Pregnant Women	99-01	481	0.1	27	-	<LOD	1.1	Agricultural area, Second trim, GW 26	Castorina et al. (2010)
Mt. Sinai, New York, USA	Pregnant women	98-01	307			-	18.3	126.9*	Third trim, *90th pct Residential use	Berkowitz et al. (2003)
CHMS, Canada	Children 6-11 yrs All, 6-79 yrs	07-09	1032 5604	0.01	99.3 99.4	 0.25	0.20 0.23			Oulhote and Bouchard (2013); Ye et al. (2015)
ELEMENT, Mexico	Pregnant women	97-01	187	0.25	56	0.26	<LOD	0.85	third trimester	Watkins et al. (2016)
Caribbean	Pregnant women	08-11	297	0.01	100	0.54	-	3.51	third trimester	Dewailly et al. (2014)
PROTECT, Puerto Rico	Pregnant Women	10-12	54	0.1		0.2	<LOD	2.3	second trimester	Lewis et al. (2014)
Asia										
Japan	Pregnant Women	09-11	231	0.02	97.8	0.33	0.35	-	GW 10-12	Zhang et al. (2013)
China	Pregnant Women	10-12	322	0.1	82	0.37	0.50	2.6		Ding et al. (2015)

GM: geometric mean; GW: gestational week

Table 1-4: HBM data on OPs. Urinary concentrations of total dialkyl phosphate metabolites (ΣDAP; molar sum of DEPs and DMPs). The values represent volume based concentrations (nmol/L) in spot urine samples unless otherwise stated.

Study	Population	Sampling Year	N	GM	50th pct (median)	95th pct	Remark	Ref.
Europe								
OCC, Denmark	Pregnant women	10-12	564	58.7	56.5	253	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children,	Children10-16 yrs	11-13	141	89.7	85.6	506		Andersen et al. (in publication)
Greenhouse Cohort Children,	Children 6-11 yrs	07-08	172	160.4	153.7	1252	First-morning-voids	Andersen et al. (2012)
DEMOCOPHES DK-part, Denmark	Women 31-52 yrs Children 6-11 yrs	11	145 144	84.8 111	92.3 106		First-morning-voids	Mørck et al. (2016)
PELAGIE	Pregnant women	02-06	254		38.8		First-morning-voids	Debost-Legrand et al. (2016)
Generation R, Holland	Pregnant women	04-06	100	183	200	659	GW 20	Ye et al. (2008)
MoBa. Norway	Pregnant women	99-04	10	87*			10 pools, each consisting of pooled urine from 11 women * Calculated from μg/L	Ye et al. (2009)
Greece, Crete	Adults	08-09	86	-	15	-	Agricultural area	Kokkinaki et al. (2014)
America								
NHANES, USA	Children 8-15 yrs	00-04	1139	68.3				Bouchard et al. (2010)
HOME, Ohio, USA	Pregnant women	03-06	327	73.7*	96.7*		*nmol/g creatinine, two spot urine samples during preg,	Donauer et al. (2016)
NYC HANES, USA	Adults >20 yrs	04	876	-	114.9	1321.8		McKelvey et al. (2013)

Study	Population	Sampling Year	N	GM	50th pct (median)	95th pct	Remark	Ref.
Mount Sinai, USA	Pregnant women	98-02	342	75.5*	77.9*	894.7*	*nmol/g creatinine, residential use	Harley et al. (2016)
CHAMACOS, U.S.	Pregnant women Children 5 yrs	99-00 04-05	348 320	109 92.6	-	-		Marks et al. (2010)
MIREC, Canada	Pregnant women	08-11	1884	78	78	538	First trim	Sokoloff et al. (2016)
CHMS, Canada	Children 6-11 yrs All, 6-79 yrs	07-09	1035 5604	- 76.7	99.2 71.4	-		Oulhote and Bouchard (2013); Ye et al. (2015)

GM: geometric mean; GW: gestational week

Table 1-5: HBM data on chlorpyrifos/chlorpyrifos-methyl. Urinary concentrations of the specific metabolite TCPY. The values represent volume-based concentrations (μg/L) in spot urine samples unless otherwise stated.

Study	population	Sampling year	LOD	%>LOD	N	GM	50th pct (median)	95th pct	Remark	Ref
Europe										
OCC, Denmark	Pregnant women	10-12	0.3	93.2	858	1.67	1.74	8.15	Fasting, GW 28	Dalsager et al. (2018)
Greenhouse Cohort Children	Children10-16 yrs	11-13	0.3	95.8 94.5	143 128	1.42 1.52	1.43 1.55	6.05 7.31	Non-fasting Fasting	Andersen et al. (in publication)
MoBa, Norway	Pregnant women	99-04			10*	0.99			second trim, *pooled samples	Ye et al. (2009)
Generation R, The Netherlands	Pregnant women	04-06	0.15	100	100	1.2	1.2	6.4	> GW 20	Ye et al. (2008)
Spain	Children 6-11 yrs	10	0.80	86	125	3.36*	3.40*	12.97*	*μg/g creatinine	Roca et al. (2014)
America										
NHANES, USA	Children 6-11 yrs Adults 20-59 yrs	09-10	0.1		386 1309	1.12 0.71	1.46 0.97	5.81 4.18		CDC (2015)
CHAMOCOS, USA	pregnant women	99-01	0.3	81.9	481	-	3.2	17.9	Agricultural area, Second trim, GW 26	Castorina et al. (2010)
Mt. Sinai, USA	pregnant women	98-01			365	-	7.5	61.2*	Third trim, *90th perc Residential use	Berkowitz et al. (2003)
ELEMENT, Mexico City	pregnant women	97-05	0.1	>90	187	1.76	1.78	11.6	Third trim	Fortenberry et al. (2014)
Puerto Rico	Pregnant women	10-12	0.1	86.2	54	0.4	0.5	2.0	4 samples per women	Lewis et al. (2015)
Australia	Children 2.5-6 yrs	03-06		92.2	115	-	12.5*	71.1*	*μg/g creatinine	Babina et al. (2012)
Asia										

Study	population	Sampling year	LOD	%>LOD	N	GM	50th pct (median)	95th pct	Remark	Ref
China	Children 3-6 yrs	2014		44.1	406	0.92*	0.63*	22.9*	*μg/g creatinine	Wang et al. (2016)

GM: geometric mean; GW: gestational week

Table 1-6: HBM data on glyphosate and AMPA. Urinary concentrations (μg/L) in various European countries

Reference	Country	Period	Population	N	Definition of average	Value average**	Calculation high values	High value**
3e Flemish Center of Expertise on Environment and Health (Steunpunt M&G)	Belgium (Flanders)	2013-2014	Adults (50-65 years), general population	269	GM (95% CI)	Gly: <LOQ AMPA: 0,109 (0,098-0,120)a	P90 (95% BI)	Gly: 0,230a AMPA: 0,344 (0,135-0,553)a
Paulussen, 2013	Belgium (Flanders)	2012-2013	Teenagers (14-15 years), general population	11	P50	Gly: 0,30 AMPA: 0,33	P75	Gly: 0,40 AMPA: 0,60
BUND, 2013 (Hoppe 2013)	Belgium	2013		11	average	Gly: 0,18 AMPA: 0,29	max	Gly: 0,57 AMPA: 1,26
	Netherlands	2013		8	average	Gly: 0,34 AMPA: 0,25	max	Gly: 1,02 AMPA: 0,64
	France	2013		10	average	Gly: 0,12 AMPA: 0,14	max	Gly: 0,23 AMPA: 0,41
	Germany	2013		10	average	Gly: 0,25 AMPA: 0,23	max	Gly: 0,49 AMPA: 0,70
	Great-Britain	2013		10	average	Gly: 0,47 AMPA: 0,23	max	Gly: 1,64 AMPA: 0,56
	Switzerland	2013		12	average	Gly: 0,09 AMPA: 0,08	max	Gly: 0,16 AMPA: 0,08
	Spain	2013		10	average	Gly: 0,12 AMPA: 0,17	max	Gly: 0,22 AMPA: 0,82
Danish part of DEMOCHOPHES, Knudsen et al. (2017)	Denmark	2011	Children 6-11 yrs	14	Mean	Gly: 1.96	max	Gly: 3.31
	Denmark	2011	mothers	13	Mean	Gly: 1.18	max	Gly: 3.22

Reference	Country	Period	Population	N	Definition of average	Value average**	Calculation high values	High value**
Conrad et al. (2017)	Germany (Greifswald)	2011	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 0.51 AMPA: 0.65
	Germany (Greifswald)	2012	20-29 yrs	40	P50	Gly: 0.11 AMPA: 0.12	max	Gly: 0.63 AMPA: 0.66
	Germany (Greifswald)	2013	20-29 yrs	40	P50	Gly: 0.11 AMPA: <LOQ	max	Gly: 2.80 AMPA: 1.88
	Germany (Greifswald)	2014	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 1.78 AMPA: 0.97
	Germany (Greifswald)	2015	20-29 yrs	40	P50	Gly: <LOQ AMPA: <LOQ	max	Gly: 0.57 AMPA: 0.41

[a]*corrected for age, sex, smoking and urine density*

** *Gly = glyphosate*

Annex 2: HBM4EU-suggested pesticide biomarkers/metabolites and reference to established analytical methods

Pesticide group	Metabolite/biomarker (abbereviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Pyrethroid	3-phenoxybenzoic acid (3-PBA)	3739-38-6	Common metabolite of most pyrethroids, e.g,: cypermethrin, deltamethrin, permethrin, lambda-cyhalothrin, d-phenothrin, tau-fluvalinate, esfenvalerate, fenpropathrin, (not cyfluthrin or bifenthrin)	Urine	LC-MS/MS, 0.03 (Davis et al. 2013); UPLC-MS/MS, 0.008 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.8 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	4-fluoro-3-phenoxybenzoic acid (F-3PBA)	77279-89-1	Cyfluthrin	Urine	LC-MS/MS, 0.03 (Davis et al. 2013); UPLC-MS/MS, 0.003 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.2 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DCCA)	55701-05-8	Cis-permethrin, cis-cypermethrin, cis-cyfluthrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.07 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.4 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Trans-DCCA)	55701-05-6	Trans-permethrin, trans-cypermethrin, trans-cyfluthrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.01 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.4 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK
Pyrethroid	cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (Cis-DBCA)	63597-73-9	Deltamethrin	Urine	LC-MS/MS, 0.4 (Davis et al. 2013); GC-MS/MS, 0.07 (Viel et al. 2015); GC-MS, 0.1 (Becker et al. 2006; Wielgomas and Piskunowicz 2013); LC-MS/MS, 0.8 (Roca et al. 2014); GC-MS, 0.1 (0.5 nM) (Bevan et al. 2013)	OK

Pesticide group	Metabolite/biomarker (abbereviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Pyrethroid	4-chloro-alpha-isopropyl benzene acetic acid (CPBA)		Esfenvalerate, fenvalerate	Urine	GC-MS, 0.04(Tao et al. 2013)	Probably not relevant, likely low DF*
Pyrethroid	2-methhyl-3-phenylbezoic acid (MPA)	115363-11-6	Bifenthrin	Urine	GC_MS, 0.04(Tao et al. 2013)	?
Pyrethroid	3-(chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylic acid (HCVA)		Lambda-cyhalothrin	Urine	GC-MS, 0.08(Tao et al. 2013)	?
Pyrethroid	Chlorotrifluorovinylcyclo-propane carboxylic acid (CIF-3-CA)		Bifenthrin and cyhalothrin	Urine	GC_MS, 0.5 nM(Bevan et al. 2013)	Might be relevant?
Pyrethroid	Parent compounds		Permethrin, cypermethrin, cyhalothrin, deltamethrin	Human breast milk	GC–NCI–MS–MS analysis, 2.8-1100 pg/g lipid weight (Corcellas et al. 2012).	Pilot study?
Pyrethroid synergist (co-formulant)	Piperonyl butoxide (PBO)		Piperonyl butoxide	Urine	UHPLC-QqQ-MS/MS, 0.047/0155.(Cazorla-Reyes et al. 2011) Below LOD in all urine samples analyzed by LC-MS/MS at SDU (unpublished).	?
Organophosphate	3,5,6-trichloro-2-pyridinol (TCPY)		Chlorpyrifos and chlorpyrifos-methyl	Urine	LC-MS/MS, 0.1 (Davis et al. 2013); GC-MS/MS, 0.15 (Ye et al. 2008); UPLC- HRMS, 0.8 (Llop et al. 2017b)	OK
Organophosphate	Dimethoate and omethoate		Dimethoate	Urine	LC-MS/MS, 0.03 and 0.05 (Llop et al. 2017a)	Not sensitive - out
Organophosphate	Diethyl phosphate (DEP), diethyl thiophosphate (DEDTP), diethyl dithiophosphate (DEDTP)		Unspecific metabolite of ethyl-organophosphates e.g., chlorpyrifos, diazinon, ethion, coumaphos, terbufos	Urine	LC-MS/MS, 0.1-0.5 (McKelvey et al. 2013); GC-MS/MS, 0.65, 0.59, 0.05 nM (Ye et al. 2008); UPLC- HRMS, 3.2-10 (Llop et al. 2017b)	OK

Pesticide group	Metabolite/biomarker (abbereviation)	Cas no	Parent pesticide/compound	Matrix	Analytical methods, reported LOD/LOQ in ng/ml (Reference)	Status
Organophosphate	Dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP)		Unspecific metabolite of methyl-organophosphates, e.g., dimethoate, chlorpyrifos-methyl, azinphos-methyl, malathion, fenthion, phosmet, naled	Urine	LC-MS/MS, 0.1-0.5 (McKelvey et al. 2013); GC-MS/MS, 0.79, 0.70, 0.63 nM (Ye et al. 2008); UPLC- HRMS, 1.6 (Llop et al. 2017b)	OK
Glyphosate	Glyphosate (Gly) and aminomethylphosphonic acid (AMPA)		Glyphosate and AMPA	Urine	GC-MS/MS, 0.1(Conrad et al. 2017); LC-MS/MS, 0.1 (Parvez et al. 2018)	OK
Co-formulant with Glyphosate	?		Polyethoxylated tallowamine (POEA)	?	?	?
Fipronil	Hydroxyl-fipronil		Fipronil	Urine	LC-MS/MS, 0.4 (rat urine)(Vasylieva et al. 2017)	OK
Fipronil	Fipronil sulfone, fipronil desulfinyl		Fipronil	Serum/plasma or breast milk	LC/TOF-MS, 0.1 (McMahen et al. 2015)	?

**DF: detection frequency*

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