**Report "Test-Achats": exposure to pyrethroid and organophosphate residues in children's urine**

## (1) General remarks

(i) In the spring of 2019, the Belgian non-profit organisation "Test-Aankoop" (TA) analysed the urine of 84 children between 2 and 15 years old. Traces of recent exposure to one or more insecticides were found in all children. The general statement states: "*with regard to some substances, especially the disruptors of the hormonal system and the reproductive system, no dose is free of risk*".

It may be noted that the assimilation of pyrethroids (P) and organophosphates (OP) with "*endocrine disruptors*" is scientifically incorrect. During the European revision program, a comprehensive analysis is carried out of the possible endocrine character of all active substances (a.s.), and the above substances are of course also included, at least the a.s. placed on the European market. Based on existing evaluations, there are no indications that P or OP would have consistent or specific endocrine *disrupting* capacity (even if endocrine *effects* were occasionally observed). Moreover, there is no scientific consensus for the claim that safe doses cannot be established for endocrine disorders.

(ii) TA reports that all children who participated in the study were exposed to at least one OP shortly before sampling, that 83 of them were exposed to at least one P-based insecticide, and that therefore no urine sample was free of metabolites of pesticides.

It is clear that the presence of metabolites in the urine can of course be caused by the intake of P or OP, but it must also be taken into account that the children may simply have been also exposed to the metabolites themselves. For food intake, which is probably the largest source of contamination (Schettgen *et al.*, 2002), it is known that foods effectively contain breakdown products (Lu *et al*, 2005) that are well absorbed by mammals (Timchalk *et al.*, 2007). In addition, it should be noted that one a.s. can form different metabolites (*e.g.*: cypermethrin degrades in PBA, c-DCCA and t-DCCA; β–cyfluthrin forms both FPBA and DCCA). For DBCA and FPBA, the origin can only be deltamethrin and β–cyfluthrin. An excessive emphasis on excretion of metabolites and double counting can be a source of risk overestimation (Krieger *et al*, 2003, Sudakin and Stone, 2011).

(iii) TA also claims that there is no legal norm that sets the maximum concentrations in the urine for these pesticides. This is of course correct, but the question then arises as to why measures should be taken to regulate this, since the authorisations already take into account the internal exposure, and therefore excretion, of pesticide metabolites.

(iv) Furthermore, TA claims that "*it is (not possible) to predict whether the concentrations observed in the urine will have an adverse effect on the health of the children*". This is not entirely correct. During the toxicological assessment of the a.s. an estimation of food intake is performed, the content of a.s. and / or metabolites is determined, and the internal doses calculated therefrom are compared with the toxicological reference values. Based on the result, it is decided whether or not there is safe use. An a.s. is only approved if the reference values, such as the acceptable daily intake and the acute reference dose (ADI, ARfD) are not exceeded.

(v) The final remark, being that: *"The association with some condition is all the more difficult to establish because children are exposed to various chemicals on a daily basis that may also have toxic effects"* is related to the cumulative risk assessment, for which EFSA is very intensively developing a methodology. For all details, see: https://www.efsa.europa.eu/en/press/news/faq-cumulative-risk-assessment-pesticides

It is obvious that there is no doubt about the observation that humans are exposed to numerous chemical and non-chemical influences.

## (2) Specific remarks

(i) We have been able to consult the limited dataset from TA on exposure to the various metabolites and glyphosate. First of all, it should be noted that the values obtained for P and OP metabolites do not differ meaningfully from those previously measured internationally. The data set from TA does not add anything essential to the data we had, and which, in turn, did not raise any particular concern.

(ii) By way of example, the urine values of the most common pyrethroid metabolite 3-phenoxybenzoic acid (3-PBA) were used to estimate the active substance content to which the individuals were exposed. This is a calculation performed on the entire population of 84 children and 5 adults (N=89). As no ages were communicated, an accurate calculation could not be performed.

For the children it was communicated that this is an age group "*from 2-15 years*" and a realistic worse-case default value (11-12 years) of the urinary output of 22.2 mL/ kg body weight / 24 hours was used (Miller and Stapleton, 1989). The urinary excretion of metabolites was believed to be only about 25%, which is in line with published data (a.o. Hays *et al*, 2009). Since for a *common* metabolite such as 3-PBA, it cannot be determined which a.s. was at the origin of it, we assumed that the lowest ADI value had to be taken, but the use of those a.s. in Belgium was also considered, taking into account the Belgian sales statistics (fytoweb, 2015). These are (in decreasing order) cypermethrin (30%), tefluthrin (29%) and lambda-cyhalothrin (19%), followed by deltamethrin and beta-cyfluthrin (both 10%), tau-fluvalinate and zeta-cypermethrin (5%), and marginal or no sales for alpha-cypermethrin, bifenthrin, gamma-cyhalothrin and esfenvalerate (<2%). Thus, from the 3 most commonly used a.s. generating the generic metabolite 3-PBA, the lowest ADI (in mg/kg b.w./d) was chosen as the reference value (λ–cyhalothrin, 0.0025 *vs*. cypermethrin and tefluthrin, both 0.005).

For the *specific* metabolites that are unambiguously representative of the a.s. deltamethrin and β–cyfluthrin, respectively DBCA and FPBA, the ADIs of those a.s. were used, i.e. both 0.01 mg/kg b.w./d.

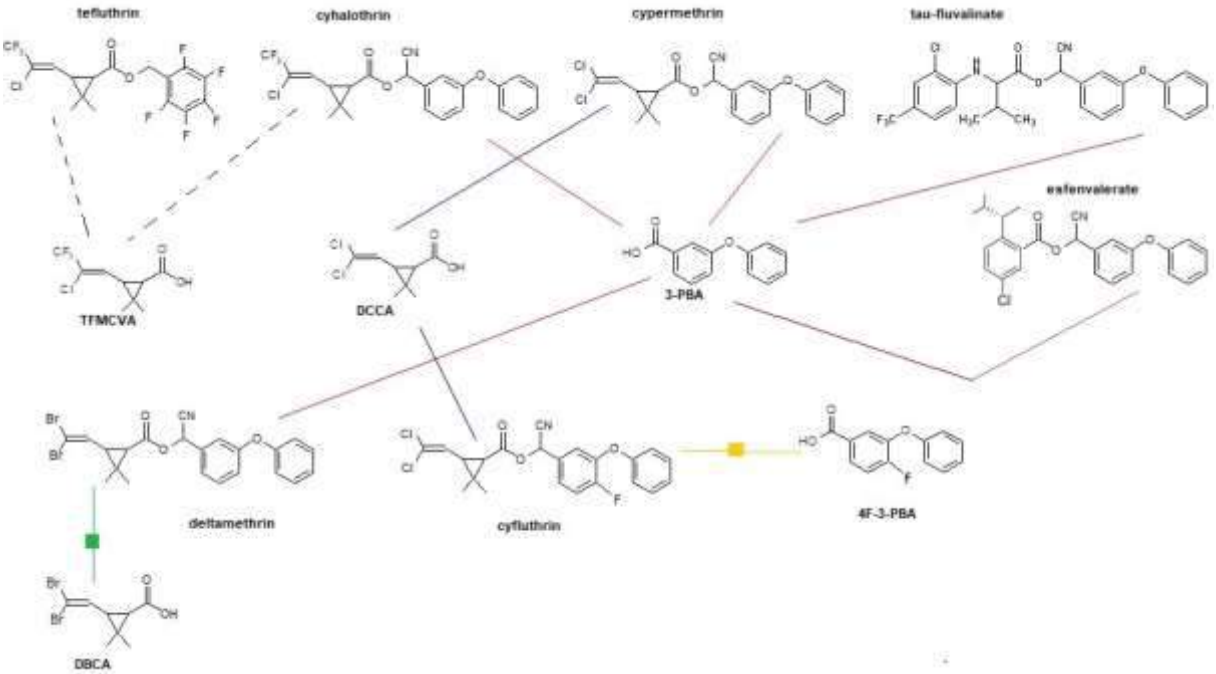
For the metabolites whose levels fell below the limit of quantification (LOQ), it was cautiously assumed that the value was 50% of this LOQ, which is a standard method of dealing with so-called 'non-detects' (Helsel, 1990).

Scheme 1 represents the metabolism of the most common pyrethroids used in crop protection, including the measured degradates DCCA, 3-PBA, DBCA and 4-F-3-PBA (FPBA). Tefluthrin does not form any of those metabolites, but rather the fluorinated form of DCCA, for which no measured values were however reported.

*(Note that for the determination of the ADI's, the most recent values mentioned by EFSA in its conclusion were taken into account. This is in particular the case for β-cyfluthrin, where the EU Commission Pesticide Database mentions an old value of 0.003 mg / kg b.w. bw / d, which was recently revised upwards to 0.01 mg/kg bw/d, see EFSA, 2018a).*

(iii) Assuming that the above parameters would be acceptable, and based on the maximum levels of the (most) common metabolite 3-PBA (possibly formed from 5 a.s. including cypermethrin, cyhalothrin, tau-fluvalinate, esfenvalerate or deltamethrin) measured in the urine, a conversion to the most toxic pyrethroid λ–cyhalothrin was calculated, and it appears that approximately 26% of ADI is consumed. The same exercise based on the breakdown product DCCA (*cis* + *trans*), now taking into account the most toxic pyrethroid (ADI = 0.005) among the 2 a.s. (cypermethrin and β–cyfluthrin) that can form this, yields an estimated exposure corresponding to 25% of the ADI.

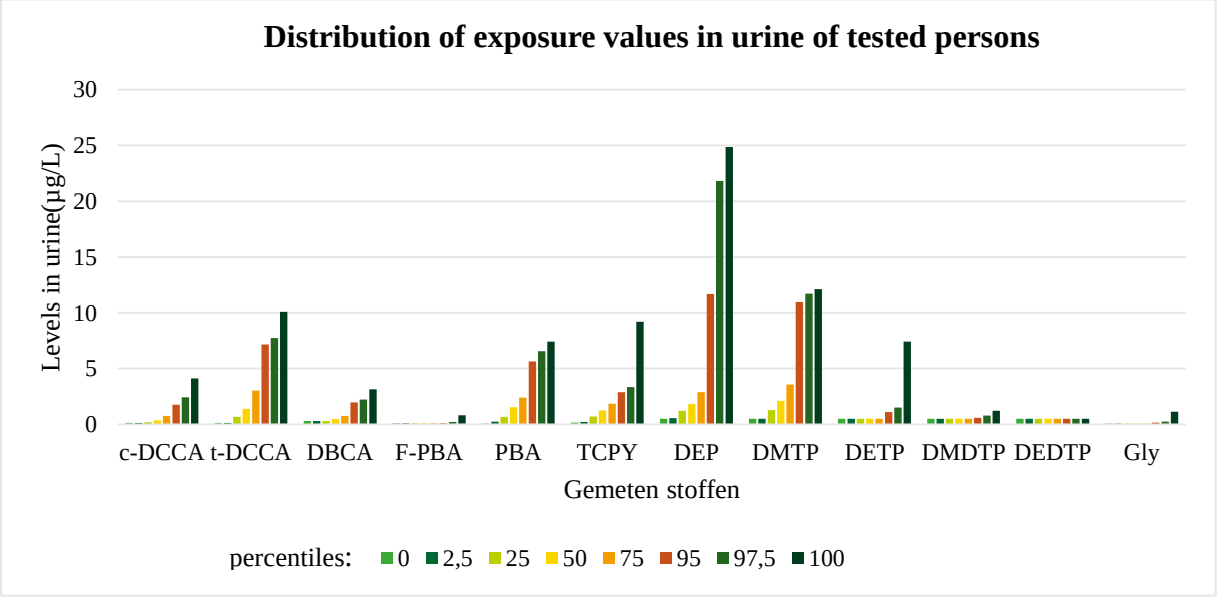
(iv) Based on the levels of the specific metabolites FPBA and DBCA of the a.s. that produce these (resp. deltamethrin and β–cyfluthrin), the estimated exposures are estimated at 2.8% and 0.7% of ADI respectively. If we would make the sum of all calculated exposures, the summed values provide approximately 55% of the ADIs.




*(Scheme 1: formation of both generic metabolites of pyrethroids and specific ones (indicated by ■ and ■))*

(v) However, refining this coarse estimate is desirable, since it is unlikely that children will be exposed to the maximum residue value during their whole lifetime, and that a comparison with the lowest reference value, which is probably a lot lower than that of the metabolite(s) themselves, is also not opportune. Summing up the exposure to 3-PBA and DCCA will already lead to some overestimation of the exposure to (the most common) cypermethrin, since both degradation substances are formed in equimolar quantities starting from 1 mole of cypermethrin.

The graph below (scheme 2) shows the distribution of the exposure values per percentile. The highest percentiles correspond to values that, at least if we limit ourselves to an assessment of residues in the food (which makes the highest contribution), come from so-called "high-consumers" for a food component that would deliver the most residues. It is worth noting that of the 12 substances studied, 1/12 in all cases and 5/12 in the categories up to and including the 75$^{th}$ percentile are ≤ the LOQ.




*(Scheme 2: distribution of exposure values (in µg / L) in the urine of the tested persons)*

(vi) As regards the *common* dialkyl phosphates (DAP's) formed from the organophosphate insecticides, the 6 metabolites sought can be linked to 21 OPs (according to the TA study). Of these, 4 are still authorised in Belgium for the time being: chlorpyrifos-ethyl, chlorpyrifos-methyl, phosmet and pirimiphos-methyl (for the chlorpyrifos substances the non-renewal of the EU approval was recently published). Only one of the metabolites sought, namely DEDTP, cannot be linked to any OP contained in an authorised product, and this metabolite was never recovered. The most common substance is diethyl phosphate (DEP), among others, which may come from OPs such as chlorpyrifos-ethyl, diazinon, disulfoton, phorate, phosalone, sulfotep and terbufos. None of those substances has been approved (or is not approved any more) in the EU, and therefore less relevant for the authorised products in Belgium. However, they can still be present in certain non-agricultural biocides or in raw materials and food imported from outside the EU. A concordance between the levels of DAP's and 28 known OPs from which they can be can formed can be found, for example, in Bravo *et al* (2002).

The only reported *specific* metabolite trichloropyridynol (TCPY) is that of the OP chlorpyrifos-ethyl, which is no longer authorised from 31 January 2020, and for which an EU ADI value of 0.001 mg / kg bw / d is stated in the EU Pesticide Database. Where appropriate, the exposure to the maximum value (0.00082 mg / kg b.w./d) would correspond to approximately 82% of the previously determined ADI value.

For the 2 organophosphates phosmet and pirimiphos-methyl still authorised in Belgium, the respective ADIs are 0.01 and 0.004 mg / kg bw / d, and the lowest value may possibly serve to put into context the only reported common metabolite DMTP (dimethylthiophosphate). For example, at a maximum level of 12.13 µg/L, the estimated amount of pirimiphos-methyl is 0.0011 mg / kg b.w./d, which corresponds to approximately 28% of the ADI.

(vii) As it is the case of all common pesticide metabolites, DAP metabolites are generally considered rather as biomarkers of exposure to OPs. Their measurement can give an impression of both cumulative and aggregate exposures (taking into account the aforementioned direct exposure to DAPs in food as well as indoor / outdoor pollution). However, because the individual OPs differ greatly in toxicity, these data are not necessarily good indicators of a cumulative toxic dose. These data can therefore be used to "screen" for total OP exposure and can be a point of departure for follow-up analyses, where the levels of more specific metabolites could be performed should the DAPs appear elevated. All that does not alter the fact that for the currently authorised pyrethroids further research is needed to gain a better insight into the toxicological profile of the common and specific metabolites.

(viii) An evaluation can be made for the herbicide glyphosate, which was also measured. The maximum value is 1.13 µg/L, corresponding to 0.0001 mg/kg b.w./d, which represents 0.02% of the ADI of glyphosate. This is also of the same order of magnitude as the value for chronic exposure to glyphosate, set at 0.05-0.16% of the ADI in the EU assessment (EFSA, 2017).

## (3) Conclusion

(i) TA's report describes the presence of metabolites in the urine of children and some adults, and the levels reported are in line with previously published values abroad. A mere consideration of the exposure values in urine says nothing about the toxicological relevance of the measured substances. The measured metabolites of pyrethroid (P) and organophosphate (OP) insecticidal active substances (a.s.) may not only originate from the degradation in humans themselves, but also from direct absorption from food or the environment.

(ii) One can assume with a reasonable degree of certainty that the reported metabolites are less harmful than the a.s., as they are not or much less neurotoxic than pyrethroids or organophosphates, although it may be highlighted that the set of the metabolite toxicity data is less complete, certainly regarding potential long-term effects.

(iii) A quick but conservative calculation based on the reported urine levels of the metabolites shows that long-term reference values will probably not be exceeded. During the recent EU review of cypermethrin (see also EFSA, 2018b), dozens of epidemiological studies were investigated, and compared with the numerous regulatory studies in the dossier and in the open scientific literature. Existing animal studies at first sight suggest some biological plausibility with regard to anti-androgenicity, neurotoxicity and DNA damage, but the study data in humans (epidemiology) currently do not allow to confirm these effects unequivocally.
Remarkably, potentially weak endocrine and DNA damaging effects, occasionally described in animal studies, are by no means systematically observed, and potential confounders such as high toxicity are not excluded. Similarly, the intrinsic neurotoxicity of (alpha-) cypermethrin, albeit an important and critical effect that determines the reference values, and explained by a number of mechanisms of action, is insufficiently characterised to explain all inconsistent effects in the human population (see also Burns and Pastoor, 2018).
As rightly noted by Saillenfait *et al.* (2015), further epidemiological research is desirable to arrive at definitive conclusions, especially in sensitive groups.

(iv) The claim that pyrethroids or their metabolites in general would be endocrine disruptors is, based on a limited number of studied a.s., therefore so far insufficiently confirmed by the facts during their re-evaluation. The hypothesis that safe threshold values could not be established for endocrine effects is, on the basis of current knowledge, by no means the subject of a general consensus. The hypothesis that the exposure period ("most sensitive window") can be important for endocrine substances is neither confirmed nor contradicted with the current data, since the level of detail of the observation is insufficient to make statements about this.

(v) The TA report therefore does not give rise to particular concerns or adjustments to existing approvals and authorisations at substance and product level. This is all the more so given the phasing out of most OPs on the EU market. However, given the import of raw commodities and food from countries outside the EU, and the possible contribution by some biocides, the human population remains exposed to residues of many neurotoxic substances and glyphosate. Further research should reduce as much as possible the existing uncertainties regarding the differential toxicity between the neurotoxicants still present and their specific metabolites.

(vi) These findings are without prejudice to the recommendations to minimise exposure to pesticides (of agricultural or other origin), as these will lead to an increase in the safety margin obtained as a result of the regulatory risk assessment.

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