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Assessment of occupational exposure to pesticide mixtures with endocrine disrupting activity

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Abstract

Occupational exposure to pesticide mixtures comprising active substance(s) and/or co-formulant(s) with known/possible endocrine disrupting activity was assessed using long-term activity records for 50 professional operators representing arable and orchard cropping systems in Greece, Lithuania, and the UK. Exposure was estimated using the harmonised Agricultural Operator Exposure Model, and risk was quantified as a point of departure index (PODI) using the lowest no observed (adverse) effect level. Use of substances with known/possible endocrine activity was common, with 43 of the 50 operators applying at least one such active substance on more than 50% of spray days; at maximum, one UK operator sprayed five such active substances and ten such co-formulants in a single day. At 95th percentile, total exposure was largest in the UK orchard system (4.1×10^{-2} mg kg bw^{-1} day^{-1}) whereas risk was largest in the Greek cropping systems (PODI 5.3×10^{-1}). All five cropping systems had instances indicating potential for risk when expressed at a daily resolution (maximum PODI 1.2-10.7). Toxicological data are sparse for co-formulants so combined risk from complex mixtures of active substances and co-formulants may be larger in reality.

Keyword: professional operator, active substance, co-formulant, combined effect, risk, regulation

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1. Introduction

Agricultural operators can be exposed to complex mixtures of pesticides when applying tank mixes of two or more products or when making sequential applications of different products (Panizzi et al. 2017). Complexity of mixtures to which operators are exposed may be further increased because pesticide products comprise both the declared active substances that control the target pests/plant diseases and co-formulants that aid application and/or improve the effectiveness of the product (Yusoff et al. 2016). To date, little is known about the risk from cumulative exposure to different combinations of pesticides in mixtures (Kienzler et al. 2016).

Pesticides with endocrine disrupting activity are of particular health concern because the endocrine system regulates the secretion of almost all hormones that control the metabolism and function of the body, influencing almost every cell, organ and function of an organism (EFSA 2013a). They can interfere with the function of the hormone system, thus dysregulating homeostatic mechanisms, reproduction and development (Sidorkiewicz et al. 2017). Numerous studies have suggested effects from occupational exposure to endocrine disrupting pesticides on the reproductive system including reduced semen quality and lower luteinizing hormone (Hossain et al. 2010; Mehrpour et al. 2014; Cremonese et al. 2017). Other studies suggest higher risk of hypospadias, and allergic and non-allergic wheeze (Rocheleau et al. 2009; Mesnage et al. 2017). Pesticides with endocrine disrupting activity can instigate effects at very low doses that are not always predicted from tests at higher doses (Futran Fuhrman et al. 2015). Similarly, chemicals that are present individually at ineffective doses can produce substantial effects when combined in mixtures (Christiansen et al. 2012; Hass et al. 2012).

Cumulative risk from exposure to mixtures of pesticides that can produce common adverse effects on the same target organ or organ system is a particular concern (EFSA 2013b); concentration/dose addition is generally used as the default first tier approach for hazard quantification (Sarigiannis and Hansen 2012). For instance, good agreement was found between observed and predicted effects on sexual development in rats based on dose-additivity for a mixture of five low-dose endocrine disrupting pesticides comprising epoxiconazole, mancozeb, prochloraz, tebuconazole and procymidone (Hass et al. 2012). Generally, the concentration/dose addition approach is considered sufficiently conservative to assess the risk from combined exposure to multiple chemicals, irrespective of the similarity and dissimilarity of their mechanisms or modes of action in the mixtures (Kienzler et al. 2016).

European pesticide regulations require risk assessments that usually focus on the declared active substances with additional, but generally fewer, data requirements for commercial product formulations (Kienzler et al. 2016). Regulation (EC) 1107/2009 concerning the placing of plant protection products on the market requires that

individual active substances to be included in pesticide products should have no harmful effect on human health nor the environment on the basis of harmonised criteria at Community level. Meanwhile, pesticide co-formulants are authorised in the Member States with responsibility for characterising toxicological hazard transferred to industry under the CLP Regulation (EC) 1272/2008 on the classification, labelling and packaging of substances and mixtures (Hernandez and Tsatsakis 2017). The potential for mixture effects from different combinations of pesticides applied in multiple products is not covered within pesticide regulation and has rarely been tested (Kienzler et al. 2016).

Professional agricultural and horticultural operators often handle large amounts of pesticides and thus have high potential for exposure to multiple products with similar toxicological endpoints. They thus represent a vulnerable group for combined effects of pesticide mixtures. This study investigates actual scenarios of pesticide use for professional operators in order to: determine the pesticide mixtures to which individuals are potentially exposed; quantify the exposure to and risk from pesticide active substances with known/possible endocrine disrupting activity; and investigate whether co-formulants in pesticide products might be an additional source of exposure to endocrine disruptors. To do this, we analyse usage of known and possible endocrine disrupting substances over an agricultural season for a total of 50 professional operators from different cropping systems in Greece, Lithuania, and the UK. Exposure of operators is assessed on a daily basis using the Agricultural Operator Exposure Model (AOEM; Groβkopf et al. 2013) and potential risk is assessed using the lowest no observed (adverse) effect levels (NO(A)ELs) for endocrine disrupting effects and an assumption of concentration addition. We analyse results to determine gaps in knowledge in the current risk assessment.

2. Methodology

2.1. Pesticide application data

We used a dataset of pesticide applications made by professional operators that was collected on behalf of the European Food Safety Authority (EFSA) with the purpose of addressing cumulative exposure and potential for combined, non-dietary effects of pesticide products (Garthwaite et al. 2015). The dataset comprises long-term records of all pesticide handling activities for a large number (> 400) of professional operators, including details on the pesticide products used, application methods, and personal protective measures. This allows in-depth investigations of operators' exposure during mixing/loading and application tasks. Based on an earlier study Wong et al. (Wong et al. 2018), a total of 50 professional operators were randomly selected to give ten individuals each from arable and orchard farming systems in the UK and Greece, and a further ten from arable agriculture in

Lithuania. These countries were selected as having robust data quality (Garthwaite et al. 2015). Data for each operator covered all pesticide spraying and handling activities over an agricultural season (2012/13) and comprised crop, pesticide product, area applied, mass applied, volume applied, spray equipment and personal protective equipment.

2.2. Identification of pesticides with endocrine disrupting activity

An endocrine disruptor is defined as "an exogenous substance or mixture that alters the functions of the endocrine system and consequently cause adverse effects in an intact organism, or its progeny, or (sub) populations" whilst a possible endocrine disruptor is "an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub) populations" (WHO/IPCS 2002). We classified the declared active substances of products applied in our dataset for their known or possible endocrine disrupting activity based on the Pesticide Properties Database (PPDB 2018), which is an international database for pesticide risk assessments and management that is endorsed by the International Union of Pure and Applied Chemistry and promoted by major organisations including the Food and Agricultural Organisation (Lewis et al. 2016). Four triazole fungicides had no relevant data available (i.e. difenoconazole, metconazole, paclobutrazol, and tebuconazole; Table S1), but were included here because studies have identified that triazoles and structurally similar chemicals are potential endocrine disruptors (Marx-Stoelting et al. 2014; Lv et al. 2017; Teng et al. 2018).

Determination of whether or not co-formulant chemicals have potential for endocrine disrupting activity was undertaken for a single, exemplar scenario (UK orchards). A total of 93 pesticide products that were applied by at least one operator from the UK orchard system were identified for their co-formulants based on individual material safety data sheets (MSDS). Where no MSDS was found, the most similar product from the same manufacturing company and formulation type was substituted. Afterwards, individual co-formulants were assessed for their potential endocrine disrupting activity based on their chemical abstract service numbers (CAS No.) in accordance with the Hazardous Substances Data Bank in the Toxicological Data Network (TOXNET, https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm) and the PPDB (2018). We extracted all endocrine-relevant data from animal-based studies including information on different routes and durations of exposure as there is limited toxicological data for co-formulants (Table S2). Co-formulants where no data were found to indicate endocrine disrupting properties were assumed not to be active as endocrine disruptors.

2.3. Quantification of exposure

Professional operators are mainly exposed to pesticide products during mixing/loading and application tasks via two major routes, namely dermal absorption and respiratory inhalation (Damalas and Abdollahzadeh 2016). These exposure scenarios are included within the harmonised Agricultural Operator Exposure Model (AOEM) to reflect agricultural practices in the EU (Groβkopf et al. 2013). The AOEM is based upon empirical data from 34 exposure studies conducted between 1994 and 2009. The model allows the adjustment of a range of exposure parameters including the formulation type (liquid, wettable powder, wettable granule), personal protective equipment (PPE; gloves, face shield, coverall), and application equipment (knapsack, vehicle-mounted tractors, cabin status) (Groβkopf et al. 2013). Here, we employed the AOEM to assess the median exposures of operators to individual active substances with known/possible endocrine disrupting activity during mixing/loading and application tasks across individual spray days (Table S3).

In the AOEM algorithms, the total mass of active substance handled during a day is the dominant input parameter to the exposure modelling. However, pesticide products consist of the declared active substance plus co-formulants that may be hazardous in themselves. The AOEM algorithms were also adopted to assess the occupational exposure to any co-formulants that were identified on the MSDS for the respective product and that were identified as having possible endocrine disrupting activity. The MSDS rarely gives precise information on the exact proportions of different co-formulants, so we used the mean value where a range was given (e.g. 3% for "1-5%") and the defining number for compositional formulations (e.g. 5% for "<5%", "≤5%" or ">5%"). Exposure to individual co-formulants was calculated as for active substances, considering exposure to the hands, body, head, and via inhalation; the influence of any personal protective equipment and/or equipment design was included in the calculation and adjustments for dermal and inhalation absorptions were based on the content of individual co-formulants in the products. Full details of the exposure model are provided in Wong et al. (2018). The total exposures to active substances and co-formulants with known/possible endocrine disrupting activity were summed separately for each individual spray day.

2.4. Risk estimation

According to EFSA (2013b), the combined effects of individual pesticide active substances should be determined based on their toxicological profiles where experimental measurements of combined effects are not available. To estimate risk from exposure to multiple active substances with known/possible endocrine disrupting activity

handled on a single spray day, we adopted an application of the concept of concentration addition to calculate the combined dosages in the mixture based on the point of departure index (PODI) (Christiansen et al. 2012):

$$\boldsymbol{PODI} = \sum_{i=1}^{n}\left[\frac{EL_i}{\left(\frac{POD_i}{UF}\right)}\right] \qquad \textbf{(Eqn. 1)}$$

where *EL* is the estimated exposure level (mg kg bw^{-1} day^{-1}) and POD is the point of departure for endocrine disrupting effects (NO(A)ELs in mg kg bw^{-1} day^{-1}). *UF* is the default uncertainty factor of 100, frequently characterised as a factor of 10 for interspecies extrapolation and a further factor of 10 for different sensitivities among humans (Bang et al. 2012). A PODI >1 indicates that significant effects are possible.

For the POD, we extracted the short-term NO(A)ELs (subacute or subchronic) for endocrine disrupting effects from six established toxicological databases, namely the EFSA Draft Risk Assessment Report and Assessment Report (http://dar.efsa.europa.eu/dar-web/provision), the Joint Meeting on Pesticide Residues of the International Programme on Chemical Safety, http://www.inchem.org/pages/jmpr.html), the Hazardous Substances Data Bank of TOXNET, the Integrated Risk Information System (https://www.epa.gov/iris), the EPA Endocrine Disruptor Screening Program Tier 1 screening determinations and associated data evaluation records (https://www.epa.gov/endocrine-disruption/endocrine-disruptor-screening-program-tier-1-screening-determinations-and) the European Commission (EC) Endocrine Disruptors Database (EDS, http://ec.europa.eu/environment/chemicals/endocrine/strategy/substances_en.htm), ECHA Classification and Labelling report, and other open literature (Table S4). Active substances that lacked a short-term NO(A)EL were assessed against individual chronic NO(A)ELs for endocrine disrupting effects; this was necessary for captan, chlorothalonil, flusilazole, linuron, paclobutrazol, propiconazole and pyriproxyfen. When neither short-term nor chronic NO(A)ELs were available (i.e. for deltamethrin and s-metolachlor), the lowest observed (adverse) effect levels (LO(A)ELs) for endocrine disrupting effects were applied with an adjusted uncertainty factor of 1000 (Bullock et al. 2006) (Table S4).

A major challenge was encountered during the identification and extraction of NO(A)ELs for endocrine disrupting effects. As the disrupting process may affect different endpoints due to an alteration of function of the endocrine system, it is often difficult to assess the endocrine mediated mechanism or mode of action (Marx-Stoelting et al. 2014). The endocrine system communicates with the nervous and immune systems via multiple common pathways, so chemical exposure may affect the function of these systems together (Liu et al. 2006). For instance, observed effects on testicular and uterine weight in test organisms could be due to endocrine disruption even though no mechanistic evidence is available (Ewence et al. 2013). The problem associated with determining adversity and risk from endocrine disruptor compounds remains unresolved (Futran Fuhrman et al. 2015). Hence,

we extracted NO(A)ELs for any observed (adverse) effects on the thyroid, adrenal, pancreas, pituitary, prostate, gonad (testes and ovaries), hormones, spleen, and growth retardation for the current assessment (Table S4).

3. Results

3.1. Pesticide application data

The pesticide programmes used across five cropping systems included eight active substances that are known to have endocrine disrupting activity (Table 1), comprising bifenthrin, bromoxynil, deltamethrin, fenoxycarb, ioxynil, picloram, tau-fluvalinate, and triadimenol (PPDB 2018). All systems included applications of at least one such substance, with a maximum of six active substances with known endocrine disrupting activity applied in the UK arable system. More than half (48-67% across the different cropping systems) of active substances with known/possible activity were fungicides, with 13-35% insecticides and 10-28% herbicides (Table 1).

Overall, the UK cropping systems were treated with a larger number of active substances with known/possible endocrine disrupting activity during the survey period (medians of 11 and 10 chemicals for arable and orchard systems, respectively) than the Greek cropping systems (6 and 5 chemicals for orchard and arable systems, respectively) and the Lithuanian arable system (4 chemicals) (Fig. 1a). The masses of identified active substances applied were also largest in the UK (medians of 305 and 256 kg a.s. for orchard and arable systems, respectively) (Fig. 1b). Active substances with known/possible endocrine disrupting activity were handled relatively frequently with 86% of the 50 professional operators handling at least one such substance on more than 50% of total spray days during the period investigated (Fig. 1c), and up to five identified active substances applied on a single day in the UK orchard system (Fig. 1d).

3.2. Predicted exposure and risk from active substances with known/possible endocrine disrupting activity

Fig. 2 shows that the estimated exposure to active substances with known/possible endocrine disrupting activity on single spray days varied greatly across the 50 selected professional operators. Overall, all operators had at least one spray day with predicted exposure to such active substances over the survey period. At median level, the predicted daily exposure was generally larger amongst the orchard operators from the UK ($1.1x10^{-3}$ - $5.1x10^{-2}$ mg kg bw^{-1} day^{-1}) and Greece ($2.4x10^{-4}$ - $2.2x10^{-2}$ mg kg bw^{-1} day^{-1}) compared to individuals working in arable systems in Greece ($8.3x10^{-5}$ - $2.0x10^{-2}$ mg kg bw^{-1} day^{-1}), the UK ($1.1x10^{-4}$ - $3.7x10^{-3}$ mg kg bw^{-1} day^{-1}), and Lithuania ($8.7x10^{-5}$ - $1.6x10^{-3}$ mg kg bw^{-1} day^{-1}). Over the survey period, the Greek arable operators had relatively larger variance around mean daily exposure (coefficients of variation 103-340%), whilst variance was

intermediate for those from the orchard systems in the UK and Greece (78-232% and 88-180%, respectively), and relatively smaller amongst the arable operators from Lithuania and the UK (51-148% and 62-116%).

Fig. 3 shows the predicted risk per spray day from exposure to active substances with known/possible endocrine disrupting activity across the 50 selected operators. Generally, the Greek and UK orchard operators had larger risk estimates (medians of PODI $5.0x10^{-3}$ - $5.5x10^{-1}$ and $7.6x10^{-3}$ - $2.2x10^{-1}$, respectively) than those from arable systems of Lithuania and the UK ($8.6x10^{-4}$ - $2.4x10^{-1}$ and $1.1x10^{-3}$ - $3.3x10^{-2}$, respectively). Overall, 14 of the 50 operators had at least one spray day with PODI >1; the largest number of individuals meeting this criterion were from the Greek cropping systems (five and four operators for arable and orchard systems, respectively) and the least for the UK cropping systems (only one operator in each system). Individuals with maximum PODIs >1 generally had larger variance around mean daily PODI over the survey period; for example, three Lithuanian arable operators with maximum PODIs of 3.5, 5.6 and 4.1 had estimated coefficients of variation 233, 398 and 263%, respectively (Fig. 3a).

Fig. 4 shows cumulative frequency distributions for estimates of total exposure and total risk on single spray days and for individual operators from the five cropping systems. Across all of the operators, at least one active substance with known/possible endocrine disrupting activity was applied on ca. 60 to 80% of the total spray days that were recorded in the database. On single spray days, the total exposure to such active substances varied greatly across all operators, ranging between $6.7x10^{-6}$ and $2.7x10^{-1}$ mg kg bw^{-1} day^{-1} (Fig. 4a). Estimated exposure was largest for the UK orchard system at all points on the cumulative frequency distribution (Fig. 4a). For example, at the 95th percentile, estimated exposure in the UK orchard system ($4.1x10^{-2}$ mg kg bw^{-1} day^{-1}) was more than an order of magnitude larger than that in the Lithuanian arable system ($2.6x10^{-3}$ mg kg bw^{-1} day^{-1}). Estimated risk was only largest for UK orchards up to the 60th percentile (Fig. 4b); at percentiles above this, risk was always largest in the Greek orchard system mainly due to the applications of a few relatively hazardous substances (e.g. deltamethrin and chlorpyrifos-methyl with points of departure for endocrine disrupting activity of 0.001 and 1.0 mg kg bw^{-1} day^{-1}, respectively). At the 95th percentile of the distribution, the Greek cropping system had largest estimated risk (PODI of ca. $5.3x10^{-1}$ in each system), whilst this was intermediate for the UK orchard system and the Lithuanian arable system ($3.0x10^{-1}$ and $2.5x10^{-1}$, respectively), and least for the UK arable system ($9.0x10^{-2}$; Table 2). All five cropping systems had at least one operator with a point of departure index for endocrine disrupting effects on a single spray day greater than one (maximum PODIs ranged between 1.2 and 10.7; Table 2).

3.3. Predicted exposure to pesticide co-formulants with possible endocrine disrupting activity

Fig. 5 shows that co-formulants increased the complexity of potential exposure of the UK orchard operators to mixtures of chemicals with possible endocrine disrupting activity. At maximum, one operator applied five such active substances and ten such co-formulants on a single spray day. Only one active substance classified as having known endocrine disrupting activity was applied by any of the ten operators working in UK orchards. Fig. 6 shows that estimated exposure of operators to co-formulants classified as having possible endocrine activity was at a level lower than that for active substances; exposure to co-formulants contributed up to ca. 0.1 mg kg bw^{-1} and 46% of an individual's total exposure to pesticides with endocrine disrupting activity over the survey period.

4. Discussion

Professional agricultural operators across five agricultural systems in three European member states were potentially exposed on single spray days to complex mixtures of active substances and co-formulants with known/possible endocrine disrupting activity (Figs. 1d and 5). The majority of active substances identified as having known/possible endocrine disrupting activity were fungicides (48-67% of total active substances across the five agricultural systems; Table 1). In a review of recent literature on the effects of pesticide mixtures in human and animal models based on 78 studies published between 2000 and 2014, mixture effects of fungicides were associated predominantly with endocrine regulation and/or reproduction (Rizzati et al. 2016). Fig. S1 compares the relative contributions of fungicides, herbicides, and insecticides to the use, exposure and risk associated with endocrine disrupting activity. Overall, fungicides made the largest contribution to total usage and associated exposure across all cropping systems (48-67% and 58-99%, respectively) compared to herbicides (10-28% and 0.7-38%) and insecticides (13-35% and 0.2-26%; Figs. S1a and S1b). In contrast, insecticides and fungicides contributed similarly to risk across the five systems as a whole (Fig. S1c). Fungicides were the major component of risk in the Greek arable system and the UK cropping systems (64% and ca. 50% of total PODI in each system, respectively), whereas insecticides dominated the risk profile in the Lithuanian arable system and the Greek orchard system (94% and 79% of total PODI, respectively). Herbicides contributed least to the risk associated with endocrine disrupting activity, representing at maximum, 22% of the PODI in the UK orchard system.

Figs. 1d and 5 indicate that the professional operators in our dataset can be exposed to up to five active substances with known/possible endocrine disrupting activity on a single spray day, with predicted exposure ranging between $6.7x10^{-6}$ and $2.7x10^{-1}$ mg kg bw^{-1} day^{-1} (Fig. 4a). Table 2 indicates that all cropping systems had at least one operator with potential risk from exposure to active substances with known/possible endocrine disrupting activity

indicated by a point of departure index greater than 1 on a spray day. The instances with potential risk are primarily due to uses of deltamethrin where the LO(A)EL had to be used as the point of departure, and uses of mancozeb and copper oxychloride where the AOEM estimates larger exposure because they are formulated as wettable powders. Many of the copper oxychloride formulations are no longer approved as plant protection products, although growers can continue to use copper oxychloride based products as foliar feeds. Predicted concentrations below individual points of departure do not mean that there is no risk as the NOAELs cannot be equated with zero-effect levels (Kortenkamp et al. 2007). The endocrine system usually responds to hormone concentrations of parts-per-trillion and parts-per-billion and endocrine disruptors can coexist in the system to cause low-dose effects that are not predicted at higher dose (Vandenberg et al. 2012). A minor change in the concentration of an endocrine disrupting chemical can induce significant changes in biological endpoints even though the dose is small (Futran Fuhrman et al. 2015). Currently, risk assessment methodologies do not sufficiently assess the hazard associated with low-dose exposure to endocrine active substances (Melching-Kollmuss et al. 2017) and the lack of a universal definition for "low dose" is one obstacle to this.

Based on the UK orchard system, Figs. 5 and 6 indicate that professional operators can be simultaneously exposed to multiple co-formulants with possible endocrine disrupting activity; levels of exposure are generally lower than for the declared active substances, with co-formulants accounting for up to 46% of total exposure at maximum due to their relatively smaller proportions in the products. The AOEM was developed to simulate active substances and the algorithms of the model might require modification for co-formulants such as surfactants that have an amphiphilic structure consisting of a long-chain hydrocarbon and an ionic or highly polar group (Castro et al. 2014). Co-formulants are usually assessed for acute ocular and dermal properties, but there is no specific requirement for medium- and long-term regulatory experiments on mammals and acceptable daily intake values are not required to be established (Defarge et al. 2016). It was not possible to estimate risk from exposure to co-formulants here because of the lack of appropriate experimental endpoints; the total risk associated with use of the products will thus be greater than that reported here based on the active substances alone.

In the EU, pesticide formulations are typically registered at the national level and require more risk assessment data for the declared active substances than for the authorisation of co-formulants (Kienzler et al. 2016). It is usually the responsibility of industry to classify the co-formulants and this may result in different classifications, labelling, and levels of protection for substances with identical CAS numbers (Lichtenberg et al. 2015). The lack of complete disclosure of identity and concentrations of co-formulants and formulation ingredients coupled with

inadequate analytical methods constrain a comprehensive risk assessment for commercial plant protection products (Mullin et al. 2016).

There is currently no consensus on a science-based approach to the assessment of endocrine disrupting properties (Marx-Stoelting et al. 2016). The assessment is affected by different issues including the existence of safe thresholds for adverse effects, the significance of dose-response relationships, and the influence of different modes of action (Solecki et al. 2017). The adoption of scientific criteria for endocrine disruptors needs a clear definition of the hazard as the first step to developing test methods, identifying hazardous chemicals, and managing risk for regulatory purposes (Slama et al. 2016). Typically, chemicals with observed endocrine effects in experimental animals based on the test guidelines of Organisation for Economic Co-operation and Development need to be addressed for their relevance to humans including consideration of species, strain, exposure route OECD (2012), and species-specific differences such as endocrine signalling, toxicokinetics, and bio-transformation (Testai et al. 2013). The dose thresholds/guidance values for "Specific Target Organ Toxicity Repeated Exposure" were used to determine whether the hazardous property of endocrine disruption should be identified for regulatory purposes in accordance with the CLP Regulation (Ewence et al. 2015). Nevertheless, the OECD framework is inadequate for the identification of all aspects of endocrine disrupting effects, because it mainly focuses on estrogenicity, anti-androgenicity, and thyroid disruption (Manibusan and Touart 2017).

5. Conclusion

Professional agricultural operators handling plant protection products can be exposed to complex mixtures of chemicals comprising both the declared active substances and co-formulants, and some of these chemicals have known/possible endocrine disrupting activity. At the extremes, our results show that exposure to pesticide active substances can result in risk quotients for mixtures handled on a single day that indicate potential for risk (i.e. point of departure index greater than 1). Additional risk might also be expected from simultaneous exposure of operators to pesticide co-formulants with endocrine disrupting activity. This study suggests the need for clarity on the identification of endocrine disrupting activity, particularly as many of the substances considered in this study were classified as having "possible" rather than "known" endocrine disrupting activity. Further work is also required on risk assessment for pesticide co-formulants, particularly where complex mixtures can occur with multiple active substances and co-formulants that have similar toxicological endpoints. There is a specific need for review of European Union policy in this field as refined risk assessment methodologies become available.

Conflict of interest The authors declare that they have no conflict of interest.

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Table 1 Summary of pesticide active substances (AS) with known/possible endocrine disrupting activity (PPDB 2018) used in the different cropping systems and classified by pesticide type

Cropping system	Number of AS with endocrine activity		Number of AS with known/possible endocrine activity used on different targets		
	Known	Possible	Fungicides	Herbicides	Insecticides
Lithuania arable	2	15	9	3	5
UK arable	6	23	14	8	7
Greece arable	1	10	6	2	3
UK orchard	1	14	10	3	2
Greece orchard	2	18	11	2	7
All systems combined	8	40	25	11	12

Table 2 Distribution of predicted total risk (expressed as the PODI) from exposure to active substances with known/possible endocrine disrupting activity. Different percentiles and the maximum are given for the five cropping systems based on 10 operators and all spray days with at least one active substance applied

	Total PODI per spray day (percentile)				
Cropping system	25th	50th	75th	95th	Maximum
Lithuania arable	-	9.53×10^{-4}	6.29×10^{-3}	2.47×10^{-1}	5.58
UK arable	-	1.27×10^{-3}	1.80×10^{-2}	9.02×10^{-2}	2.61
Greece arable	-	3.25×10^{-3}	2.15×10^{-2}	5.32×10^{-1}	1.74
UK orchard	3.37×10^{-4}	1.05×10^{-2}	3.44×10^{-2}	3.03×10^{-1}	1.15
Greece orchard	-	5.28×10^{-4}	1.06×10^{-1}	5.28×10^{-1}	10.72

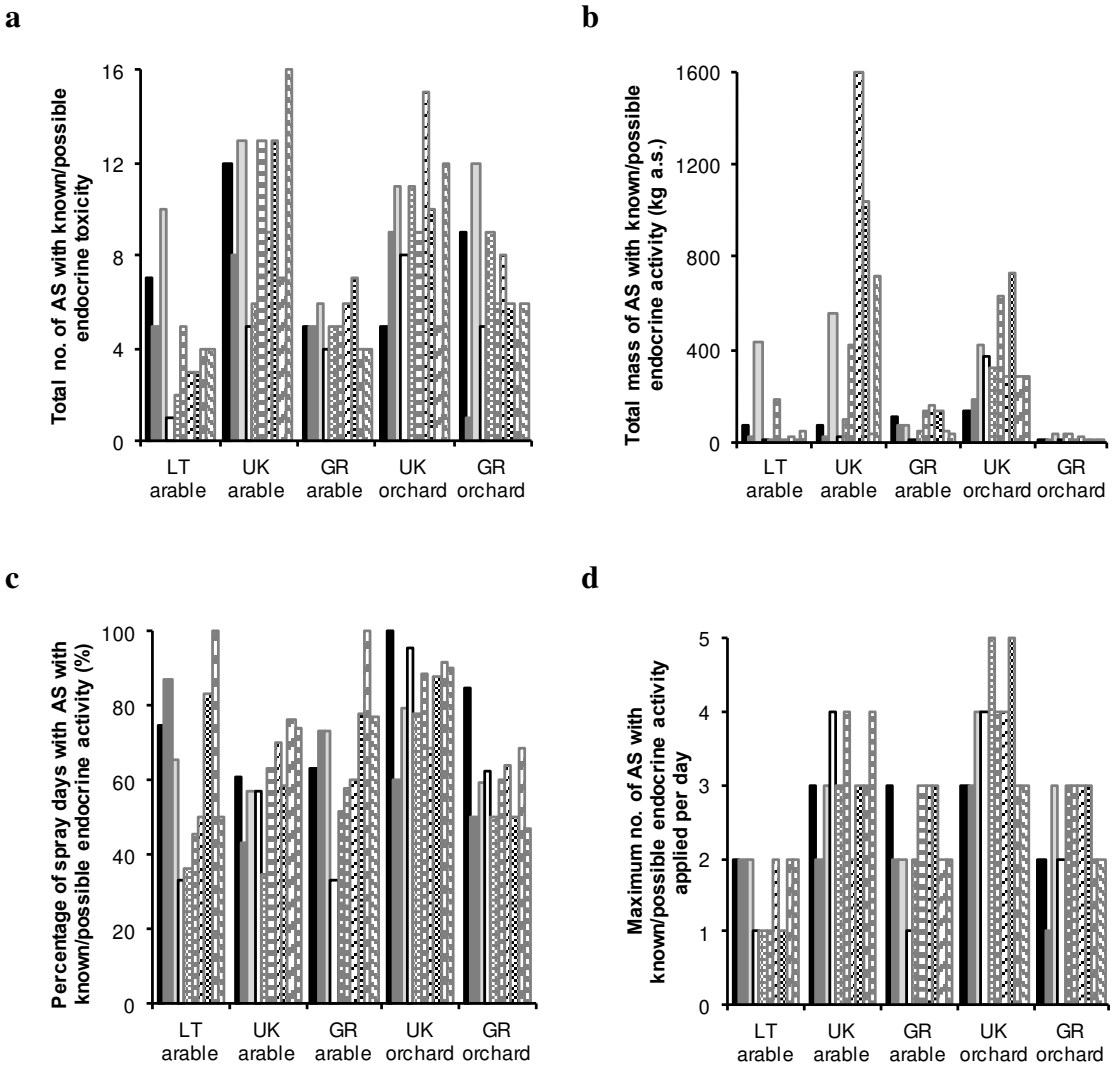


Fig. 1 Application data for 50 professional operators from the cropping systems in Lithuania, the UK and Greece expressed as total number (a), total mass (b), percentage of spray days (c), and maximum number applied on a single day (d) of active substances (AS) with known/possible endocrine disrupting activity

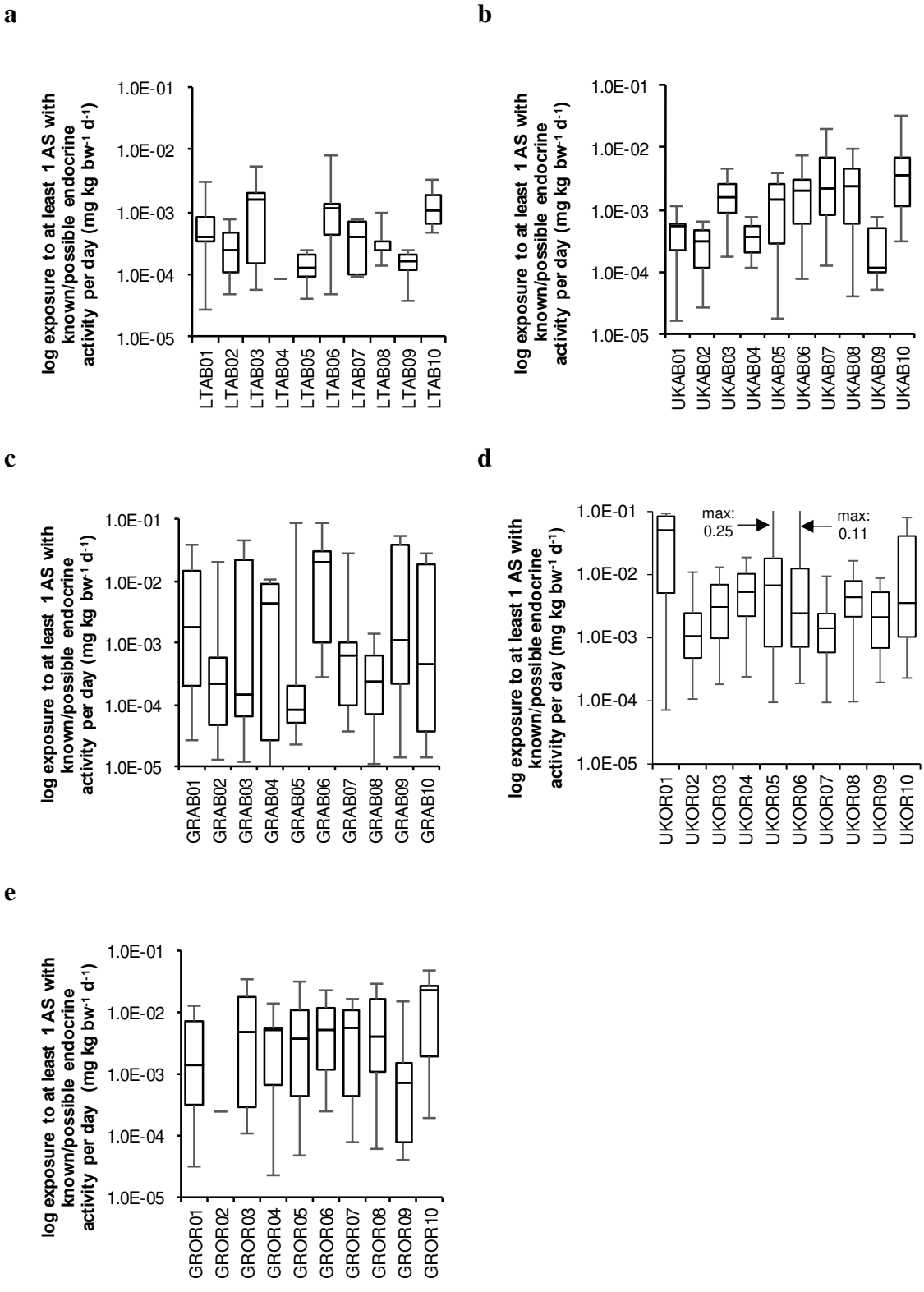


Fig. 2 Estimated total exposure on individual spray days when at least one active substance with known/possible endocrine disrupting activity was applied. Data are shown for individual operators from the arable systems in Lithuania (a), the UK (b) and Greece (c), and the orchard systems in the UK (d) and Greece (e). Boxes show the median and quartiles, and whiskers show the range

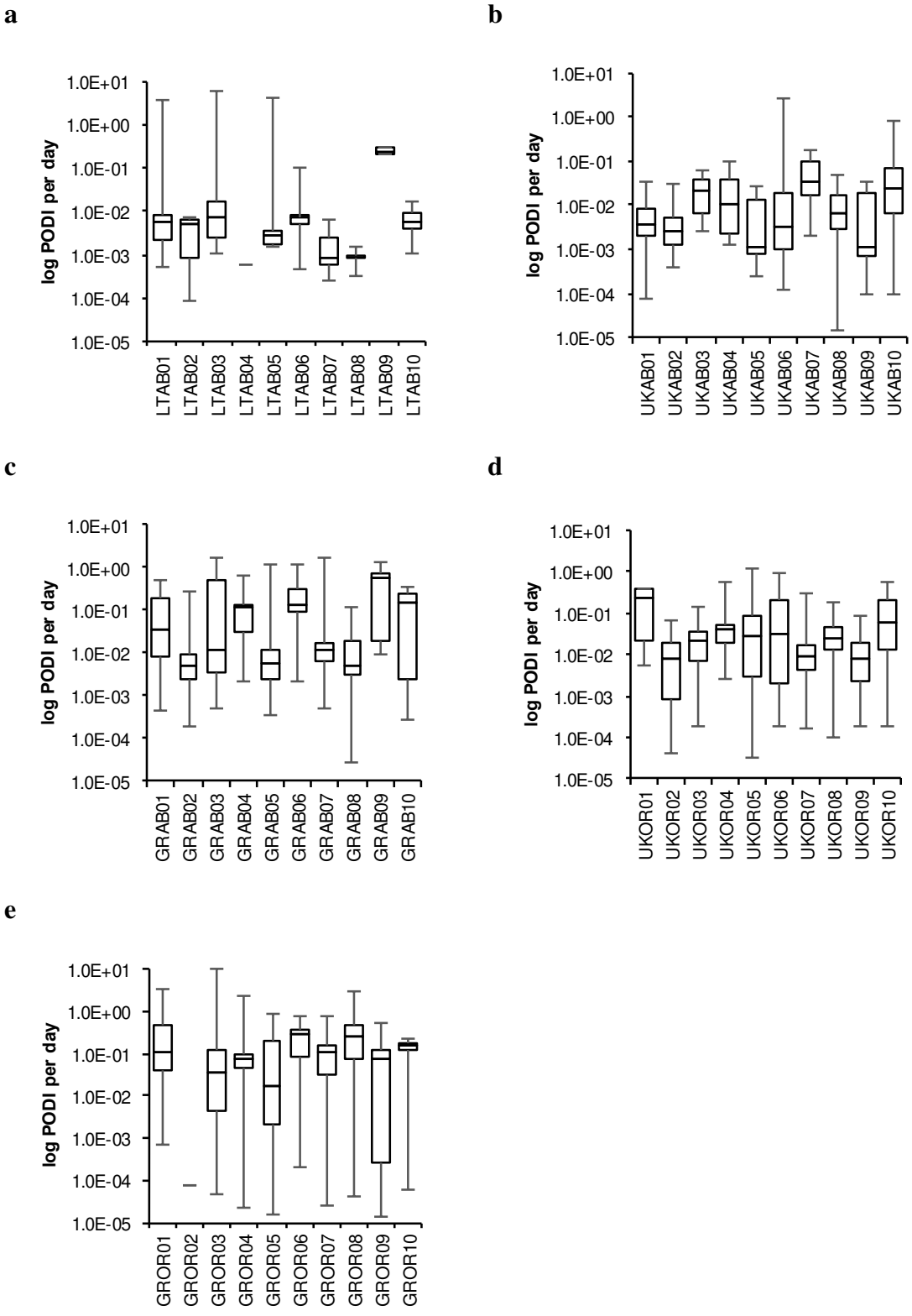
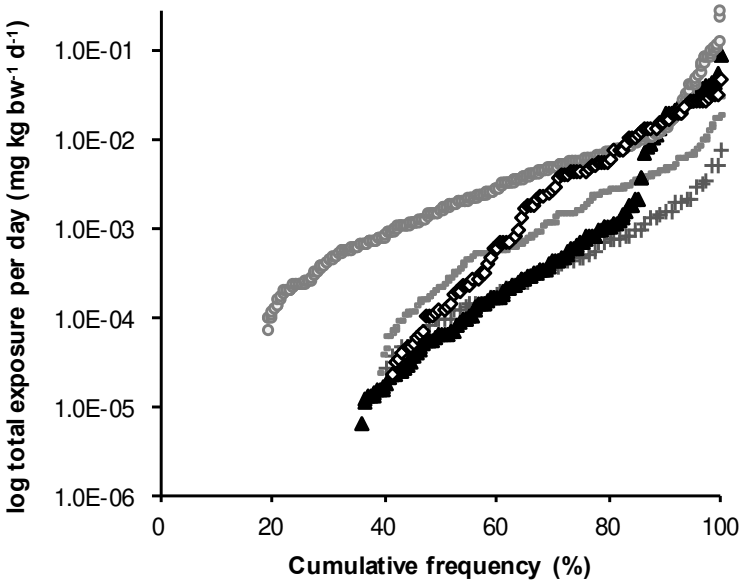


Fig. 3 Estimated risk from exposure on individual spray days when at least one active substance with known/possible endocrine disrupting activity was applied. Data are shown for individual operators from the arable systems in Lithuania (a), the UK (b) and Greece (c), and the orchard systems in the UK (d) and Greece (e). Boxes show the median and quartiles, and whiskers show the range

a



b

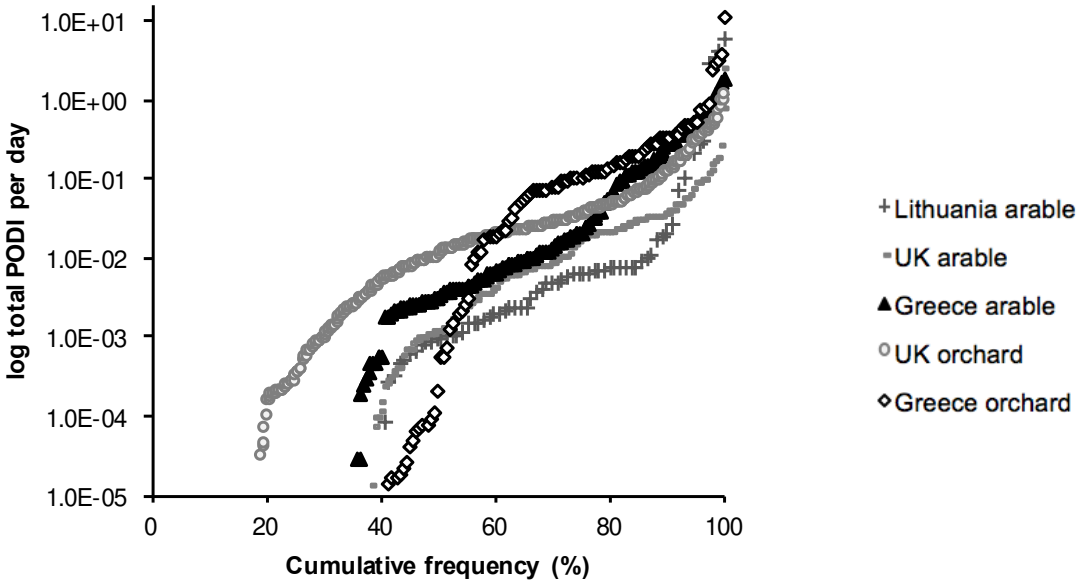


Fig. 4 Cumulative frequency distributions of total exposure (a) and total risk expressed as the PODI (b) on single spray days with at least one active substance with known/possible endocrine disrupting activity applied by 50 individual operators across the cropping systems. Each data point represents the value for an individual operator on a single day

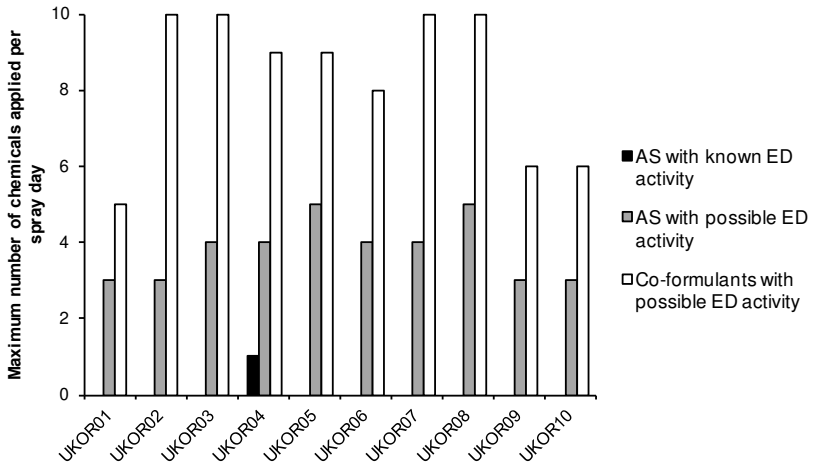


Fig. 5 Maximum number of active substances and co-formulants with known or possible endocrine disrupting (ED) activity applied on a single spray day for ten operators working in UK orchards

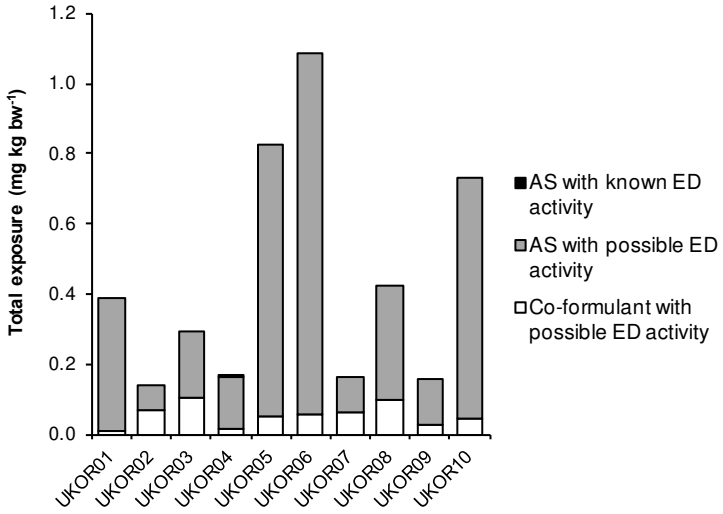


Fig. 6 Predicted total exposure to active substances and co-formulants with known/possible endocrine disrupting activity over the survey period for 10 operators working in UK orchards