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US EPA's regulatory pesticide evaluations need clearer guidelines for considering mammary gland tumors and other mammary gland effects

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Abstract

Breast cancer risk from pesticides may be missed if effects on mammary gland are not assessed in toxicology studies required for registration. Using US EPA's registration documents, we identified pesticides that cause mammary tumors or alter development, and evaluated how those findings were considered in risk assessment. Of 28 pesticides that produced mammary tumors, EPA's risk assessment acknowledges those tumors for nine and dismisses the remaining cases. For five pesticides that alter mammary gland development, the implications for lactation and cancer risk are not assessed. Many of the mammary-active pesticides activate pathways related to endocrine disruption: altering steroid synthesis in H295R cells, activating nuclear receptors, or affecting xenobiotic metabolizing enzymes. Clearer guidelines based on breast cancer biology would strengthen assessment of mammary gland effects, including sensitive histology and hormone measures. Potential cancer risks from several common pesticides should be re-evaluated, including: malathion, triclopyr, atrazine, propylene oxide, and 3-iodo-2-propynyl butylcarbamate (IPBC).

Keywords

Breast cancer; Estradiol synthesis; Progesterone; Endocrine disrupting chemicals; Triazines; Malathion

1. Introduction

Regulations typically require extensive toxicological testing of pesticides prior to their legal use because of their expected toxicity and widespread exposure. Since breast cancer is a significant public health concern, it is important to ensure that pesticide testing is designed to detect chemicals that could increase breast cancer risk, for example through hormonal

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Authorship statement

BC: Methodology, data curation, validation, visualization, editing and writing. RAR: conceptualization, methodology, data curation, funding acquisition, writing and review.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mce.2020.110927.

as well as genotoxic mechanisms. In general, human and rodent evidence suggests at least three overlapping classes of chemicals that might increase breast cancer risk: chemicals that cause mammary gland tumors in animal cancer bioassays, primarily by damaging DNA; endocrine disrupting chemicals (EDCs) that promote the growth of mammary tumors through estrogenic or other pathways; and developmental toxicants—often EDCs—that can alter development of the mammary gland in ways that permanently increase susceptibility (Rodgers et al., 2018).

It has been previously reported that chemically-induced effects on the mammary gland are not assessed in the types of guideline toxicology studies required for pesticide registration (Fenton, 2006; Makris, 2011; Rudel et al., 2011), and that when mammary tumors are observed in two-year rodent cancer bioassays they are often dismissed and not carried forward into risk assessments (Rudel et al., 2014; Rudel et al., 2007). Some of these decisions may reflect limited appreciation for the interaction of endocrine pathways in breast carcinogenesis. For example, body weight reductions from high dose toxicity can reduce tumor incidence at the high dose, causing tumors at low and mid doses to be dismissed for lack of dose-response (Haseman et al., 1997). Additionally, a 2007 workshop at the US National Toxicology Program reviewed rodent models for hormonally-mediated carcinogenesis and noted several limitations in assessing breast cancer risk, including that mice are relatively insensitive to mammary tumors, and that some strains of rats have high and variable background rates of mammary tumors reducing the ability to detect a treatment-related increase (Thayer and Foster, 2007).

Furthermore, developing organisms are known to be sensitive to hormonal influences, for example from EDCs, and these exposures can alter susceptibility to tumors in hormonally-responsive tissues or affect lactation (Gore et al., 2015). Despite this, guideline toxicology studies have historically not been designed to detect these effects on developing tissues, since dosing is often to mature animals and endocrine-responsive endpoints aren't thoroughly assessed, especially if they don't manifest until later in life. Thus, guideline toxicity studies have historically been relatively insensitive to effects of EDCs. To correct this gap, some guideline study protocols have been modified over the past 10–15 years to include dosing during gestation (in some cases prior to gestation) and assessment of reproductive system development and puberty timing. However even these updated protocols, such as the Organization for Economic Cooperation and Development (OECD) Extended One-Generation Reproductive Toxicity Study which evaluates reproductive and developmental effects due to pre- and postnatal chemical exposures (OECD, 2018), still don't require assessment of altered mammary gland development despite the observation that in some cases these changes result in altered susceptibility to carcinogens or impaired lactation, and that they can occur at lower doses than the standard endpoints (Rudel et al., 2011).

Based on these observations, we hypothesize that mammary tumors have been dismissed inappropriately from pesticide risk assessments and that effects of pesticides on mammary gland development have not been measured or considered. In order to evaluate this hypothesis, we reviewed US EPA pesticide Reregistration Eligibility Decisions (REDs), which document the agency's health effect assessments. From these REDs, we identified

pesticides for which mammary tumors were reported, and then noted whether mammary tumors were carried forward into the carcinogenicity classification (EPA, n.d.). We also identified pesticides that cause other mammary gland effects from several review articles and, if applicable, noted the discussion of these effects in the EPA assessments.

Finally, we compiled mechanistic data for these pesticides from EPA's ToxCast program to identify biological activities that may be related to their *in vivo* effects. For example, since estrogen and progesterone play an important role in the development of breast cancer (Brisken et al., 2015), we hypothesized that chemicals that increase production of estradiol, estrone or progesterone might increase tumor incidence. Similarly, chemicals that alter xenobiotic metabolizing enzymes, such as CYPs (cytochrome P450), may also alter endogenous hormone levels since some of these enzymes control levels of endogenous hormones. Activity at nuclear receptors is also likely to be important for breast cancer, for example the estrogen receptor (ER), progesterone receptor (PR), and others. US EPA's ToxCast chemical screening program has tested many pesticides for these endpoints using high and medium-throughput *in vitro* assays that use a variety of human cell lines such as MCF-7 breast cancer and H295R adrenocortical carcinoma cells (Thomas et al., 2019). These publicly-available data are an important resource despite the known limitations of *in vitro* testing such as the lack of metabolic capability of cells tested, influence of chemical properties affecting bioavailability, uncertain relevance of cell lines used in *in vitro* assays to normal tissue, and uncertainty in extrapolating effects across tissues or even multiple cell types in a single tissue (Ginsberg et al., 2019; Krewski et al., 2020).

Our objectives in this paper are 1) to identify pesticides with reported mammary gland effects, including tumors; 2) to review interpretation of mammary gland effects in the context of pesticide registration processes; and 3) to identify mechanistic evidence from high throughput *in vitro* assays of endocrine disruption for these pesticides that may aid in interpreting the observed mammary gland effects.

2. Methods

2.1. Identify REDs that describe mammary gland effects

To identify pesticides with reported effects on the mammary gland, we downloaded all available REDs (n = 449), including Tolerance Reassessment Eligibility Decisions (TREDs) and Interim Reregistration Eligibility Decisions (IREDs), from EPA's Pesticide Chemical Search website (EPA, 2016). This represented approximately 529 pesticides (some pesticides did not have available REDs) with dates of the REDs ranging from 1990 to 2008. To download the REDs, we first extracted the links for the PDFs from the website using the R package rvest (Wickham, 2019a), and then we downloaded the PDFs using the R package curl (Ooms, 2019). This download was completed in 2017 which was shortly after the website was archived and maintenance by EPA stopped. The downloaded PDFs were converted to text files using the pdf to text editor Xpdf (Noonburg, 1995) with R. Using the R package stringr (Wickham, 2019b) we text searched the text files to identify the REDs that mentioned the word "mammary."

2.2. Manual review of REDs

Once we identified the REDs that mentioned “mammary”, we manually inspected them to identify the context in which mammary was used. Pesticides whose RED did not describe significant effects on the mammary gland were removed from further analysis (n = 7). For the pesticides that did report mammary effects we noted the effect on the mammary gland and EPA’s comment on the reported effect, such as reason for dismissal or assignment of a cancer classification. We also took note of the MRID (or Master Record Identifier, a unique number assigned to each study submitted to EPA) for the study reporting the effect and additional study details if included in the RED, such as the species affected, doses administered, dose(s) with the reported effect(s), and route of administration.

2.3. Identify pesticides with mammary gland effects from review articles

In addition to identifying pesticides with effects on the mammary gland through REDs, we used three major review articles that compiled chemicals that cause mammary tumors or alter mammary gland development and identified any pesticides. These review articles include: Rudel et al. (2007), Rudel et al. (2011), and Rodgers et al. (2018). We recorded the type of effect in the mammary gland as well as the review article reporting the effect. Furthermore, we downloaded the associated RED if it had not been downloaded already; to download we used both the archived website we used to download the REDs and EPA’s new Pesticide Chemical Search website (EPA OPP, 2020).

2.4. Collect and review overall carcinogenicity classification and non-cancer risk assessment

We noted the cancer risk classification as reported by the RED and the basis for this classification. If a RED was not available for a pesticide, we used EPA’s *Chemicals Evaluated for Carcinogenic Potential Annual Cancer Report 2018* (EPA OPP, 2018) which reported up-to-date classification of many pesticides. For the pesticides with a RED, we checked that the cancer classification assigned was the most recent, or updated accordingly. A few pesticides did not have a RED and were not in the cancer report so for these we included the cancer risk assessment of other authoritative sites (such as IRIS, IARC, or NTP ROC) which we attained through EPA’s Toxicity Value Database, or ToxValDB (R. Judson, 2018). This database is free for public download at ftp://newftp.epa.gov/COMPTOX/STAFF/rjudson/datasets/ToxValDB/. For chemical classifications attained through ToxValDb, we also went directly to the website of the corresponding authoritative site to find the basis for the cancer classification. We then searched the EPA web site to fill any remaining gaps and noted the specific source of the risk assessment information. For pesticides with non-cancer mammary effects, we reviewed the RED and noted if the no observed effect level (NOEL) was set below the dose where the mammary effect was observed.

2.5. Collect usage and registration information

For each pesticide in our list, we determined if federally approved pesticide products contain these chemicals in the United States, summarized estimated usage in the US, and noted whether they were approved for use in Europe. To determine whether a chemical was

an active ingredient in pesticide products currently approved for use in the US, we used the National Pesticide Information Retrieval System, *i.e.* NPIRS (CERIS, 1998–2020), and manually searched for chemicals in our list by name or CASRN. Information about products approved for use in the US is updated weekly by the NPIRS. To summarize estimated usage in the US, we used the *2008–2012 EPA Pesticides Industry Sales and Usage* report (Atwood and Pairsley-Jones, 2017) and the 2016 USGS pesticide national synthesis project database (USGS, 2016). In both, we manually searched for the chemicals in our list by name and, where available, gathered the reported estimated usage. To compare with Europe, we used the Pesticide Properties Data Base (Lewis et al., 2016), which notes whether a pesticide is approved for use by the European Commission, and manually searched for each pesticide by name.

2.6. Compile relevant mechanistic data

We used EPA's CompTox Chemicals Dashboard (Filer et al., 2017), and recent papers from EPA's computational toxicology group, to gather mechanistic data that may be relevant to the pesticide's effect on the mammary gland. Specifically, we compiled information from *in vitro* screening assays that measure effects on estrogen or progesterone synthesis, estrogen receptor-mediated activity, and other gene targets if they showed activity with an AC50 below 1 μM.

We used data from the H295R high-throughput steroidogenesis assay to identify pesticides that affect levels of progesterone (P4) and the estrogens estradiol (E2) and estrone (E1). This assay uses human adrenocortical carcinoma (H295R) cells to measure the concentrations of 11 steroid hormones – starting from cholesterol and including the androgens, estrogens, and glucocorticoids – following chemical exposure at six doses (Haggard et al., 2019; Karmaus et al., 2016); chemicals were only tested in dose-response if they had a significant effect on the levels of at least four hormones at a single maximum tolerated concentration (MTC). A chemical screened in dose-response was defined as having an effect on a hormone if it significantly affected the concentration at two consecutive non-cytotoxic doses or at the highest tested non-cytotoxic dose. Directionality of this significance—indicating whether a chemical increased, decreased or had no effect on the hormone analyte— was determined based on the slope of the fitted data. We gathered information on each pesticide's effect on E2, E1 or P4 using supplementary data 2 from Haggard et al. (2019). For those chemicals not tested in dose-response we also indicated whether they had been tested at the single dose and whether they affected any of the 11 hormones; this information was acquired from Supplementary Table S4 in Karmaus et al. (2016). The dose-response data from this H295R experiment was also processed through the ToxCast tcpl data processing pipeline (Filer et al., 2017; Williams et al., 2017) and corresponding AC50 values were calculated (Williams et al., 2017). We downloaded AC50 values from the ToxCast & Tox21 Summary Files for invitroDBv3.2 (EPA, 2019) and have included the values in Table S1. However, data analysis by Haggard (2019) was developed specifically for the underlying H295R data, whereas tcpl is an automated pipeline for all of EPA's ToxCast data. Therefore, the Haggard analyses may be more reliable than tcpl for the H295R data.

To estimate the potential for a pesticide to activate the estrogen receptor, we compiled the estrogen receptor area under the curve (ER AUC) for each pesticide. The ER AUC is an integrated measure of *in vitro* estrogen receptor activity developed by EPA's Computational Toxicology group (Browne et al., 2015; R. S. Judson et al., 2015) and is presented as a number between 0 and 1 that indicates the probability a chemical is a true ER agonist or antagonist. This score was calculated by integrating the response of a chemical in 18 *in vitro* assays that tested various interactions on the ER such as binding, dimerization, and transcriptional activation, as well as estrogen-sensitive cell proliferation (Browne et al., 2015; R. S. Judson et al., 2015). To provide additional insights into biological activity that may relate to pesticide mechanism of action, we noted any genes or gene family targets that showed activity with an AC50 below 1 μM from the ToxCast data, and classified these according to the target gene family.

3. Results and discussion

3.1. Pesticides that affect mammary gland

Using REDs or review articles, we identified 35 pesticides that affect the mammary gland by causing mammary tumors or other mammary gland effects. Table 1 describes the reported effects for pesticides that target the mammary gland and use of these data in EPA risk evaluations, and additional details are in Table S1.

Based on searching 449 pesticide REDs we identified 32 that included the word "mammary" and described effects. From these we found 25 pesticides with reported mammary effects, including 20 with mammary tumors and five with other mammary gland effects, such as swelling or atrophy. From the review articles, we identified 13 pesticides with mammary gland effects, including nine with mammary tumors and five with other mammary gland effects, especially altered mammary gland development. Thus, from the REDS and review articles, we identified 35 unique pesticides with three of the pesticides – atrazine, malathion, and parathion— having effects on the mammary gland listed in both the RED and a review article.

3.1.1. Dismissing of mammary gland tumors—In all we identified 28 pesticides with mammary tumors. Of these, the overall carcinogenicity assessment included the mammary tumors for nine, while the mammary gland tumors for the remaining 19 pesticides were dismissed for a variety of reasons (Table 1, Table 2a, Table 2b). This raises concerns that useful information about the potential for a pesticide to cause breast cancer is not being fully considered, since the cancer evaluation did not mention mammary tumors for 19 of 28 pesticides that reported those tumors.

Common reasons for dismissing the mammary tumors reported for these 19 pesticides include: effects not considered treatment related (n = 7), effects only seen at excessively toxic doses (n = 2), mechanism asserted to be not relevant to humans (n = 3; this was for the triazines), or there was simply no additional discussion about the mammary tumors (n = 6); see Tables 1 and 2.

Pesticides whose effects were not considered treatment related include: clonitralid, IPBC, paraquat dichloride, propylene oxide and triclopyr. Reasons cited for this dismissal include lack of a dose-relationship, effects were within historical control range or control group range, no reason was given or the reason was unclear (Table 2). For some of these chemicals, these reasons for dismissing may be inappropriate, or merit further review. For example, the effects of triclopyr –which included mammary tumors in both mice and rats with dose-response trend—were considered “marginal” where marginal was defined as “not entirely negative, but yet not convincing” (EPA OPPTS, 1998e). Mammary tumors from propylene oxide—which included fibroadenomas and tubulopapillary carcinomas that were significantly higher than both concurrent and historical controls, and increased multiplicity at every dose—were dismissed because the study went on for longer than the usual of 2 years (EPA OPPTS, 2006f). The EPA assessment did not mention the structural similarity to ethylene oxide, which also causes mammary tumors, or acknowledge the well-established connection between epoxide-forming chemicals and mammary tumors (Dunnick et al., 1995). In fact, recent epidemiology studies report associations between ethylene oxide exposure and breast cancer (reviewed in Rodgers et al., 2018). Fenvalerate was dismissed because tumor incidence was within the historical control range (Parker et al., 1984). In general, EPA cancer risk assessment guidelines do not support using comparison with historical controls to dismiss tumors (EPA Risk Assessment Forum, 2005). Finally, IPBC and paraquat dichloride were both dismissed based on the lack of dose-response (EPA OPPTS, 1997a; 1997b), whereby tumor incidence is expected to increase as the dose increases. However, because mammary tumor incidence is strongly influenced by body weight (Haseman et al., 1997), toxicity at the higher doses that reduces body weight may also reduce tumor incidence. Thus it is important not to dismiss mammary tumors simply because fewer tumors are observed at the higher doses, especially if body weight is reduced.

Pesticides whose effects were not considered relevant to humans were the class of chemicals called triazines (which include atrazine, propazine and simazine) because the mechanism for tumor formation in the rodent model – proposed to involve induction of persistent estrous via attenuation of luteinizing hormone (LH) surge— is hypothesized not to be applicable to humans (EPA OCSPP, 2018). One consideration in these cases is that mammary tumors may indicate more generally that the pesticide is an EDC and should be evaluated as such. For example, atrazine and its degradates also alter mammary gland development following gestational exposure (Enoch et al., 2007; Rayner et al., 2005), and these effects may be relevant to humans. The relevance of triazine mammary tumors to humans is further discussed below in the context of *in vitro* data on steroidogenesis.

Chemicals with no discussion about the observed mammary gland tumors in the RED include dichlorvos, folpet and malathion. Review of these decisions is warranted, especially in light of new insights about endocrine-related mechanisms in cancer. In some cases, documentation from EPA pesticide Cancer Assessment Review Committee (CARC) panels provides additional information. For example, for malathion, the EPA CARC assessment in 2000 reports mammary gland tumors in 2-year rat studies, as well as liver, adrenal, thyroid, and uterine tumors, but doesn’t discuss mammary tumors any further and dismisses most of the others, retaining a concern about liver and nasal tumors (CARC, 2000). The reasons mammary tumors are dismissed remain unexplained.

3.1.2. Dismissing of other mammary gland effects—Ten pesticides reported effects other than tumors on the mammary gland (Table 1). From the REDs, five reported mammary swelling, atrophy or pathological changes; abnormal vacuolization of mammary epithelial cells, or impaired lactation. These endpoints appeared to be considered in the non-cancer risk assessments because NOEL levels were set below the doses where these effects were reported.

From the review articles, five pesticides reported altered mammary gland development: atrazine, chlorpyrifos, malathion, methoxychlor, and parathion. This outcome was not discussed in the risk assessments, likely because altered mammary gland development is not routinely assessed in guideline toxicity studies. It remains a priority area for future work to make this assessment more routine, to conduct this testing on pesticides and other commercial chemicals, and to better understand the implications of altered development for lactation and tumor susceptibility. We have previously noted that diverse chemicals, including many not considered primarily estrogenic, alter mammary gland development in rodents (Fenton, 2006; Rudel et al., 2007). Inconsistent reporting methods hinder comparison across studies, and relationships between altered development and effects on lactation or carcinogenesis are still being defined. In some studies, altered MG development is the most sensitive endocrine endpoint.

3.1.3. Limitations in reviewing mammary effects—One limitation of our approach is that our search for “mammary” was conducted in the REDs rather than in the technical reports that are the basis for the REDs— reports include the Cancer Assessment Review Committee (CARC) reports for each pesticide, the original study reports, and the Data Evaluation Records that summarize each study report. Thus, there may be additional explanation in these documents about interpretation of the mammary tumors that we did not review. In addition, we likely missed many instances where mammary tumors were observed and dismissed but not mentioned in the RED. These technical documents are not readily available to be web scraped and text mined as we did with the REDs since they have to be requested via a Freedom of Information Act request.

Another limitation is that the REDs reviewed covered years from 1990 to 2008 and thus some assessments may have been updated, since EPA periodically reviews registrations of active pesticides though the Registration Review program and incorporates new data that becomes available. While we did not systematically search all registration review documents post 2008 for mentions of “mammary,” we did check whether any of the 35 pesticides we included in this review had updated information related to mammary tumor findings. We found assessments for 6 of our 35 mammary-active pesticides: terbuthylazine, oryzalin, ethalfluralin, etridiazole, triclopyr and diphenylamine. Only the risk assessment for terbuthylazine had updated information related to mammary tumors—in this case the mechanism for the mammary tumors is stated as being similar to the triazines, and thus proposed to be not relevant to humans. Additionally, the risk assessment for oryzalin originally attributed its carcinogenicity to thyroid and mammary gland tumors, but the updated cancer classification only includes the thyroid tumors; no reason is provided for not including mammary tumors in the classification but the mammary tumors are noted elsewhere in the risk assessment. In future work it would be useful to review these risk

assessments for other pesticides to identify additional pesticides that may cause mammary tumors.

Although the purpose of this paper was to review the reasons pesticide risk assessments dismiss observed mammary gland effects, we noticed that other types of tumors besides mammary are also dismissed, often using similar logic. For example, in dismissing the mammary tumors observed for difenzoquat due to the effects not being treatment related (with no discussion as to the reasons), tumors in the adrenals, lungs, pituitary, ovaries, and uterus, were also dismissed (EPA OPPTS, 1994a). Malathion and alachlor also had other tumors dismissed (CARC, 2000; EPA OPPTS, 1998b). We hypothesize that because mammary tumors result from the interaction of conventional genotoxic cancer process and hormonal effects, mammary gland effects may be more commonly dismissed as not treatment-related since endocrine disrupting effects are not comprehensively assessed. However, we did not evaluate this systematically in this study.

3.2. In vitro endocrine disruptor activity

Mechanistic data indicating endocrine disruption may inform decisions about whether mammary tumors in cancer bioassays are related to treatment, so we reviewed activity of these 35 pesticides in EPA's ToxCast testing program. Many of the pesticides that caused mammary gland tumors or other mammary effects also alter steroidogenesis in the H295R adrenocortical carcinoma cell line, activate nuclear receptors or CYP enzymes, or are estrogenic (Figs. 1 and 2).

We found that 17 of the 35 mammary-active pesticides affected the synthesis of estradiol (E2), estrone (E1) or progesterone (P4) (Fig. 1, Table S1). Most of these chemicals increased synthesis of E2 (n = 10), E1 (n = 11), or P4 (n = 12) and few decreased their synthesis, (n = 3, 1, 1 respectively), reinforcing the idea that an increase in these three hormones may cause mammary gland effects. Pesticides where mammary effects were considered versus not considered in cancer classifications showed a similar likelihood of affecting steroidogenesis. Only three pesticides had no activity in dose-response while the rest were either not tested (n = 7) or were not active at the high dose and so were not tested in dose-response format (n = 8). It is possible that some chemicals will be responsive in this assay at lower doses and not at high doses, as has been reported for some endocrine system effects, and so this initial high dose screening approach would miss those.

Few of the 35 pesticides with mammary effect appear to be strongly ER active – methoxychlor was the most active agonist, and chlopyrifos, chlordane, trifluralin, and terbuthylazine were much weaker ER agonists (Table S1).

We identified *in vitro* activity below 1 μM in other ToxCast targets for 22 of the 35 mammary active pesticides. Common active targets include xenobiotic metabolism, diverse nuclear receptors, and transcription factors (Fig. 2, Tables S1 and S2). The most common effects were on CYPs (n = 14), which are involved in xenobiotic metabolism including hormone synthesis and breakdown; on the cell cycle (n = 11, these assays measure effects on cell proliferation or regulation of DNA repair); on nuclear receptors (n = 11); and on DNA binding (n = 6). There were 5 pesticides that had not been tested in ToxCast assays

and 8 which had no *in vitro* activity below 1 μM. As with hormone activity, pesticides whose mammary effects were considered in the cancer classification versus not showed a similar likelihood of affecting the observed gene family targets.

3.2.1. Relevance of in vitro data—While we have not attempted to use the *in vitro* data to systematically evaluate every *in vivo* observation, bringing these data together can inform weight of evidence discussions about carcinogenicity and generate new hypotheses that can be tested. For example, all the triazines – especially atrazine—are active in the H295R steroidogenesis assay, increasing E2, E1, and P4. These findings are consistent with previous reports of increase in E2 production in ovarian granulosa cells and H295R cells (Tinfo et al., 2011), and *in vivo* in male rats and in ovariectomized female rats (Cooper et al., 2007). These effects represent an important mechanism of action that may be operative in humans and increase local or systemic hormone levels and breast cancer risk. Additional research to better characterize the significance of these effects is a priority.

Malathion is another pesticide with findings relevant to mammary gland effects, and its activity in the H295R assay as an inducer of progesterone synthesis suggests a possible mechanism. Although the 2009 RED for malathion mentions a rat bioassay that did not report mammary tumors and no mammary effects were otherwise discussed, the 2000 EPA CARC assessment reports mammary gland tumors in 2-year rat studies but doesn't discuss mammary tumors any further. Malathion is also reported to increase mammary tumors via a promotion mechanism when co-treated with the carcinogen DMBA (Okey, 1972), and mammary tumors are reported for malaoxon, the primary metabolite (National Toxicology Program, 1979). Malathion has also been reported to alter mammary gland development, possibly via an acetyl cholinesterase (AChE) mechanism (Cabello et al., 2001; Okey, 1972), and the 2009 RED notes that since the exposure limits are derived to prevent AChE inhibition, that should protect from the mammary gland development effects. A 2-generation reproduction study for malathion reports decreased pup weight and lactation issues, supporting the findings of altered mammary gland development in Cabello 2001 and Calaf 2012.

Taken together, these observations indicate a need for more thorough investigation of potential effects from these two widely used pesticides – atrazine and malathion— on breast health. More broadly, they highlight gaps in current testing and risk assessment approaches. In order to learn the *in vivo* implications of these *in vitro* effects on steroidogenesis, it is necessary to add careful assessments of mammary gland and local and systemic hormone measurements to regulatory studies that currently do not include them. For example, in Cooper 2007, sensitive measurements of hormone levels in males and ovariectomized females showed that E2 and P4 were increased with atrazine treatment, but these types of measurements are not typically conducted as part of regulatory guideline studies. In the context of endocrine disruption as a mode of action, it is also important to include dosing within the range of human exposures and to verify internal exposure levels in rodent studies so they can be directly compared with human exposures. Improvements are needed in risk assessment as well, to integrate diverse types of data and develop more comprehensive assessments of risk.

3.2.2. Limitations of in vitro data—While high throughput *in vitro* assays may be helpful for prioritization, there are many limitations that should be kept in mind (Ginsberg et al., 2019; Krewski et al., 2020). For example, the assays typically don't have metabolic capacity, which can lead to false positive and negative results. The assays may not reflect *in vivo* sensitivity that depends on life stage, co-exposures, and tissue context. They may also only cover a part of the biological space of endocrine disruption and carcinogenesis, and many common chemicals cannot be readily tested because they are volatile, for example. Additionally, they are highly reductive since they usually capture a very specific biological space and don't integrate effects across tissues or even multiple cell types in a single tissue. For example, the H295R steroidogenesis assay uses adrenocortical carcinoma cells, whose aromatase promoter profile differs from that of breast adipose tissue and gonadal tissue (Bulun et al., 2003; Caron-Beaudoin et al., 2016), so it is not known whether mammary tissue responses to aromatase inducers will be the same as those observed in the H295R assay. While it would be useful to observe the effects of these chemicals on hormone synthesis in breast adipose tissue or the gonads, to date there is no other high-throughput *in vitro* assay that measures chemical effects on the entire steroidogenesis pathway, thus the H295R assay gives us the best *in vitro* prediction of effects on E2, E1 and P4.

3.3. Registration status and current uses for pesticides that affect mammary gland

Twenty-four of the thirty-five pesticides that affect mammary gland endpoints are still found in products approved for use in the US (Table 3). Of these 24 pesticides, 19 reported tumors (6 of which were considered in the risk assessment) while 7 reported other effects, such as altered mammary gland development; and two reported both (Table 2). In comparison, only 9 of the 35 pesticides (information was not available for five) are known to be approved for use in the EU by the European Commission (Table 3); 3 other pesticides were not approved by the European commission but appear to be used in some EU countries. All nine products that were approved by the European commission are used in the US; 6 reported tumors, 2 reported other effects, and 1 reported both tumors and other effects.

We found use data for 16 of the 24 pesticides that are still in use in the US (Table 3). Based on 2016 USGS data, the pesticides with the highest use per year were atrazine (~75 million pounds), dichloropropene (~45 million pounds), paraquat dichloride (~8 million pounds), chlorpyrifos (~5 million pounds) and metribuzin (~5 million pounds). Based on a 2012 EPA pesticide use report, atrazine was the 2nd most commonly used pesticide in the agricultural sector, dichloropropene was 4th, chlorpyrifos was 14th, ethalfluralin was 19th and paraquat dichloride ranked in 23rd with 2–6 million pounds of use reported. Of the organophosphates used across all sectors, chlorpyrifos was the most used, malathion was 3rd and phosmet was 9th with 1–4 million pounds (Atwood and Pairsley-Jones, 2017). Malathion was the 3rd most commonly used pesticide ingredient in the home and garden sector at an estimated 1 million pounds (Atwood and Pairsley-Jones, 2017), and may be associated with high exposures because of its use in treating lice. Triclopyr (one million pounds/year (USGS, 2016),) is commonly used for brush control along right of ways, and so may be commonly applied in residential areas and near water supplies.

It is important to note that while these usage data may not be up to date – representing data from 2012 to 2016—more recent reports do not appear to be available to estimate current pesticide use.

4. Conclusions

We identified 35 pesticides that affect the mammary gland—causing mammary tumors or other mammary gland effects—from EPA REDs or review articles. These are of interest because they are potential breast carcinogens and so are priorities for exposure reduction. Twenty-four of these pesticides are still in use. For example, IPBC, an antifungal, is used in cosmetics and malathion is used in lice treatments and has other home and garden uses (Table 3). Atrazine, dichloropropene, paraquat dichloride, chlorpyrifos, and triclopyr are some of the most widely used pesticides in the U.S.

Of the 28 pesticides that reported mammary tumors in a cancer bioassay, only 9 carried the mammary tumors into the overall carcinogenicity assessment, while the mammary gland tumors for the remaining 19 pesticides were dismissed for a variety of reasons (Tables 1 and 2). Our comparison of bioassay results with cancer risk evaluations raises concerns that useful information from the bioassays about the potential for a pesticide to cause breast cancer is not being fully considered, since the cancer evaluation did not mention mammary tumors for 19 of 28 pesticides that reported those tumors. It would facilitate more robust inquiry using text mining and other systematic investigation if the technical reports that underlie the REDs were readily available.

In addition, 10 pesticides reported effects other than tumors on the mammary gland (Table 1), and five of these reported that gestational or early life exposure altered mammary gland development (atrazine, chlorpyrifos, malathion, methoxychlor, and parathion). This outcome was not discussed in the risk assessments, likely because altered mammary gland development is not routinely assessed in guideline toxicity studies. It remains a priority area for future work to make this assessment more routine and to better understand the implications of altered development for lactation and tumor susceptibility. Specifically, additional measures in guideline toxicology should include careful mammary gland assessments, sensitive hormone measurements, and environmentally-relevant dose levels for endocrine disruptors, with internal dose measurements that allow direct comparison to human exposures.

About one-third of the mammary-active pesticides increased synthesis of estradiol or progesterone in the H295R assay that measures effects on steroidogenesis (Fig. 1). In addition, 17 of the 35 mammary-active pesticides show activity in xenobiotic metabolism, diverse nuclear receptors, and/or transcription factors at concentrations below 1 μM (Fig. 2). Taken together, these findings are consistent with endocrine modes of action for many of these pesticides. It may be helpful to integrate *in vitro* activity data such as this when considering whether mammary tumors are treatment-related. Also, pesticides and commercial chemicals that show similar endocrine activity in these assays may be priorities for *in vivo* testing and risk assessment, since activity in these assays may indicate likelihood that the chemical is an EDC.

Overall, clearer guidelines are needed for testing and interpreting the effects of pesticides on the mammary gland in order to reduce exposure to pesticides and other chemicals that may increase breast cancer risk. Considering evidence of endocrine activity could lead to better decisions. The potential cancer risks from several common pesticides should be re-evaluated, including malathion, triclopyr, atrazine, propylene oxide, and 3-iodo-2-propynyl butylcarbamate (IPBC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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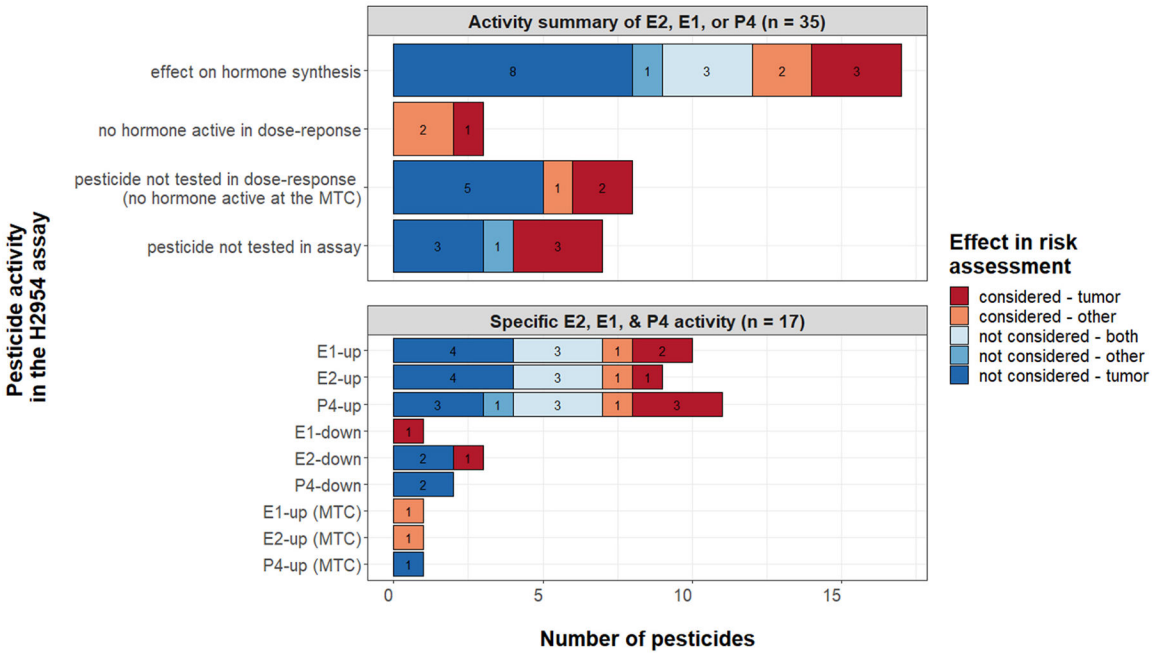

Fig. 1.

Pesticide effects on the synthesis of E2, E1, or P4 in the H295R steroidogenesis assay. Data from Haggard et al. (2019) supplemental and Karmaus et al. (2016) supplemental. Top: number of pesticides active for E2, E1, or P4, or that had no hormone activity either in dose-response or at the MTC (if the pesticide was not tested in dose-response), or that were not tested in the assay. Bottom: specific hormone activity observed; "-up" indicates increased hormone synthesis and "-down" indicates decreased hormone synthesis. For each activity, we highlight the number of pesticides whose effects on the mammary gland (tumor or other effect) were either considered in the risk assessment or not considered in the risk assessment. Abbreviations: E1 = Estrone; E2 = Estradiol; P4 = Progesterone; MTC = Maximum tolerated concentration.

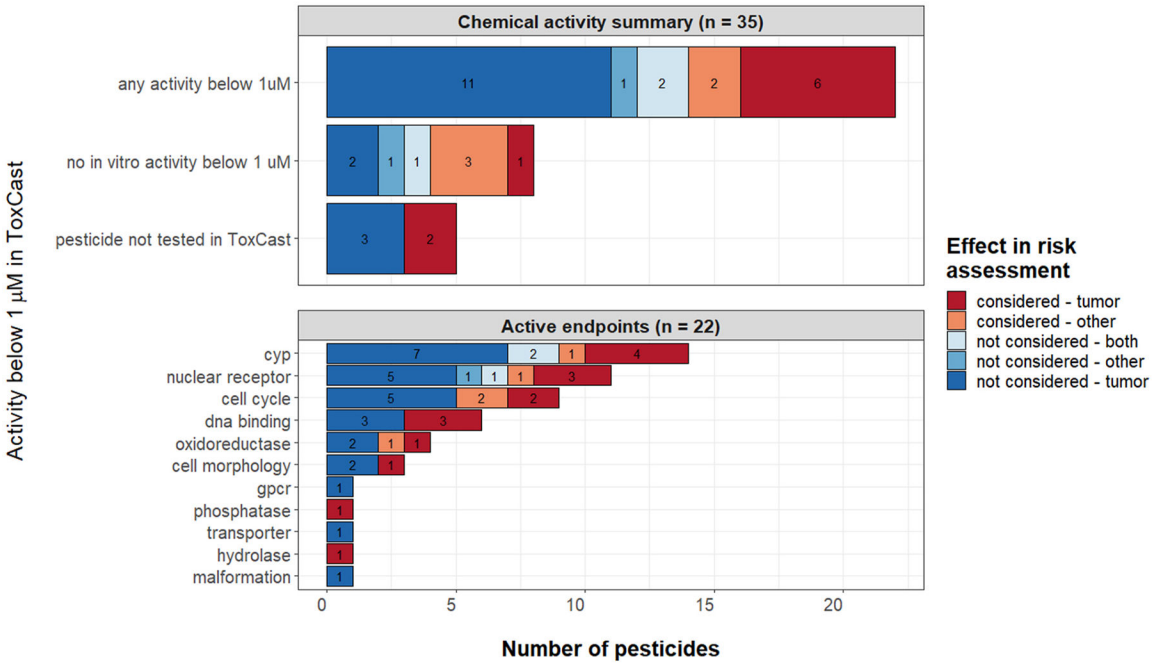

Fig. 2.

Low dose biological activity of mammary-active pesticides in EPA ToxCast *in vitro* assays. Top: number of pesticides that had *in vitro* activity below 1 µM, or had no *in vitro* activity below 1 µM, or were not tested in ToxCast. Bottom: endpoints that were active below 1 µM and the number of pesticides active for these endpoints. For each activity, we highlight the number of pesticides whose effects on the mammary gland (tumor or other effect) were either considered in the risk assessment or not considered. All data is available online on EPA's CompTox Dashboard. Abbreviations: cyp = Cytochrome P450; gpcr = G protein-coupled receptor.

Table 1

Reported effects for pesticides that target the mammary gland and use of these data in EPA risk evaluations.

1a. Flagged for mammary tumors

Pesticide	Reason included	Summary of mammary effects in RED or review article	Study/EPA conclusion in RED re: mammary effects	Cancer risk assessment summary from EPA OPP or other authoritative source[a]	Comment on EPA risk evaluation
Alachlor	Mammary tumors noted in RED (EPA OPPTS, 1998b)	Significantly increased incidence of mammary gland adenofibromas, fibroadenomas, and/or papillary adenocarcinomas combined at the low and high doses relative to control	Doses with significant effects on the mammary gland were considered to be excessively toxic so effects on mammary tumors were not considered treatment related	"Likely" to be a human carcinogen at high doses, but "not likely "at low doses Basis: increased incidence of malignant and combined benign/ malignant nasal, stomach, and thyroid tumors in rats	Mammary tumors at highest dose were dismissed in RED based on the dose being "excessively toxic" but the increase at the low dose is not discussed.
Ametryn[b]	Mammary tumors noted in RED (EPA OPPTS, 2005)	Mammary tumors in female rats at high dose (500 ppm)	Dose with significant effect on the mammary gland considered to be excessively toxic so mammary gland tumors not considered treatment related	Classification updated in 2017 (EPA OPP, 2018): suggestive evidence of carcinogenic potential	Mammary tumors at highest dose were dismissed in RED based on the dose being "excessively toxic"
Atrazine[b]	Mammary tumors in RED (EPA OPPTS, 2006a); effects on mammary gland development in review article (Rodgers, 2018)	Mammary gland tumors observed in female rats (RED) Rats exposed in utero to atrazine or its degradates in drinking water experienced delayed mammary gland development that persisted into adulthood (Rodgers, 2018)	Mechanism of tumor formation considered not relevant in humans. The proposed POD for non-cancer effects, based upon attenuation of the LH surge, appears to be protective against adverse reproductive/ developmental outcomes such as delays in onset of puberty, disruption of ovarian cyclicity and inhibition of suckling-induced prolactin release.	Not likely to be carcinogenic to humans Basis: cancer mode of action considered not relevant to humans	EPA's conclusion that the rodent mammary tumors induced by atrazine are not relevant to humans has been generally well-accepted based on atrazine's induction of persistent estrus in that model. However atrazine's ability to alter mammary gland development remains a concern because the mechanism may be independent of the attenuated LH surge that is the basis for the POD. This question is not addressed in the atrazine risk assessment (EPA OCSPP, 2018). EPA also notes a study that did not find altered mammary gland development from atrazine.
Captafol	Mammary tumors noted in review article (Rudel, 2007)	Increased incidence of tumors in mammary gland of rats	EPA RED not available. EPA reports mammary and other tumors in rats and lymphosarcomas in mice (EPA OPPTS, 1987).	Probable human carcinogen (Group B) Basis: lymphosarcoma in mice (EPA OPPTS, 1987).	Mammary tumors in rats are considered in the EPA 1987 cancer risk assessment (EPA OPPTS, 1987)
Chlordane	Mammary tumors noted in review article (Rudel, 2007)	Limited evidence of mammary gland tumors (Rudel, 2007).	EPA RED not available. EPA IRIS document does not mention mammary tumors.	EPA IRIS classified chlordane as a probable human carcinogen (B2) Basis: human epidemiology studies showing non-Hodgkin's lymphoma, liver tumors in mice, liver toxicity, and structural similarity to other rodent liver carcinogens (EPA IRIS, 1998).	Mammary tumors are not considered in EPA cancer risk assessment

Clonitralid (Niclosamide)	Mammary tumors noted in review article (Rudel, 2007)	Increased mammary adenocarcinomas in male and female rats - findings considered equivocal based on life-table analysis		Since this is not a food use pesticide, carcinogenicity evaluation was not required (EPA OPPTS, 1999a). A 1978 NCI cancer study in rats and mice concluded there was no convincing evidence of carcinogenicity but did note elevated mammary tumors with dose-response compared with historical controls (NCI, 1978).	Mammary tumors dismissed in risk evaluation because of higher than usual incidence in controls.
1,2-dibromo-3-chloropropane (DBCP)	Mammary tumors noted in review article (Rudel, 2007)	Induced carcinomas in mammary gland of female rats following administration by gavage	No RED, but in a chemical summary published in 2000 EPA notes that high incidences of tumors of the nasal tract, tongue, adrenal cortex, and lungs of rodents were reported in a National Toxicology Program (NTP) inhalation study, and that a gavage study showed tumors of forestomach and mammary gland (EPA, 2000a)	NTP RoC classified as reasonably anticipated to be a human carcinogen Basis: sufficient evidence of carcinogenicity from studies in experimental animals. When administered by stomach tube caused cancer of the forestomach (squamous-cell carcinoma) in rats and mice of both sexes and mammary-gland cancer (carcinoma) in female rats (NTP, 2016a).	Mammary tumors from gavage study are acknowledged. Emphasis is on inhalation study, which reported other tumors.
Dichlorvos (DDVP)	Mammary tumors noted in RED (EPA OPPTS, 2006d) and review article (Rudel, 2007)	Increased incidence of mammary gland fibroadenoma in female rats at administered dosages; one female rat with fibroma (EPA OPPTS, 2006d). Increased incidence of mammary gland fibroadenomas/adenomas in a group of female rats upon oral administration (Rudel, 2007)	No additional discussion of mammary tumors	Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential. Basis: increased incidence of forestomach tumors in mice and mononuclear cell leukemia (MCL) in rats	Mammary tumors are described in RED with no additional discussion
Dichloropropene (1,3-D)/Telone	Mammary tumors noted in RED (EPA OPPTS, 1998a)	Increased tumors (including mammary) in both sexes of rats and mice. An impurity of 1,3-D (1,2-D) was shown to have a dose-related trend in mammary adenocarcinomas in female rats	Classified as a probable human carcinogen.	Probable human carcinogen (B2) Basis: increased tumors in both sexes of rats and mice including tumors of the forestomach, liver, mammary, thyroid, adrenal, urinary, and lung.	Mammary effects mentioned in RED and considered in carcinogen risk assessment. This chemical is genotoxic. Many other epoxide-forming chemicals also cause mammary tumors.
Difenzoquat	Mammary tumors noted in RED (EPA OPPTS, 1994a)	Neoplastic lesions observed in the mammary glands of female rats including adenocarcinomas and fibroadenomas	Neoplastic lesions in mammary glands considered not treatment related	Evidence of non-carcinogenicity for humans Basis: lack of evidence in adequate studies with rats and mice	Mammary tumors were dismissed in RED as not treatment related. Tumors observed in the following tissues were also dismissed as not treatment related: adrenals (cortical adenoma), lungs (reticulum cell sarcoma), pituitary (adenoma), ovaries (adenoma) and uterus (polyps). Thyroid follicular adenocarcinoma in the mid-dose and high-dose male rats was determined to be below historical controls. Ultimately EPA concluded that none of the neoplasms were treatment-related.

Diuron	Mammary tumors noted in RED (EPA OPPTS, 2003a)	Increased incidence of mammary gland carcinomas in female mice at doses exceeding 600 mg/kg/day	Classified as a “known/likely” human carcinogen	Known/likely mammary carcinogen Basis: urinary bladder carcinomas rats, kidney carcinomas in male rats, and mammary gland carcinomas in female mice	Mammary tumors noted in RED and considered in carcinogen risk assessment
Ethalfluralin (Trifluralin)	Mammary tumors noted in RED (EPA OPPTS, 1995a)	Mammary gland fibroadenomas and adenomas/fibroadenomas combined in female rats at mid and high doses	Classified as a possible human carcinogen	Possible human carcinogen (C) Basis: increased mammary gland fibroadenomas and adenomas/ fibroadenomas combined in female rats	Mammary tumors noted in RED and considered in carcinogen risk assessment
Etridiazole (Terrazole)	Mammary tumors noted in RED (EPA OPPTS, 2000)	Increased incidence of mammary tumors in rats	Classified as a probable human carcinogen	Probable human carcinogen (B2) Basis: increased incidence of multiple tumors types in rats, including the liver, bile duct, mammary gland, thyroid and testes	Mammary tumors noted in RED and considered in carcinogen risk assessment. Positive for mutagenicity.
Fenvalerate (Pydrin)	Mammary tumors noted in review article (Parker et al., 1984 in Rudel, 2007)	Significantly increased incidence of benign mammary tumors in female rats upon oral administration	EPA RED not available. Authors of study concluded effects were not treatment related because effects were within historical control range (Parker et al., 1984)	EPA 1996 classified as Group E — Evidence of non-carcinogenicity for humans based on studies in rats and mice; mammary tumors are not mentioned in this document although Parker (1984) was reviewed (EPA OPP, 1996)	Parker et al., (1984) dismissed mammary tumors and mammary tumors are not discussed in the EPA 1996 risk evaluation (EPA OPP, 1996).
Folpet	Mammary tumors noted in RED (EPA OPPTS, 1999b).	Increased incidence of mammary benign fibroepithelial tumors observed in female rats at the highest dose compared to control	No additional discussion	Classification updated in 2010 (EPA OPP, 2018): not likely to be carcinogenic to humans at doses that do not cause an irritation response in the mucosal epithelium	Mammary and thyroid C-cell adenomas noted at high dose in RED with no additional comment about carcinogenicity. NOAEL set below doses where tumors were observed.
3-Iodo-2-propynyl butylcarbamate (IPBC)	Mammary tumors noted in RED (EPA OPPTS, 1997a)	Incidence of mammary gland fibroadenoma and combined fibroadenoma/ carcinoma significantly increased at the lowest dose relative to control by pairwise comparison. Mid-dose not reported and high dose reported as similar to controls. Body weight reduced at high dose.	Except for the lowest dose (20 mg/kg/day), incidence of tumor was within historical control range and because incidence at the highest dose tested (80 mg/kg/day) was almost equal to control incidence of mammary fibrodenomas declared unrelated to treatment	Not likely to be carcinogenic in humans Basis: lack of evidence in carcinogenicity studies	Mammary tumors at low dose dismissed in RED because of lack of a dose-relationship. Lower mammary tumor incidence at higher doses may be due to body weight decreases, which are known to reduce mammary tumors (Haseman et al., 1997).
Malathion	Mammary tumors and developmental effects noted in review article (Rodgers, 2018) No mention of mammary effects in RED (EPA OPPTS, 2009)	Rats in late puberty exposed to levels high enough to inhibit AcE had increased TEB formation and mammary tumors (Cabello et al., 2001). Another study in rats found that alone and combined with E2, malathion altered mammary gland structure and induced mammary tumors (Calaf and Echiburú-Chau, 2012). These were not regulatory toxicology studies.	EPA in 2009 RED describes malathion carcinogenicity as “suggestive” and mammary tumors are not mentioned. RED also notes the CARC review of Cabello et al. (2001) – which reports mammary effects and tumors—concluded that the paper provided insufficient basis for revising the cancer classification for malathion, however this CARC report is not currently public. EPA RED also notes that the risk assessment is	Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential. Basis: Liver tumors in rats and mice at excessive doses as well as rare tumors (nasal) that cannot be distinguished as treatment induced or random occurrence Note that 2000 CARC assessment concluded malathion is a likely human carcinogen based on liver and nasal tumors (CARC, 2000)	Mammary tumors and effects on mammary gland development reported in two studies were summarized in the Rodgers (2018) review. Also, the 2000 EPA CARC assessment reports mammary gland tumors in 2-year rat studies (as well as liver, adrenal, thyroid, uterine) but doesn’t discuss mammary tumors any further and dismisses most of the others, retaining a concern about liver and nasal tumors. Effects on mammary gland development are not discussed in the 2009 RED, except for a note that by protecting for

			considered protective of any potential carcinogenic effects.		AChE inhibition they protect for effects reported in Cabello et al. (2001).
Oryzalin	Mammary tumors noted in RED (EPA OPPTS, 1994c)	Increased incidence of mammary gland tumors in female rats at 300 ppm.	Classified as a possible human carcinogen based on mammary, skin, and thyroid tumors.	Classification updated in 2003 (EPA OPP, 2018): Likely human carcinogen	Mammary tumors noted in RED and considered in carcinogen risk assessment.
Parathion (parathion-ethyl)	Mammary tumors and developmental effects noted in review article (Rodgers, 2018)	Rats in late puberty exposed to levels high enough to inhibit AcE had increased TEB formation and mammary tumors (Cabello, 2001).	No RED	Limited data on carcinogenicity in animals available; increased adrenal cortical tumors and benign pancreatic tumors were observed in rats orally exposed to parathion. EPA has classified parathion as a Group C, possible human carcinogen. EPA notes that cholinesterase inhibition is caused by doses far below those eliciting carcinogenic effects (EPA OPP, 2000).	Mammary tumors were reported in 2001, and so are not discussed in cancer risk assessment conducted in 1989 (EPA OPPTS, 1989) Non-cancer risk discussion is focused on acute toxicity from cholinesterase inhibition.
Paraquat dichloride	Mammary tumors noted in RED (EPA OPPTS, 1997b) (	Frequent lesions observed in various organs in rats including the mammary glands (cysts, adenomas, fibromas, fibroadenomas and adenocarcinomas)	Lesions did not appear to be treatment related (either a dose-relationship was lacking or the incidence was similar in the controls and treated groups)	Evidence of non-carcinogenicity for humans Basis: lack of evidence of carcinogenicity in studies with rats and mice	Mammary tumors were dismissed in RED due to lack of a dose-relationship and/or tumors within control range.
Phosmet	Mammary tumors noted in RED (EPA OPPTS, 2006e)	Increased mammary gland tumors in female mice	Classified as having suggestive evidence of carcinogenicity but not sufficient to assess carcinogenicity potential in humans	Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential. Basis: Increased liver carcinomas/ adenomas in male mice and mammary gland tumors in female mice but not carcinogenic in rats. CARC suggested using RfD approach as for non-cancer risks.	Mammary tumors mentioned in RED and considered in carcinogen risk assessment.
Propazine[b]	Mammary tumors noted in TRED (EPA OPPTS, 2006b)	Mammary and pituitary tumors noted as well as similarity to atrazine MOA.	Mentions similarity to atrazine for which mechanism of mammary tumor formation not relevant to humans	Cancer risks have not been assessed but considered not likely to be a human carcinogen due to having a mechanism of action similar to atrazine	Mammary and pituitary tumors noted in RED and considered not relevant to humans based on MOA similarity to atrazine.
Propylene oxide	Mammary tumors noted in RED (EPA OPPTS, 2006f)	Significant incidences of fibroadenomas and tubulopapillary carcinomas in the mammary glands of female rats at the high dose. Multiplicity of fibroadenomas significantly increased at all doses	While incidence at the high dose exceeded the historical control range, higher incidence of mammary tumors were expected because the study went 28 months. So EPA questions whether this evidence supports propylene oxide being a systemic carcinogen.	Probable human carcinogen Basis: forestomach tumors in rat and nasal tumors in mice	Mammary tumors in rats (EPA OPPTS, 2006f) and mice (EPA OPPTS, 1991b) were dismissed in RED despite the fact that PrO is structurally similar to ethylene oxide, which causes mammary gland tumors in rodents and is associated with elevated breast cancer in humans (Rudel, 2014). Also PrO and EtO are epoxide-forming chemicals, a class that is known to induce mammary tumors.

Silicon dioxide (diatomaceous earth)	Mammary tumors noted in RED (EPA OPPTS, 1991a)	Nine incidences of mammary fibroadenomas in rats treated with 20 mg/day diatomaceous earth compared to five instances in controls (significance not given)	No additional comment	IARC expressed opinion that silicon dioxide was not classifiable as to its carcinogenicity in humans	mammary effects mentioned in RED with no additional comment
Simazine[b]	Mammary tumors noted in RED (EPA OPPTS, 2006g)	Mammary and pituitary tumors noted as well as similarity to atrazine MOA.	Mentions similarity to atrazine for which mechanism of mammary tumor formation not relevant to humans	Cancer risks have not been assessed but considered not likely to be a human carcinogen due to having a mechanism of action similar to atrazine	Mammary and pituitary tumors noted in RED and considered not relevant to humans based on MOA similarity to atrazine.
Sulfallate	Mammary tumors noted in review article (Rudel, 2007)	Induced mammary adenocarcinomas in female rats and mice after oral (diet) administration	EPA RED and risk assessment not available	NTP RoC classified as reasonably anticipated to be a human carcinogen Basis: sufficient evidence of carcinogenicity from studies in experimental animals: Dietary administration of sulfallate caused cancer of the mammary gland (adenocarcinoma) in female rats and mice, cancer of the forestomach (squamous-cell carcinoma) in male rats, and benign lung tumors (alveolar/ bronchiolar adenoma) in male mice (NTP, 2016b)	Mammary tumors are considered in the NTP ROC carcinogenicity assessment (NTP, 2016b)
Terbuthylazine[b]	Mammary tumors noted in RED (EPA OPPTS, 1995b)	Increase in mammary gland carcinomas relative to the control group at the highest dose	Dose with observed mammary effects considered to be that with excessive systemic toxicity (exceeding the MTD) and tumor incidence said to be of “uncertain relevance to humans”	Inadequate evidence to determine carcinogenicity in humans Basis: Mammary and testis tumors only observed at a dose that exceeded the MTD and were only seen in one species, so considered of uncertain relevance to humans.	Mammary tumors dismissed in RED because they were observed only at the highest dose, which was described as an excessively toxic dose. Note decreased BW at all doses which may mask mammary tumor effects from the treatment (Haseman et al., 1997).
Triclopyr	Mammary tumors noted in RED (EPA OPPTS, 1998e)	Female mice showed a significant increasing trend in mammary gland adenocarcinomas. Additionally, female rats showed significant increasing trends in mammary gland adenocarcinomas and in adenomas and/or adenocarcinomas combined. Significant pair-wise difference of gland adenomas and/or adenocarcinomas combined at the high dose compared to control.	Increases in mammary tumors considered to be marginal and there was an absence of support from structural analogs or genotoxicity	Not classifiable as to human carcinogenicity Basis: carcinogenicity in animals considered to be marginal (“not entirely negative, but yet not convincing”)	Mammary tumors were dismissed in RED despite being observed in female mice and rats with dose-response trend. Body weight reductions at higher doses may have masked a mammary tumor response (Haseman et al., 1997).

1b. Flagged for other mammary effects

Pesticide	**Reason included**	**Summary of mammaryeffects in RED or review m article**	**Study/EPA conclusion in RED re: ammary effects**	**Cancer risk assessment summary from EPA OPP or other authoritative source**[a]	**Comment on EPA risk evaluation**

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Amitrole	Mammary effects mentioned in RED (EPA OPPTS, 1996)	A high incidence and/or severity of acinar and/or ductular epithelial cell vacuolation in the mammary gland at the highest dose	No additional comment; LOEL for reproductive toxicity set to 112.5 ppm and the NOEL to 15 ppm	Probable human carcinogen Basis: thyroid tumors in rats and mice; liver tumors in mice	Mammary effects mentioned in RED as occurring in 2-gen repro study at highest dose. NOEL was set below this dose.
Atrazine (see above)					
Chlorpyrifos	Mammary gland developmental effects are described in review article (Rodgers, 2018) No mention of mammary effects in RED (EPA OPPTS, 2006c)	A study reported altered mammary gland development and circulating hormone levels in adult rats in 2016	No mention of mammary in 2011 EPA risk evaluation or 2016 EPA Revised Human Health Risk Assessment (EPA OCSPP, 2016)	Group E: Evidence of non-carcinogenicity for humans	No mention of mammary effects in RED. Chlorpyrifos altered mammary gland development and circulating hormone levels at doses below US Environmental Protection Agency's (EPA) benchmark dose for AChE inhibition, indicating AChE may not be the most sensitive endpoint. Effects on mammary gland development are not captured in 2016 EPA evaluations for chlorpyrifos (summary from Rodgers, 2018).
Diphenylamine	Mammary swelling mentioned in RED (EPA OPPTS, 1998c)	Swelling of the mammary glands at the highest dose in female rats	Swelling of mammary gland at 5000 ppm; NOEL set to less than 500 ppm.	Not likely to be carcinogenic to humans Basis: lack of evidence in two acceptable carcinogenicity studies	Mammary swelling mentioned in RED at high dose with no additional comment, and NOEL set at lower dose.
Malathion (see above)					
Meta-cresol	Mammary atrophy mentioned in RED (EPA OPPTS, 1994b)	Mammary, ovarian, and uterine atrophy observed in female mice at 30,000 ppm	No additional comment; LOEL set to 10,000 ppm and NOEL set to 3000 ppm	EPA IRIS classified as possible human carcinogen Basis: Increased incidence of skin papillomas in mice in an initiation-promotion study. The three cresol isomers produced positive results in genetic toxicity studies both alone and in combination (EPA IRIS, n.d.).	Mammary atrophy mentioned in RED and NOEL set below the dose with this effect.
Metribuzin	Mammary pathology noted in RED (EPA OPPTS, 1998d)	Pathological changes in mammary glands at the highest dose.	Acknowledgement of toxicity at the highest dose but overall no evidence of carcinogenicity in either sex; LOEL for chronic toxicity set to 300 ppm and NOEL set to 100 ppm	Not classifiable as to human carcinogenicity Basis: lack of evidence for carcinogenicity	Mammary pathology (not further described) is considered in RED non cancer risk evaluation.
Methoxychlor	Effects on mammary gland development noted in review article (Rudel, 2011)	Increased area, branches, TEBs, LBs, increased cell division in epithelium at PND in rats after oral (diet) administration of 800 ppm	EPA revoked methoxychlor registration in 2004 based on EDC activity without completing a full risk assessment. A risk summary sets RfD based on fertility effects. Mammary gland is not mentioned in the document (EPA, 2000b)	Not classifiable as to its carcinogenicity to humans (IARC) Not classifiable as to human carcinogenicity (IRIS)	Mammary gland developmental effects noted in Rudel (2011) review. EPA risk assessment focuses on fertility effects and does not mention mammary gland effects (EPA, 2000b)
Oxadiazon	Mammary gland effects noted in RED (EPA OPPTS, 2003b)	Inactivation of mammary glands in female rats resulted in disrupted lactation	LOAEL appears to be based on the impaired lactation effect. EPA also notes inactivation not likely due to endocrine disruption.	Likely to be carcinogenic to humans Basis: increased incidence of hepatocellular adenoma and carcinoma in rats and mice	MG effect (impaired lactation) considered in RED non-cancer risk evaluation.
Parathion (see above)					

Blank cells indicate data not available from the sources indicated.

[a] Cancer classification and basis gathered from REDs unless stated otherwise.

[b] A triazine.

Table 2a

Mammary gland tumors

Considered in risk assessment (n = 9)	Not considered in risk assessment (n = 19)
1,2-dibromo-3-chloropropane	***Dismissed: effect not considered treatment related (n = 7)***
Captafol	Clonitralid (within control range)[a]
Dichloropropene[a]	Difenzoquat (no details)
Diuron[a]	Fenvalerate (within historical controls)
Ethalfluralin[a]	IPBC (lack of a dose response)[a]
Etridiazole[a]	Paraquat dichloride (lack of dose relationship or within control range)[a]
Oryzalin[a]	Propylene oxide (unclear)[a]
Phosmet[a]	Triclopyr (increase considered “marginal”)[a]
Sulfullate	
	Dismissed: effect only seen at excessively toxic doses (n =2)
	Ametryn[a]
	Terbuthylazine[a]
	Dismissed: mechanism not relevant to humans (n = 3)
	Atrazine[a,b]
	Propazine[a]
	Simazine[a]
	Dismissed: no reason provided (n =6)
	Alachlor (no discussion about tumors at low dose; highest dose tumors dismissed because of excessive toxicity)
	Chlordane
	Dichlorvos[a]
	Folpet[a]
	Malathion[a,b]
	Silicone dioxide
	Mammary effects reported after risk assessment (n = 1)
	Parathion[b]

[a] Pesticides that are currently approved for use in the US.

[b] Pesticides that have both mammary tumors and other mammary effects.

Table 2b

Other mammary effects

Considered in risk assessment (n = 5)	Not considered in risk assessment (n = 5)
Amitrole	**Inadequately tested and evaluated (n = 4)**
Diphenylamine[a]	Atrazine[a,b]
Meta cresol[a]	Chlorpyrifos[a]
Metribuzin[a]	Malathion[a,b]
Oxadizion[a]	Methoxychlor
	Mammary effects reported after risk assessment (n = 1)
	Parathion[b]

[a]Pesticides that are currently approved for use in the US.

[b]Pesticides that have both mammary tumors and other mammary effects.

Table 3

Use characteristics for pesticides that target the mammary gland, focusing on US and Europe.

Pesticide	Pesticide type/Class	Are US products currently labeled for use with this active ingredient?[a]	US use data[b]	Approved by European Commission?[c]
1,2-dibromo-3-chloropropane (DBCP)	Fumigant, Nematicide/ Halogenated organic	No		No
3-Iodo-2-propynyl butylcarbamate (IPBC)	Fungicide, Wood Preservative/Other Carbamate	Yes	Used in cosmetics (Johnson, 2017). Not listed in USGS 2016.	
Alachlor	Herbicide/Chloroacetanilide	No	Not in use but it is structurally similar to metolachlor-S and metolachlor, which are 3rd and 15th most commonly used pesticides in the agricultural sector; their combined use was 38–52 million pounds in 2012 (Atwood and Pairsley-Jones, 2017)	No
Ametryn	Herbicide/Triazine	Yes	Estimated 0.15 million pounds agricultural use (USGS, 2016)	No
Amitrole	Herbicide/Triazole	No		EC not approved but appears to be used in some EU countries
Atrazine	Herbicide/Triazine	Yes	Estimated 75 million pounds agricultural use (USGS, 2016). EPA report indicates 2nd most commonly used pesticide in the agricultural sector with 64–74 million pounds used in 2012 (Atwood and Pairsley-Jones, 2017).	No
Captafol	Fungicide/Thiophthalimide	No		No
Chlordane	Insecticide/Organochlorine	No		No
Chlorpyrifos	Insecticide, Nematicide/ Organophosphorus	Yes	Estimated 5 million pounds in agricultural use (USGS, 2016). EPA 14th most commonly used pesticide ingredient in agricultural sector with 4–8 million pounds used in 2012; most commonly used organophosphate pesticide across all sectors with 5–8 million pounds used in 2012 (Atwood and Pairsley-Jones, 2017);	Yes
Clonitralid (Niclosamide)	Molluscicide	Yes	Used to kill sea lamprey larvae in tributaries to the Great Lakes, the Finger Lakes, and Lake Champlain (EPA OPPTS, 1999a). Not listed in USGS 2016.	No
Dichloropropene (1,3-D)/Telone	Fumigant, Nematicide/ Halogenated organic	Yes	Estimated 45 million pounds of agricultural use (USGS, 2016); 4th most commonly used pesticide in agricultural sector with 32–42 million pounds used in 2012 but trend suggests use has gone up (Atwood and Pairsley-Jones, 2017)	No
Dichlorvos (DDVP)	Insecticide/ Organophosphorus	Yes	Registered to control insects in agricultural, commercial, institutional and industrial sites; in and around homes; and on pets (EPA OPPTS, 2006d). Not listed in USGS 2016	No
Difenzoquat	Herbicide	No		No
Diphenylamine	Fungicide, Insecticide, Plant Growth Regulator/Amine	Yes	Not listed in USGS 2016	No
Diuron	Herbicide/Urea	Yes	Estimated use of 2.5 million pounds in U.S on various crops; trend is downward (USGS, 2016)	Yes
Ethalfluralin (Trifluralin)	Herbicide/2,6-Dinitroaniline	Yes	19th most commonly used pesticide in agricultural sector with an estimated 3–7 million pounds used in 2012 (Atwood and Pairsley-Jones, 2017)	No

Pesticide	Pesticide type/Class	Are US products currently labeled for use with this active ingredient?[a]	US use data[b]	Approved by European Commission?[c]
Etridiazole (Terrazole)	Fungicide/Azole	Yes	Estimated use of less than 0.05 million pounds in U.S on various crops; trend is towards phaseout (USGS, 2016)	Yes
Fenvalerate (Pydrin)	Insecticide/Pyrethroid	No		No
Folpet	Fungicide/Thiophthalimide	Yes	Avocados in US, imported fruit and vegetables, some paints and preservatives (EPA OPPTS, 1999b). Listed in USGS 2016 but has no estimated agricultural use.	Yes
Malathion	Insecticide/ Organophosphorus	Yes	Estimated 1 million pounds in agricultural use (USGS, 2016). EPA 10th most commonly used pesticide ingredient in the home and garden sector with 1–3 million pounds used in 2012. 3rd most common organophosphate pesticide across all market sectors with 1–4 million pounds used in 2012. Trend indicates decreased use (Atwood and Pairsley-Jones, 2017). Used in lice treatments.	Yes
Meta-cresol	Microbiocide/Phenol	Yes	Orchards (EPA OPPTS, 1994b). Not listed in USGS 2016.	
Methoxychlor	Insecticide/Organochlorine	No		No
Metribuzin	Herbicide/Triazinone	Yes	Estimated 5 million pounds in agricultural use; trend is upward (USGS, 2016)	Yes
Oryzalin	Herbicide/2,6-Dinitroaniline	Yes	Estimated less than 0.5 million pounds in agricultural use; major drop in use (USGS, 2016)	Yes
Oxadiazon	Herbicide/Oxadiazole	Yes	Estimated less than 2 thousand pounds in agricultural use; downward trend (USGS, 2016)	EC not approved but appears to be used in some EU countries
Paraquat Dichloride	Herbicide/Bipyridylium	Yes	Estimated 8 million pounds in agricultural use; trend suggests increased use (USGS, 2016). EPA 23rd most commonly used pesticide in agricultural sector with 2–6 million pounds used in 2012 (Atwood and Pairsley-Jones, 2017)	No
Parathion (parathion-ethyl)	Insecticide/ Organophosphorus	No		No
Phosmet	Insecticide/ Organophosphorus	Yes	Estimated 0.6 million pounds in agricultural use (USGS, 2016). Overall 9th most common organophosphate (OP) insecticide pesticide ingredient across all market sectors with less than 1 million pounds used in 2012; overall decrease in OP use in U.S (Atwood and Pairsley-Jones, 2017);	
Propazine	Herbicide/Triazine	Yes	Estimated 0.2 million pounds in agricultural use (USGS, 2016)	No
Propylene oxide	Fumigant/Alcohol (Ether)	Yes	Fumigant used to treat herbs and spices, nuts, some other foods, cosmetics, pharmaceuticals (EPA OPPTS, 2006f). Not listed in USGS 2016	
Silicon dioxide (diatomaceous earth)	Silicone dioxide: Inorganic	No		
Simazine	Herbicide/Triazine	Yes	Estimated 3 million pounds in agricultural use (USGS, 2016) EPA 8th most commonly used pesticide ingredient in the industrial/commercial/government sector with 0–2 million pounds used in 2012 (Atwood and Pairsley-Jones, 2017);	EC not approved but appears to be used in some EU countries
Sulfallate	Herbicide/Dithiocarbamate	No	Listed in USGS 2016 but has no estimated agricultural use	No
Terbuthylazine	Algaecide, Herbicide, Microbiocide/Triazine	Yes	Used in ornamental ponds and aquaria, heating and cooling water (EPA OPPTS, 1995b). Not listed in USGS 2016	Yes

Pesticide	Pesticide type/Class	Are US products currently labeled for use with this active ingredient?[a]	US use data[b]	Approved by European Commission?[c]
Triclopyr	Herbicide/Chloropyridinyl	Yes	Estimated 1 million pounds in agricultural use and along right-of ways for brush control (USGS, 2016)	Yes

[a] Information gathered from the NPIRS Public website (CERIS, 1998–2020).

[b] Blank cells indicate pesticide is currently not in use.

[c] Information gathered from the Pesticide Properties Database (Lewis et al., 2016). Blank cells indicate data not available from the sources indicated.