UCSF
UC San Francisco Previously Published Works

Title

Rapid identification of reproductive toxicants among environmental chemicals using an in vivo evaluation of gametogenesis in budding yeast Saccharomyces cerevisiae

Permalink

https://escholarship.org/uc/item/4431z1b6

Authors

Kumar, Ravinder

Oke, Ashwini

Rockmill, Beth

et al.

Publication Date

2024-09-01

DOI

10.1016/j.reprotox.2024.108630

Copyright Information

Peer reviewed
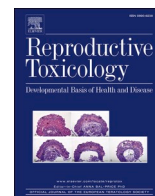
Rapid identification of reproductive toxicants among environmental chemicals using an *in vivo* evaluation of gametogenesis in budding yeast *Saccharomyces cerevisiae*

Ravinder Kumar [a,b], Ashwini Oke [a,b], Beth Rockmill [a,b], Matthew de Cruz [a,b], Rafael Verduzco [a,b], Anura Shodhan [a,b], Xavier Woodruff-Madeira [a,b], Dimitri P. Abrahamsson [a], Julia Varshavsky [e], Juleen Lam [f], Joshua F. Robinson [a,b], Patrick Allard [c,d], Tracey J. Woodruff [a], Jennifer C. Fung [a,b,*]

[a] *Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA, USA*
[b] *Center of Reproductive Sciences, University of California, San Francisco, CA, USA*
[c] *UCLA Institute for Society & Genetics, University of California, Los Angeles, CA, USA*
[d] *Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA, USA*
[e] *Department of Health Sciences and Department of Civil and Environmental Engineering, Northeastern University, Boston, MA, USA*
[f] *Department of Public Health, California State University, East Bay, Hayward, CA, USA*

ABSTRACT

Infertility affects ~12 % of couples, with environmental chemical exposure as a potential contributor. Of the chemicals that are actively manufactured, very few are assessed for reproductive health effects. Rodents are commonly used to evaluate reproductive effects, which is both costly and time consuming. Thus, there is a pressing need for rapid methods to test a broader range of chemicals. Here, we developed a strategy to evaluate large numbers of chemicals for reproductive toxicity via a yeast, *S. cerevisiae* high-throughput assay to assess gametogenesis as a potential new approach method (NAM). By simultaneously assessing chemicals for growth effects, we can distinguish if a chemical affects gametogenesis only, proliferative growth only or both. We identified a well-known mammalian reproductive toxicant, bisphenol A (BPA) and ranked 19 BPA analogs for reproductive harm. By testing mixtures of BPA and its analogs, we found that BPE and 17 β-estradiol each together with BPA showed synergistic effects that worsened reproductive outcome. We examined an additional 179 environmental chemicals including phthalates, pesticides, quaternary ammonium compounds and per- and polyfluoroalkyl substances and found 57 with reproductive effects. Many of the chemicals were found to be strong reproductive toxicants that have yet to be tested in mammals. Chemicals having affect before meiosis I division vs. meiosis II division were identified for 16 gametogenesis-specific chemicals. Finally, we demonstrate that in general yeast reproductive toxicity correlates well with published reproductive toxicity in mammals illustrating the promise of this NAM to quickly assess chemicals to prioritize the evaluation for human reproductive harm.

1. Introduction

Infertility is a surprisingly common problem affecting 10–15 % of reproductive age couples [1]. It can stem from a variety of causes including reduced quality and quantity of gametes (both sperm and eggs), physical blockage of the male or female ducts, as well as uterine abnormalities. In the broad class of infertility in which quality and quantity of gametes are reduced, problems of gametogenesis are a main contributor [2]. Gametogenesis can be divided into three major steps: sex determination, meiosis and gamete development. Failure of gametogenesis is primarily due to a breakdown in the ability of chromosomes to divide properly during meiosis, ultimately resulting in gamete aneuploidy which in turn lead a loss of gamete viability either through the reduction of gamete numbers or problems in gamete development that impede growth. Thus in addition to infertility, gamete aneuploidy results in an increased incidence of miscarriages in the mother and developmental disabilities in subsequent generations (e.g. Down Syndrome – trisomy 21) [3].

Not all underlying causes for gamete aneuploidy are known, however there is mounting evidence that environmental chemicals can contribute to its incidence [4–6]. Many of the ~40,000 chemicals that are actively manufactured, imported, or used in household or commercial products have not been evaluated for their potential toxicities towards human health. Reliable information about reproductive toxicology is particularly scarce [7], even for those chemicals commonly detected in maternal and umbilical cord sera [8,9].

In humans, an impediment to identifying reproductive toxicants is the prolonged delay between toxicant exposure and the manifestation of reproductive perturbations. Meiosis, a key molecular process leading to gametogenesis, takes place in female fetuses *in utero* and manifestation of the adverse effect is not observed until adulthood. If a woman is exposed to meiotic toxicants as an adult, they will not necessarily affect her own fertility, since a significant part of meiosis has already taken place during her fetal ovarian development. Relating chemical exposure in an individual to a fertility reduction in their children, or birth defects in their grandchildren, is epidemiologically challenging due to the need for large cohorts followed over long periods of time with adequate information about exposure during critical developmental periods. As a result, there is a relative paucity of information on human reproductive toxicity. Instead, evaluation of reproductive toxicity is most commonly performed using whole-animal rodent tests, which is costly, time consuming, require a large number of animals for transgenerational studies, thus greatly restricting the number of chemicals that can realistically be tested.

One alternative organism that has been used to rapidly elucidate reproductive toxicity related to problems in gametogenesis is the nematode C. elegans [10,11]. In *C. elegans* the appearance of males can be used to detect problems in meiosis in the mother by measuring an increase in phenotypically male eggs. Human and mammalian in vitro systems for spermatogenesis have also been developed that start with pluripotent stem cells [12–14]. However, these systems typically have a low yield of gametes and often the gametes are not fully functional. Moreover, the ability to rapidly evaluate chemicals on the order of hundreds to thousands has yet to be demonstrated. One *in vitro* spermatogenesis system using human pluripotent cells has reported reproductive toxicity for 2-bromopropane and 1,2,dibromo-3-chloropropane, but the effect was attributed to cell death and not to disruption of meiosis [14]. Another recent development used a mouse spermatogonial cells and testicular cell co-culture model to evaluate reproductive toxicity of BPA analogs [15]. Using this system, this study showed cytotoxic effects and found cytological changes, however whether these effects eventually result in aneuploidy or lower gamete numbers was not assessed.

One system that would be amenable for high-throughput discovery of reproductive toxicants is the budding yeast, *Saccharomyces cerevisiae*. Conservation is remarkably high between yeast and humans with ~60 % of yeast genes having human homologs and 87 % of yeast protein domains being present in the human proteome [16]. Yeast has long been a major workhorse of eukaryotic molecular biology. In addition to the leading role it has played in our understanding of such common core processes as transcription, chromatin, DNA replication, and the cell cycle, yeast is one of the most studied organisms for gametogenesis [17]. The use of this organism thus leverages an extensively developed trove of molecular and genetic information. Moreover, besides its ability to be easily induced to undergo gametogenesis, yeast has the added benefit that, as a single cell system, it lacks a reproductive tract, thus gametogenesis can be evaluated more directly.

2. Material and methods

2.1. Yeast strains and growth media

Yeast strains are constructed in a diploid BR1919–8B background which has high meiotic efficiency [18]. Yeast can be easily induced into meiosis via starvation of carbon and nitrogen. To induce meiosis, strains are switched from glucose rich YPD media to T-SPO media containing only 1 % potassium acetate and 0.05 % glucose. *pdr1Δ* and *pdr3Δ* strain construction and media formulations are described in supporting information (Text S1).

2.2. Chemicals

Table S1 lists the chemicals used in this study with the associated catalog numbers, manufacturer, CAS number, barcode for blinding and % purity, usage and chemical class. Table S2 lists the associated chemical structures as determined by ClassyFire [19] for each chemical. Seven replicate 100 mM stocks were made in 100 % DMSO (Sigma Aldrich, St. Louis, MO) and were stored in 0.5 ml aliquots at -80ºC. Chemicals were thawed for use and diluted to exposure concentrations while keeping the final DMSO concentration at 0.1 %. For 6 chemicals, 10 mM stocks were made due to limited chemical availability or solubility.

2.3. Chemical screening dose choice

We chose the two initial screening concentrations of 30 and 100 μM to best capture any compounds that potentially would have any reproductive effect. 30 μM was chosen since in yeast drug discovery this was the concentration used to identify compounds that target conserved proteins [20,21]. Both concentrations were also chosen as they are similar to concentrations that have been used in other chemical assays previously in C. elegans, and zebrafish and that have shown both sensitivity and predictivity [10,22–24]. Four additional doses for the positive hits were performed which will be analyzed in detail in an accompanying publication to directly compare the same chemicals in a *C. elegans* reproductive toxicity assay [25].

2.4. Yeast reproductive toxicity assay

Cell preparation: The SRK007 yeast strain was freshly streaked from frozen stock and grown on YPD plates. A saturated culture was generated by inoculating a colony into 1 ml of YPD and grown for 24 hours at 30ºC on a shaker set at 230 rpm. After 24 hours, cells were pelleted and washed three times with T-SPO media before resuspension into 1 ml of T-SPO. Cells were diluted into 50 ml of T-SPO to a final OD600 of 0.25. 100 μl of cell suspension is transferred into each well of a 96 deep-well plate using a Liquidator-96 (Mettler-Toledo Rainin, LLC, Columbus, OH) into which 400 μl chemicals and sporulation media had been dispensed such that the final doses of the chemicals were 30 μM or 100 μM in a 0.5 ml volume. The plate is covered with a Breathe-Easier membrane (Sigma-Aldrich, St. Louis, MO) before incubation in a Multitron HT shaker (Infors AG, Basel, CHE) at 30°C, 950 rpm for 72 hours.

2.4.1. Chemical dispensation

Chemicals were dispensed just prior to cell inoculation into sterile 96-deep well plates with pyramidal bottoms. Only the inner 60 wells were used due to 10 % evaporation loss in the outer wells. To prevent internal well evaporation, the outer 36 wells were filled with 0.5 ml of sterile water. 100 mM chemical stocks were first thawed and vortexed before diluting into two working solutions of 37.5 μM and 125 μM from which 400 μl was added to each well in three technical replicates. Once the cells were added, the final dose concentrations of 30 and 100 μM in 0.1 % DMSO was achieved. For each plate, three replicates of the negative control 0.1 % DMSO and positive control 0.5 μM Latrunculin B (LatB) were also included. We performed a minimum of six replicates for each chemical at each dose (30 μM and 100 μM) at least two biological replicates and three technical replicates. The biological replicates were from single isolates taken from cells grown on YPD plates for 3 days after streaking from frozen stock Technical replicates were placed in different wells spread across at least three different plates. Each replicate was treated as an individual data point. Different chemicals were randomly positioned for each plate. All chemicals were barcoded to allow for blinding of the experiments. A total of 199 different chemicals were evaluated in this study. We also included four duplicate chemicals under a different barcode as a control for measurement consistency (Figure S1).

2.4.2. Growth curve measurement

On completion of incubation, cells are pelleted in a tabletop centrifuge at 1000 x g for 5 minutes. Cells are washed with 300 μl sterile water and pelleted three times. Cells are resuspended in 100 T zymolase (31.25 μg/ml of zymolyase 100 T and 10 mM DTT) and incubated at 30 °C for 3 hours. After each hour, cells are vigorously mixed in a Mixmate (Eppendorf, Hamburg DE) at 1000 rpm for 2 minutes. After incubation, cells are washed three times with 300 μl of sterile water. Cells are then resuspended in 1 ml YPD and vigorously mixed in the Mixmate for 10 minutes. 10 μl of the cell suspension is added to 90 μl of YPD in each of the inner 60 wells of a 96-well imaging plate (Corning #3631, Corning NY). The outer wells are filled with 100 μl of YPD to limit evaporation, the plate is covered and sealed with tape before placing in the Tecan M200 plate reader (Tecan, Männedorf, CHE) with the following settings (30ºC, 432 orbital shaking,1 mm amplitude). Readings are recorded every 30 minutes for 35–45 hours using Tecan's iControl software.

2.4.3. Δt_{Hmax} calculation

OD values across all timepoints are used to fit a logistic growth curve using R package drc [26] with the following parameters (fct=l4(fixed=c (NA,NA,1,NA)) for slope, start and end of curve and intercept. Time at half-max (t_{Hmax}) is calculated as time to reach OD600 = 0.5 on the fitted growth curve. For each chemical, the shift in t_{Hmax}, Δt_{Hmax} is calculated as the difference between a chemical's t_{Hmax} and the average t_{Hmax} of the 0.1 % DMSO wells on that plate. For wells that do not reach saturation, the t_{Hmax} value is capped at 46 hr (32 hr for mitotic experiments). Wells with poor fits are flagged and removed from the data set.

2.5. Yeast proliferative growth toxicity assay

The yeast proliferative growth toxicity assay is similar to the meiotic assay except for a few exceptions. Cells were introduced to the chemicals at the same concentrations of 30 and 100 μM in 96 deep-well plates in triplicates, but in YPD rather than T-SPO media. Cells were exposed to chemicals for 3 hours in an incubator at 30ºC shaking at 950 rpm to allow for at least one round of mitotic division. Cells were washed three times with water as described for the meiotic toxicity assay before resuspending in 1 ml YPD. 10 μl of the cell suspension was added to 90 μl of YPD in a 96 well imaging plate as described for the meiotic assays. The Lat B positive control was performed at 0.7 μM. The growth curve was monitored every 30 minutes for 24 hours.

2.6. Meiotic characterization

Synapsis progression: Chromosome spreads [27] were prepared at 19 hours and 22 hours after meiotic induction and imaged on a Delta-vision (GE Healthcare) fluorescence microscope. Spreads were stained with anti-Zip1 antibodies to highlight the synaptonemal complex and anti-Rap1 antibodies to highlight the ends of the chromosomes. The experiment was repeated three times and equal number of spreads were counted (n=271). Each chromosome spread was evaluated for the state of synapsis progression [28]. Gametogenesis frequency: The frequency of cells that progress beyond meiosis I was calculated from counting the number of nuclei 3 days after cells were induced to undergo meiosis. Cells were fixed with 70 % ethanol and stained with DAPI to highlight the number of nuclei. Gamete viability: Gamete viability was determined by manually dissecting 10 mM zymolyase-digested tetrads onto YPD plates [29]. Viability was measured by determining what percentage of gametes formed colonies. Recombination: Recombination was measured in centiMorgans (cM) for *HIS4-LEU2* and *LEU2-MAT* intervals based on the number of parental (P), nonparental ditype (NPD) and tetratype (T) combination of genetic markers [30].

BPA and BPA Substitute Competitive Assays: For those BPA alternatives that at 15 μM or 30 μM shifted the t_{Hmax} without affecting either slope or saturation, we determined combination effects using the Loewe additivity model [31–33]. The Loewe additivity model tests whether two chemicals are additive by asking if the sum of each individual dose response at dose X equals, is greater or less than the individual responses at dose 2X (i.e chemical A at 10 μM + chemical B at 10 μM equals, is greater or less than chemical A at 20 μM and chemical B at 20 μM). If equal, it is considered additive; greater than it is synergistic; if less than, it is considered antagonistic. Δt_{Hmax} was calculated for BPA and a BPA alternative individually at X μM, 2X μM and mixed at X μM BPA substitute + X μM BPA doses where X could be 15 or 30 μM. A synergistic effect is concluded if Δt_{Hmax} for the X μM BPA substitute + X μM BPA doses is significantly greater than for the 2X μM doses of either BPA alone or its substitute alone. Antagonistic effects are concluded when Δt_{Hmax} for X μM BPA alternative + X μM BPA doses is less than for both 2X μM BPA or 2X μM BPA substitute.

3. Results

3.1. Yeast high-throughput screen for reproductive toxicants

To rapidly assess a large number of chemicals for reproductive toxicity, we developed a 96-well plate high-throughput screen (HTS) based on detecting gamete viability in budding yeast (Fig. 1). Because gametes are haploid, and each chromosome carries essential genes, any failure of meiotic chromosome segregation leading to chromosome loss would produce inviable gametes. Since yeast can proliferate in either the diploid or haploid state, the level of gamete viability in yeast is an easily measurable indicator of meiotic success as it relies only on absorbance measurements to assess the proliferative growth of viable gametes, making it more suitable for high throughput screening. Compounds detrimental to meiosis will cause a shift in the gamete growth curve to the right relative to the vehicle control due to the decreased number of viable gametes (Fig. 1) which delays the appearance of visible growth. In this assay, the chemical being tested is only applied while the cells are undergoing meiosis, and is then extensively washed away so that growth curves are measured in the absence of the chemical (see Material and Methods). The shift in time observed at half maximum of the growth curve (Δt_{Hmax}) reflects the number of viable cells present in the sample at the start of the growth phase, and therefore the extent of toxicity. To reduce the well-known resistance of yeast to exogenous chemicals, we constructed a *pdr1Δ pdr3Δ* double-mutant strain that codes for transcription factors needed for the MDR class drug efflux pumps [34]. The *pdr1Δ pdr3Δ* double-mutant strain has been used effectively in a HTS for drugs that affect neurodegenerative disease [20] and we found that it
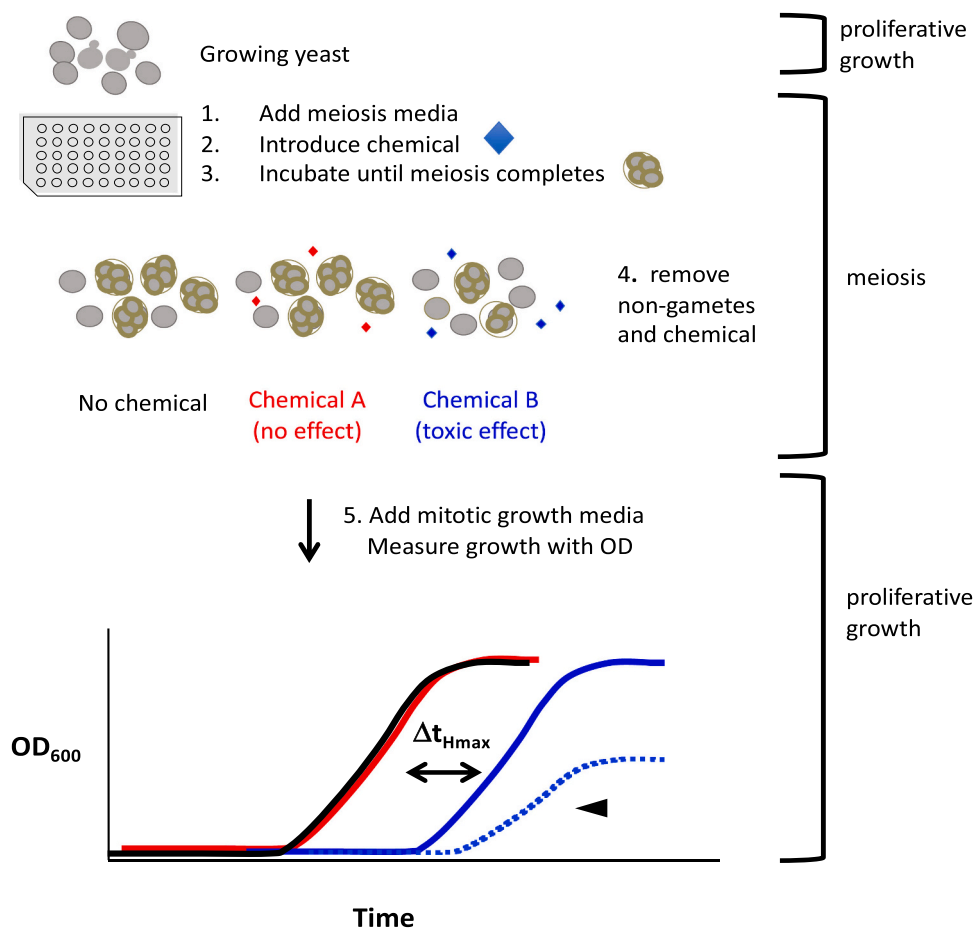
Fig. 1. Yeast high-throughput assay for reproductive toxicity. Chemicals (diamonds) are introduced to yeast in meiosis media used to initiate gametogenesis. Gametogenesis results with the formation of a four-gamete ascus Non-gametes are removed enzymatically. Several wash steps remove chemicals before gametes are reintroduced to proliferative growth. Growth curves are obtained by measurement at OD600 in a plate reader. Any toxic chemical that reduces the number of gametes or decreases gamete viability will cause the growth curve (blue – toxic chemical, red – nontoxic chemical, black – no chemical) to shift to the right relative to the no chemical control. The measured shift in time at Δt_{Hmax} reflects the extent of toxicity. Any growth curves that show a change in slope and/or lowered plateau (dotted, arrowhead) reflect acute toxicity, not solely meiotic toxicity, and t_{Hmax} is capped at 46 hours.

does not significantly affect gamete viability (Figure S2). A key aspect of the assay is the removal of any diploid cells with zymolyase [35] that fail to enter meiosis that would otherwise confound the gamete viability assessment after the cells are reintroduced to proliferative growth. Once diploids are removed and the chemicals washed away, gamete viability can be assessed using absorbance at OD_{600} in a microplate reader by measuring resumption of proliferative growth after exchange into YPD medium. Proliferation is monitored until the growth curve reaches saturation in order to accurately calculate the Δt_{Hmax} and to detect if sustained damage occurred that affects mitotic/proliferative growth (e. g. losses to mitochondrial function) and not meiotic growth which is evident by both a change in slope in the growth curve and a lowered saturation level (Fig. 1, arrowhead).

3.2. Validation of screen using meiotic mutants and bisphenol A (BPA)

As an initial validation of our approach for using growth of meiotic products to detect meiotic defects, we examined two well-characterized yeast meiotic mutants, *spo11Δ* [36] and *msh4Δ* [37], both of which are recombination mutants with known loss of gamete viability (<1 % [38] and 43 % [39], respectively). Both mutants shift the growth curves to an extent compatible with their known gamete viability (Fig. 2A – *spo11*–3 %, *msh4*–27 %) suggesting that the assay reflects meiotic perturbations in viable gamete number. To determine whether our assay is sensitive enough for HTS applications, we calculated a Z' value [40], a

robust measure of separation between hits and non-hits in a screening experiment. A Z' of 0.753 was determined from the signal dynamic range and data variation from both the negative control (0.1 % DMSO) and the positive control (0.5 µM Latrunculin B (LatB)). Z' values between 0.5 and 1.0 indicate a high quality HTS. Latrunculin B (LatB), a highly specific inhibitor of the actin cytoskeleton, was used as a positive control since it is known to disrupt the cytoskeletal elements needed for telomere-led chromosome motion in budding yeast essential to prophase I of meiosis [41]. Our negative control – 0.1 % DMSO was selected to solubilize the chemicals since it had no effect on meiosis up to 1 % DMSO (Figure S3). Similarly, no toxicity was observed for acetone, acetonitrile, methanol and toluene. Among potential solvents tested, ethanol was detrimental to meiosis, as ethanol can be used as a carbon source thus preventing meiotic entry.

To ask if our assay detects reproductive toxicants, we tested bisphenol A (BPA), a well-studied chemical with known adverse effects on mammalian reproduction [2,42,43], BPA is a component in plastics that has been found extensively in humans [39,44–46] due to its widespread use in products including food and beverage containers, thermal paper, toys, electronics, medical equipment and water pipes (reviewed in Catenza et al. [47]). We tested BPA at concentrations of 0, 30, 100, 120, 140, 160, and 180 µM. As shown in Fig. 2B, our assay exhibits sensitivity to BPA, showing greater toxicity (i.e. larger shift in Δt_{Hmax}) with greater dose.

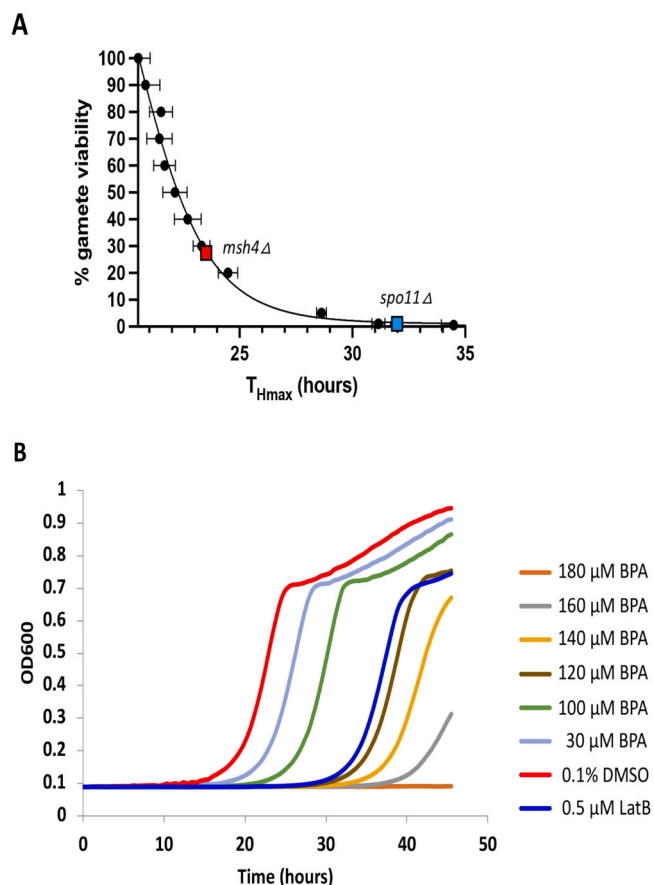Prior studies of both mammals and worms have shown that BPA

Fig. 2. Validation of screen using meiotic mutants and known reproductive toxicant BPA. A) Averaged growth curves for meiotic mutants *msh4* (n=3) and *spo11* (n=6) Error bars: STD. Mutant Δt_{Hmax} mapped onto standard curve calculated from a dilution series of sporulated cells (black diamonds). Formula for curve fit used to convert Δt_{Hmax} to % gamete viability C) Dose response curves for BPA at several increasing concentrations shown in μM. The experiments were performed in triplicate and averaged values are shown. DMSO – 0.1 % (negative control). LatB – 0.5 μM (positive control). Note that increase in absorbance after saturation is a spurious artifact of the plate reader that is not included in the fit.

disrupts meiosis [42,48]. During prophase I oogenesis in mice, Susiarjo et al. (2007) [42] observed both unsynapsed chromosomes and higher recombination that resulted in an increase in aneuploidy. In nematodes, Allard et al. (2010) [48] also found unsynapsed chromosomes and a delay in double-stranded break processing during meiosis resulting in fewer eggs and higher embryonic lethality. In yeast, we see a similar perturbation in meiosis during prophase I at 19 hours (Fig. 3**A**) and 22 hours (Fig. 3**B**) after meiotic induction. This is manifested both as a delay in chromosome synapsis progression and by the unexpected appearance of the polycomplexes, an abnormal aggregation of the synapsis protein Zip1 previously shown to accompany problems in chromosome synapsis [49]. Further microscopic evaluation of BPA effects at higher doses in yeast reveals that the overall frequency of cells progressing through meiosis is reduced (Fig. 3**C**), however gamete viability is only significantly perturbed at 100 μM BPA (Fig. 3**D**). Changes to recombination can often lead to loss of gamete viability. To determine if recombination is affected by BPA, we measured recombination in two genetic intervals, *HIS4-LEU2* and *LEU2-MAT*. Although recombination was not affected at 30 μM, we observed reduced recombination at 100 μM BPA as compared to 0 μM BPA (*HIS4-LEU2*: 23.6 cM to 12.0 cM; *LEU2-MAT*: 32.3 cM to 19.1 cM) (Fig. 3**E**). Together these results show that BPA in yeast affects recombination and progression in meiotic prophase I as observed in both mammals and worms suggesting that BPA

affects similar mechanisms in these diverse organisms.

3.3. Relative reproductive toxicity of BPA alternatives

Due to numerous studies linking BPA to reproductive toxicological effects, BPA limitations have been imposed for use in daily products (e.g. Commission Regulation (EU) 2018) resulting in the increased substitution of BPA with BPA analogs, which are not necessarily less toxic than BPA itself [50,51]. We therefore set out to test the relative toxicity to gametogenesis of BPA analogs by examining 19 BPA-related compounds (BADGE, BFDGE, BPAF, BPAP, BPB, BPC, BPE, 2,2'-BPF, 4,4'-BPF, BPAP, BPOPP-A, BPP, BPZ, HPP, diphenyl sulfone, hydroquinone, PHBB, TMBPA, 17 β-estradiol). Fig. 4**A** shows BPA analogs ranked in the order of Δt_{Hmax} using the yeast assay. Table S3 contains the Δt_{Hmax} values for each of these BPA analogs. Out of the 19 chemicals examined, ten were ranked higher and nine were ranked lower than BPA based on Δt_{Hmax}. The relative ranking of BPAF, BPA and BPS mirrors the ranking of these chemicals in both a mouse testicular co-culture model [15] and a mouse spermatogonial cell culture [12,52].

It is known that women are concurrently exposed to multiple potential endocrine disrupters with the potential to affect fertility [53]. The widespread adoption of BPA substitutes raises the concern that simultaneous exposure to BPA along with BPA analogs might lead to additive effects or even heightened synergistic effects. To explore whether each chemical mixture acts additively or whether there are synergistic or antagonistic effects between BPA and its analogs, we performed a series of competitive assays (see Methods) to elucidate whether such effects exist (Fig. 4**B**). Out of 14 BPA analogs, the majority showed additive effects, but two of the BPA analogs – BPE and 17β-estradiol, showed synergistic effects with BPA (Fig. 4C, Figure S4). None showed antagonistic effects.

3.4. Screening environmental chemicals for reproductive effects

Having demonstrated selectivity in our assay based on both known mutants and control compounds, and having shown its ability to detect meiotic defects caused by BPA, it becomes possible to apply this assay widely to measure meiotic effects of other compound classes. We thus applied our assay to an additional 179 chemicals (199 total for the entire study) (Table S1) spanning several environmentally relevant use categories (i.e. fire retardants, pesticides, pharmaceuticals, cosmetics, food additives, plasticizers, tobacco-related chemicals, flavorants, cleaners and industrial chemicals) and chemical classes (i.e. phthalates, per- and polyfluoroalkyl substances (PFAS), quaternary ammonium compounds (QAC), organophosphate esters (OPE)). Figure S4. Competitive Assays for Additive, Synergistic and Antagonistic Effects for BPA vs. BPA substitutes. Sub refers to the BPA substitute in the heading used in the assay. The numbers in the x-axis indicate concentrations used.

The majority of the chemicals were selected from a database that prioritizes chemicals for testing in order to facilitate cross comparisons of different reproductive and development assays [54]. The chemicals included those suspected to negatively impact human health and those of interest to policy makers (e.g. TSCA chemicals, chemicals under consideration for EPA's priority list). Several of these chemicals were detected in maternal and umbilical cord blood and thus are relevant to exposure during early gametogenesis [9,55]. Many chemicals that were toxic in worm and rodent reproductive assays were included (e.g. parathion-methyl [56,57], Bis(2-ethylhexyl) phosphate (DEHP) [58,59], Tris(2-chloroethyl) phosphate (TCEP) [60] and thiabendazole [11].

We designated chemicals that showed $\Delta t_{Hmax} \geq 1.5$ hours (equivalent to 20 % reduction in gamete viability) with a p-value ≤ 0.05 (t-test) as reproductively toxic (reprotox20). Of the total 199 compounds screened, 57 (29 %) compounds were classified as reprotox20 in our assay (Fig. 5, Table S3). We expected to find several reprotox20 compounds since we deliberately included many chemicals known to be reproductive toxicants in other organisms in order to assess the assay's
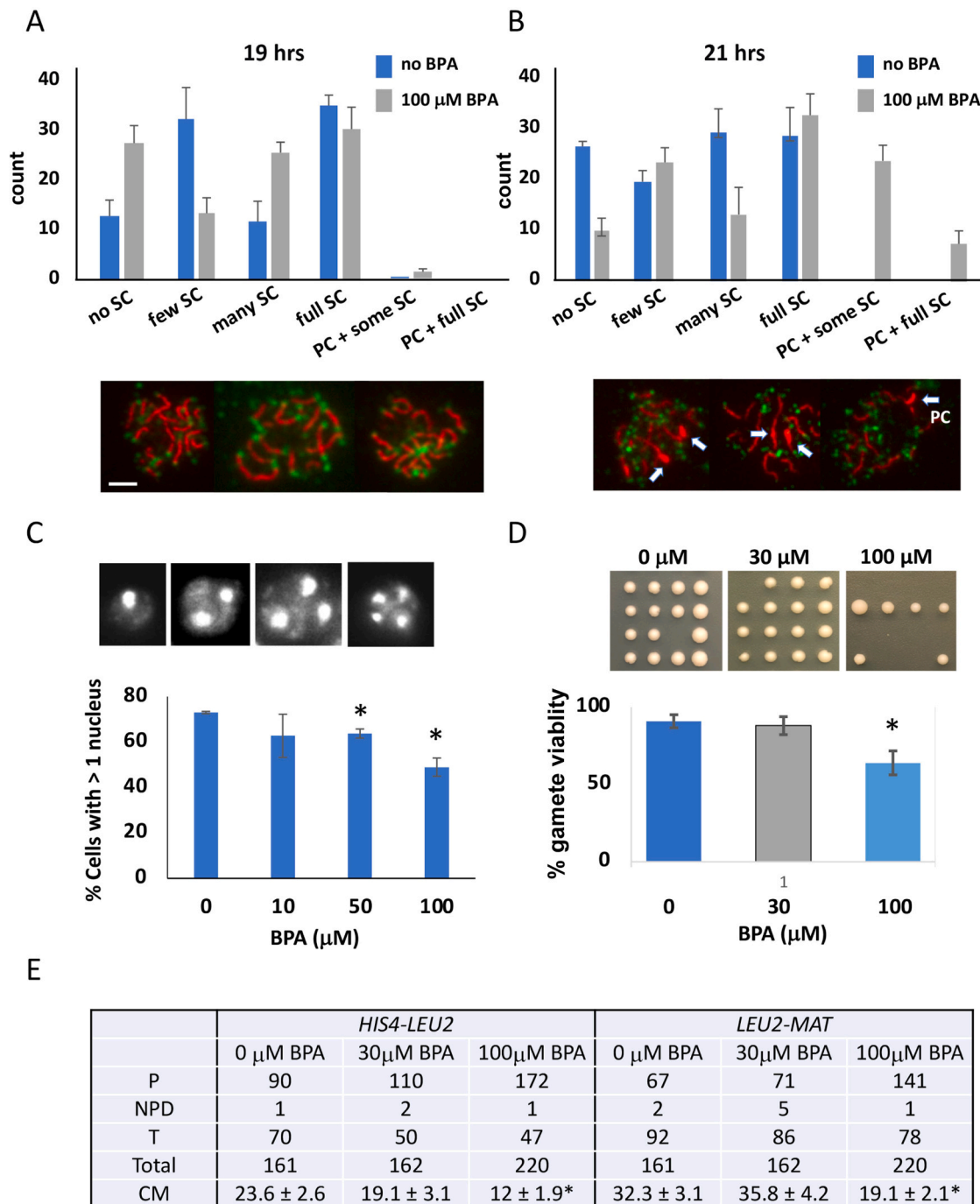
Fig. 3. BPA affects defined aspects of meiosis in yeast. Distribution of extent of synapsis as measured by immunofluorescence staining of chromosome spreads when 100 μM BPA is added during meiosis. A) 19 hours or B) 21 hours after meiotic induction. Synapsis or the incorporation of the synaptonemal complex (SC) was detected using anti-Zip1 antibodies (red) and anti-Rap1 antibodies (green) which highlight chromosome ends. The extent of observed synapsis was classified into groups of no SC, few SC, many SC and full SC. The panels below show example chromosome spreads with full synapsis. The number of spreads having polycomplexes – aggregates of Zip1 protein that occurs when meiotic progression is delayed during prophase I – is shown. 371 chromosome spreads were evaluated for each time course. Each time course was repeated two times and averaged. C) Panel shows the number of DAPI stained nuclei, which indicates whether meiosis I (> 2 nuclei) or meiosis II (>3 or 4 nuclei) has completed. The number of cells with > 1 nucleus indicates the gametogenesis frequency. D) Gamete viability determined by tetrad dissection shown in panel above. Each meiosis normally results in four gametes (tetrad). The tetrads can be dissected and arrayed in a column of four and grow to form colonies. Gamete viability is calculated by determining the percentage of gametes that form colonies. * indicates significant difference (P ≤ 0.05, t-test). E) Levels of genetic recombination can be measured in a strain with distinct genetic markers at the same locus. The strain used to measure recombination is heterozygous for three genetic markers *HIS4/his4*, *LEU2/leu2* and *MATA/MATalpha*. The level of recombination is given in centiMorgans (cM) between two markers either *HIS4* and *LEU2* (*HIS4-LEU2*) or *LEU2-MATA* (*LEU2-MAT*). Based on Mendel's law, the phenotype of the markers can report on the number of recombination events. P – parental configuration of markers (0 recombination events), T – one recombination event occurred, resulting in a tetratype configuration of markers and NPD – non parental ditype represents typically two recombination events occurring between the two markers. The number of meiosis events showing P, T, or NPD configurations of markers is given for two genetic intervals. The level of recombination calculated from the Perkin's formula is given in centimorgans (CM). At 100 μM, we see a significant reduction in recombination levels for both intervals (P ≤ 0.05, chi-square).
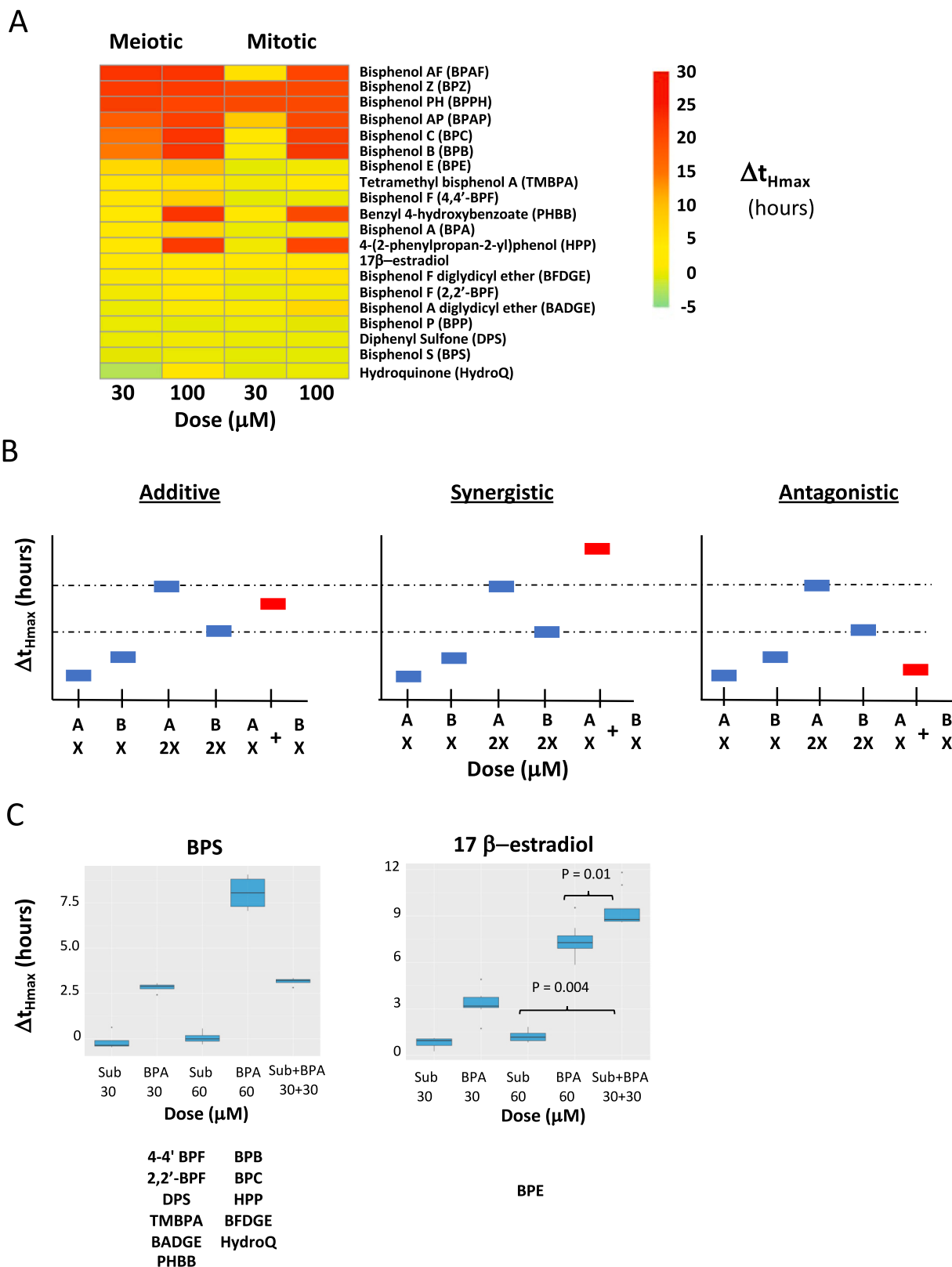
Fig. 4. Relative reproductive toxicity of BPA alternatives. A) Heat map of Δt_{Hmax} values for BPA and 19 BPA substitutes at 30 and 100 μM doses for meiotic and mitotic assay. The chemicals are ranked first by Δt_{Hmax} 30 μM (meiotic) and then by Δt_{Hmax} at 100 μM (meiotic). The values for Δt_{Hmax} are listed in Table S4 in ranked order. B) A schematic diagram of the competition assay for BPA and its substitutes. A mixture of BPA and its substitute (sub) can show a Δt_{Hmax} equivalent to (additive), higher than (synergistic) or lower than (antagonistic) that of double the dose "X" of individual chemicals. A represents BPA and B represents one of the substitutes. Note that the response to a chemical can be nonlinear so that response at dose 2 A will not be 2 times the response at A which is why it is important to measure at 2 A and 2B to obtain the boundaries. C) Actual examples of additive (BPS) and synergistic activity (17 β-estradiol) between BPA and its substitutes. Significance calculated by t-test. Additional chemicals showing additive or synergistic effects with BPA are listed under their respective examples. Graphs showing the data for each substitute with BPA can be found in Fig. S3.
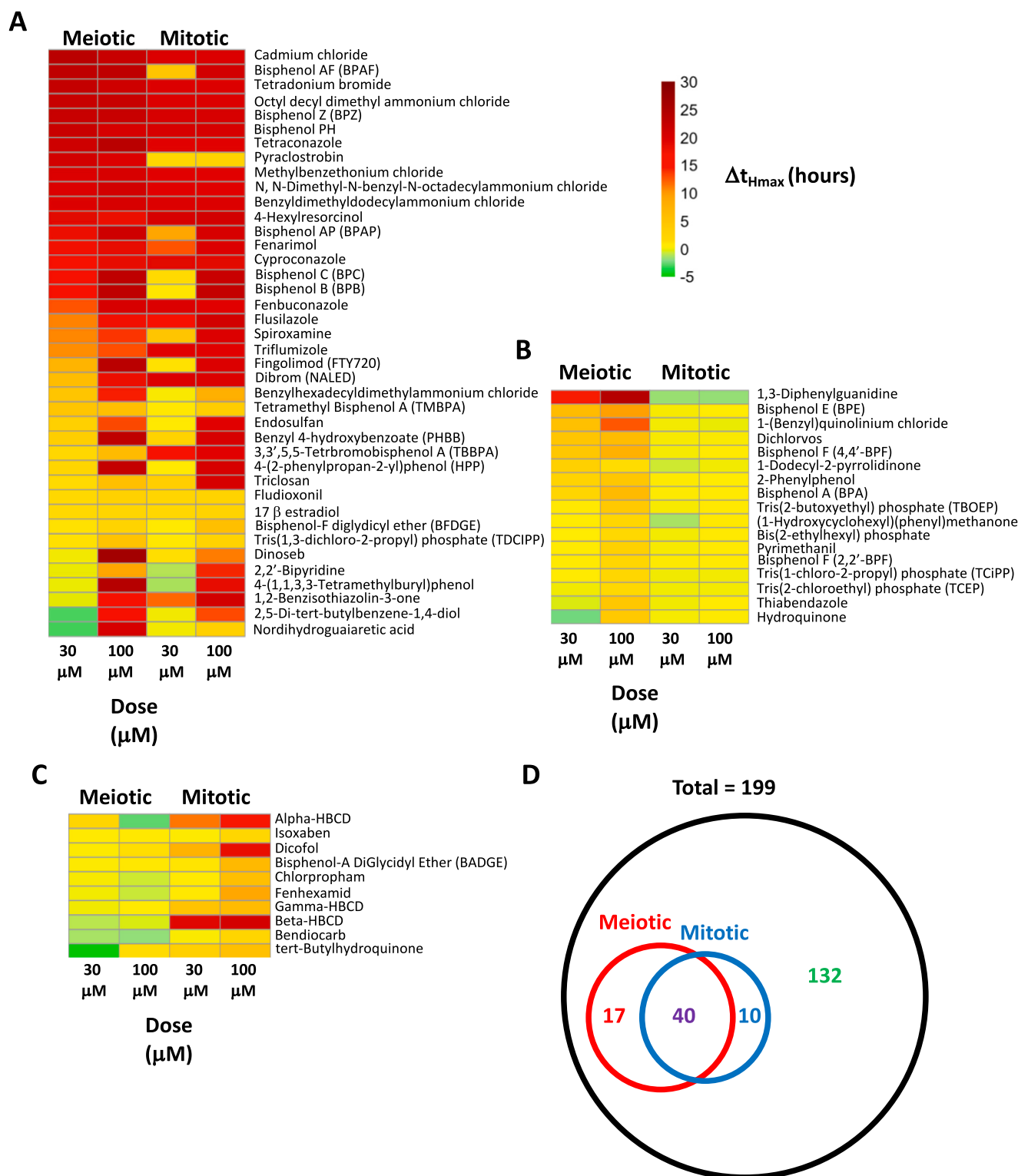
Fig. 5. Reproductive toxicants identified by yeast assay. 199 chemicals were evaluated for both their toxicity for meiosis and for proliferative growth (mitotic) at concentrations of 30 and 100 μM. Chemicals were considered hits if $\Delta t_{Hmax} > 1.5$ hours and p<0.05. A). Heat map of chemicals identified as meiotic as well as mitotic hits. Table S5 lists the Δt_{Hmax} values in ranked order. B) Heatmap of chemicals identified as only affecting meiosis Table S6 lists the Δt_{Hmax} in ranked order. C) Heat map of chemicals that solely affect mitotic growth. Table S7 lists the Δt_{Hmax} in ranked order. D) Venn diagram enumerating the number of hits in each category. The 132 chemicals showing neither a meiotic or mitotic effect are listed in Table S4. Chemicals are ranked according to the meiotic Δt_{Hmax} shifts at a dose of 30 μM.

ability to detect reproductive toxicants common across diverse organisms. We also performed a secondary HTS for diploid proliferative growth using the same chemicals, to distinguish compounds solely affecting meiosis from compounds affecting both meiosis and mitosis.

For the proliferative growth assay, cells were chronically exposed throughout the assay without chemical washout. Fig. 5 illustrates chemicals ranked from highest to lowest severity, with their toxicity categorized as affecting both meiosis and growth (Table S5), meiosis-

specific (Table S6), or growth-specific (Table S7). Out of the 57 reprotox20 compounds, 17 solely affected gametogenesis. Included in the top hits are 1,3 diphenylguanidine, dichlorvos, 2-phenylphenol, bisphenol E (BPE) and 1-(benzyl) quinolinium chloride, 1-dodecyl-2-pyrrolidinone, BPA and decanedioic acid and 1,10-dibutyl ester. Forty other reproductive toxicants showed toxicity for both reproductive and proliferative growth. Only ten of the compounds were designated as toxic to proliferative but not reproductive growth. The remaining 132 compounds did not cause a significant change based on our reprotox20 criteria (Table S3).

3.5. Bisphenol and QAC chemical classes were strongest predictors for reproductive toxicity

Certain chemical classes are of interest as potential hazards to both humans and wildlife due to their persistence in the environment and potential for chemical reactivity. We thus queried whether chemicals within a particular consumer use, chemical class or chemical structure were more likely to correlate with reproductive toxicity. Fig. 6 depicts the distribution of our chemicals within 1) various consumer usage categories (Fig. 6A); 2) chemical classes (Fig. 6B) as defined by EPA's CompTox Chemical Dashboard [61], an extensive searchable database that contains structure, property, toxicity, and bioassay data for collections of chemicals; and 3) chemical structural features as defined by the program ClassyFire, a web-based application for automated chemical structural classification [19], Table S2). In the case of the consumer usage category, each compound can span many classes, however for chemical classes. chemicals belonged to only one chemical category. For chemical structural classification, this information will be useful towards understanding the mode of action of chemicals through possible binding partners as well as form a database from which to base algorithms used to predict the extent of toxicity such as data that informs QSAR [62–64] algorithms.

We used LASSO analysis [65,66] (details described in Text S1) based on logistic regression as a preliminary multivariate analysis to rank the predictors (Fig. 6A, B) . The LASSO analysis is evaluating the relationship between members or "predictors" within either consumer use class, chemical category or chemical structure features with the outcome which is reproductive toxicity. The higher LASSO coefficient indicates higher predictability of that there is an association with reproductive toxicity. For consumer classes, there was no strong association between any specific consumer usage class with reproductive toxicity (Fig. 6A). Among the chemical classes we considered, bisphenol and QAC chemical structures were the strongest predictors for reproductive toxicity (i.e. highest coefficients) (Fig. 6B). Fig. 6C shows that in terms of chemical structural features, bisphenols, organic chloride salts, tetraalkylammonium salts and hydrocarbon derivatives were the highest predictors from the LASSO analysis.

3.6. Yeast and mammalian reproductive toxicants show significant association

One important aim of this study was to see if a yeast-based assay alone or together with other non-animal models could identify reproductive toxicants that would be relevant to mammalian gametogenesis (Fig. 7A). Mammalian endpoints that are typically measured to evaluate reproductive toxicity include animal weight, mortality, organ weight (testes, ovaries, liver and kidney), gonadal somatic index (GSI = gonad/ animal weight), sperm count, fetal adsorption, implantation success, litter size and litter viability, fetal deformation or behavioral change, mating ability and male/female sex ratio. Of these endpoints, gonad weight, GSI and sperm count are likely the most directly relevant measures of perturbations of gametogenesis, whereas litter size, fetal adsorption and implantation success are still relevant but less direct readouts from problems occurring during gametogenesis. The remaining endpoints were considered more distal and were not included here,

allowing us to focus on evaluating reproductive toxicity outcomes most relevant to gametogenesis. Comprehensive PubMed and internet searches were performed on each chemical for mammalian reproductive toxicity information (Table S8). If a chemical resulted in change within our criteria of a relevant reproductive endpoint (see above) at any dose the chemical was considered to show mammalian reproductive toxicity. Out of the 199 chemicals, 153 had publicly accessible data that evaluated mammalian reproductive toxicity (Table S8). Fig. 7B illustrates that 26 chemicals scored as meiotic toxicants in the yeast assay were also deemed reproductive toxicants based on mammalian data. The association between yeast and mammalian reproductive toxicity was considered significant using a 2 ×2 contingency table (P-value ≤ 0.008, two tailed Fisher's exact test) (Fig. 7C). However, a better assessment would be to compare dose response using benchmark dose modeling in which each chemical is tested for a full range of dose response. In the accompanying paper, Varshavsky et al. (2024) performs this assessment using data available in the Toxicity Reference Database (ToxRefDB) which contains highly-curated legacy information from guideline and guideline-like in vivo studies [67]. Together our analyses suggest that yeast is a suitable NAM to assess a subset of mammalian reproductive outcomes.

The nematode *C. elegans* is one invertebrate model that has successfully be used to identify reproductive toxicants as an alternative to mammalian studies. Of 29 chemicals in both yeast and mammals, seven chemicals (bisphenol A, endosulfan, fenarimol, tetraconazole, thiabendazole, triflumizole and triclosan) were also found in the literature to be reproductively toxic in *C. elegans* (Table S8). Since these different organisms differ in terms of development and physiology, but share a common molecular mechanism for gametogenesis, we infer that a shared reproductive effect in all three organisms increases the likelihood that gametogenesis *per se* is affected, rather than other processes involved in reproduction.

3.7. Rapid identification of affected stages of gametogenesis using yeast

Standard yeast assays can quickly evaluate a toxicant's effect on gametogenesis. To determine whether a chemical's effect occurs before the meiotic I division or before the meiotic II division or affects both stages, we monitored the number nuclei during meiotic progression (Fig. 3C) after 100 μM chemical exposure for 16 chemicals that were shown to be meiosis-specific (Fig. 5B). **Figure 8A** illustrates how the distribution of nuclei exposed to each of the 16 chemicals changes as compared to the 0.1 % vehicle control. To determine whether a chemical has an effect that occurs before meiosis I (either not allowing meiotic entry or having a problem during prophase I before the meiosis I division), we compared the ratio of the number cells having more than two nuclei divided by the number of cells having at least one nucleus, which we refer to as >1 n, in DMSO vs. chemical exposure (Table S9). We also assessed whether a problem was present after meiosis I division and before the meiosis II division by doing the same comparison but determining the number of cells that have more than 2 nuclei divided by the number of cells that have 2 or more nuclei, which we refer to as >2 n (Table S9). By using the number of cells with 2 or more nuclei as the denominator, we restrict the analysis of meiosis II defects to those cells that have progressed through meiosis I. We plotted the percentage difference from DMSO of >1 n vs. >2 n to assess relative effects for meiosis I and meiosis II (Fig. 8B). As shown in Fig. 8, certain chemicals have a greater effect before the meiosis I division (e.g. Hydroquinone), whereas other chemicals predominantly have an effect after the meiotic I division (e.g. Bisphenol E). Most chemicals appear to manifest effects both in meiosis I and II. Interestingly, no counts for nuclei for 1-Dodecyl-2-pyrrolidinone could be obtained since the nuclei appeared disintegrated.
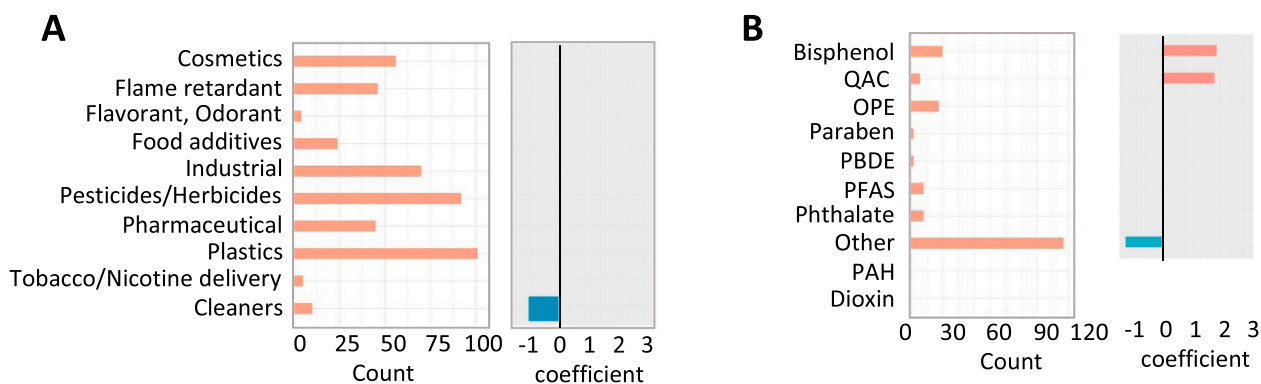
(caption on next page)

Fig. 6. Analysis for predictors of reproductive toxicity. A) Chemical classification based on their commercial use. Plot on the left indicates the number of chemicals in each category (a chemical can have multiple uses). Chemical use categories were used in a LASSO regression model to predict meiotic toxicity (LASSO outcome was whether or not a chemical is a meiotic hit). We used LASSO analysis based on logistic regression as a preliminary multivariate analysis to rank the predictors. R package 'glmnet' was used to generate a linear regression model via penalized maximum likelihood. The glmnet() function was run using parameters alpha=1 (for lasso) and family="binomial" (to indicate binary outcomes). The lambda at which the cross-validated error is within 1 standard error (SE) was used to get the coefficients for the regularized model. The coefficients (at lambda+1 standard error are listed in the plot on the right. The classes showing a higher coefficient is a more likely predictor for reproductive toxicity. The coefficients (at lambda+1SE are listed in the plot on the right. B) Similar analysis for chemical classes (non-overlapping) C) Chemical structure categories determined using the algorithm Classyfire were used in a LASSO regression model to predict meiotic toxicity. Each chemical can have multiple structural elements. Only coefficients >0 are listed in the plot.
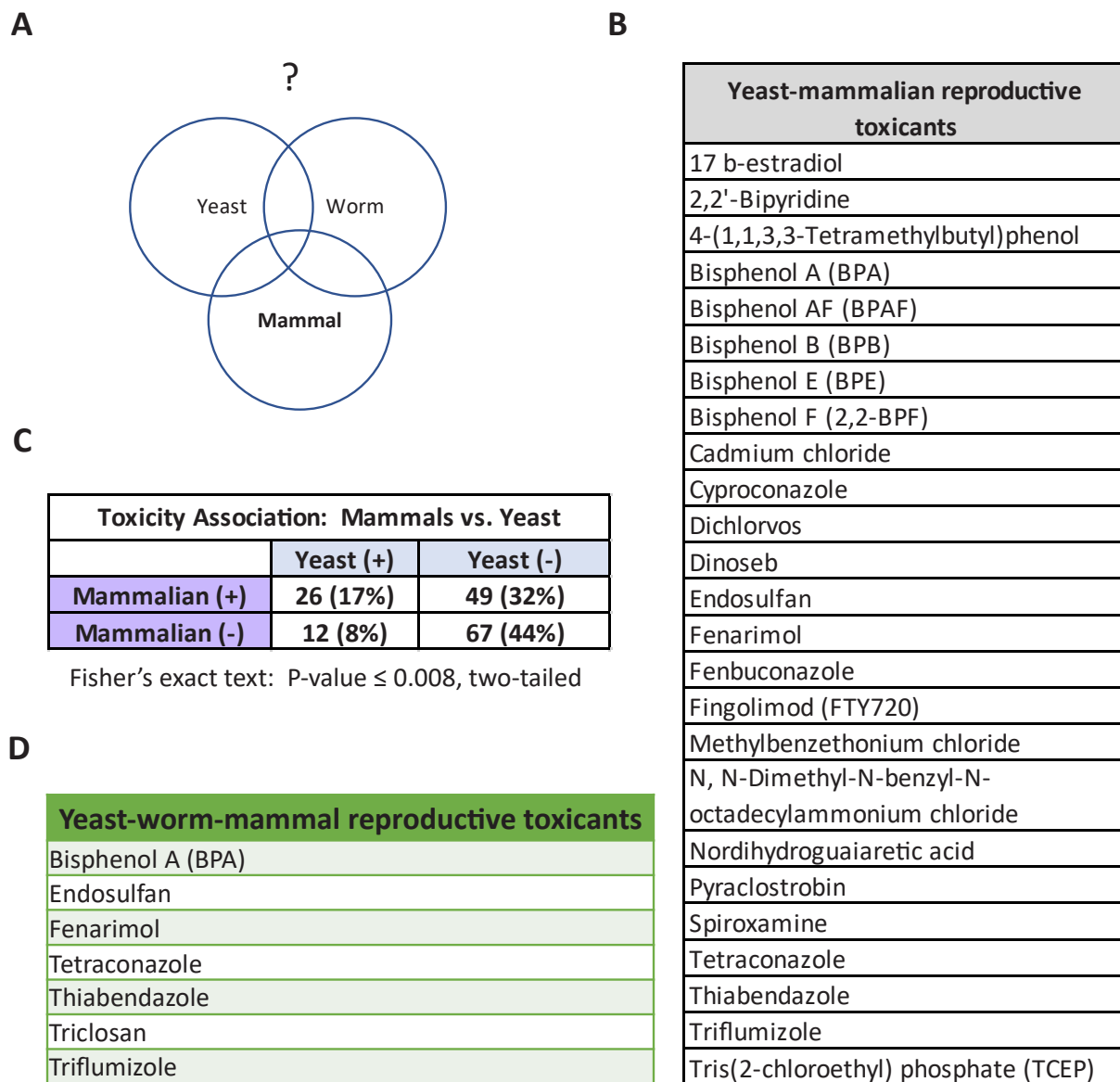
A

?

B

Yeast-mammalian reproductive toxicants
17 b-estradiol
2,2'-Bipyridine
4-(1,1,3,3-Tetramethylbutyl)phenol
Bisphenol A (BPA)
Bisphenol AF (BPAF)
Bisphenol B (BPB)
Bisphenol E (BPE)
Bisphenol F (2,2-BPF)
Cadmium chloride
Cyproconazole
Dichlorvos
Dinoseb
Endosulfan
Fenarimol
Fenbuconazole
Fingolimod (FTY720)
Methylbenzethonium chloride
N, N-Dimethyl-N-benzyl-N-octadecylammonium chloride
Nordihydroguaiaretic acid
Pyraclostrobin
Spiroxamine
Tetraconazole
Thiabendazole
Triflumizole
Tris(2-chloroethyl) phosphate (TCEP)

C

Toxicity Association: Mammals vs. Yeast		
	Yeast (+)	Yeast (-)
Mammalian (+)	26 (17%)	49 (32%)
Mammalian (-)	12 (8%)	67 (44%)

Fisher's exact text: P-value ≤ 0.008, two-tailed

D

Yeast-worm-mammal reproductive toxicants
Bisphenol A (BPA)
Endosulfan
Fenarimol
Tetraconazole
Thiabendazole
Triclosan
Triflumizole

Fig. 7. Assessment of relevance of yeast reproductive toxicants to other organisms. A) Relevance of yeast identified reproductive toxicants to mammalian gametogenesis are more probable if the same chemicals in diverse organisms overlap in their effects. B) List of chemicals that were yeast reproductive toxicants that also show mammalian reproductive toxicity based on literature search for reduction of litter size and viability, reduced gonad weights, reduced sperm count, reduced implantation and increased fetal adsorptions that could result from problems in gametogenesis. C) A Fisher's exact test was used to evaluate whether there was any association between yeast and mammalian toxicants. D) Lists reproductive toxicants that are found in all three organisms.

4. Discussion

4.1. Benefits and considerations of a non-mammalian model for rapid reproductive toxicity assessment

The yeast reproductive assay is a sensitive preliminary step to screen through large libraries of compounds to pinpoint likely reproductive toxicants which can then be further verified in other *in vivo* systems. Multiple models are needed in order to eliminate species specific toxicities that may not be relevant to human health. Thus, combining this assay with information from other model systems will be useful to add specificity for policy and regulatory purposes. Commonality between diverse organisms can provide additional evidence for human toxicity such that further examination of the effect of these chemicals on
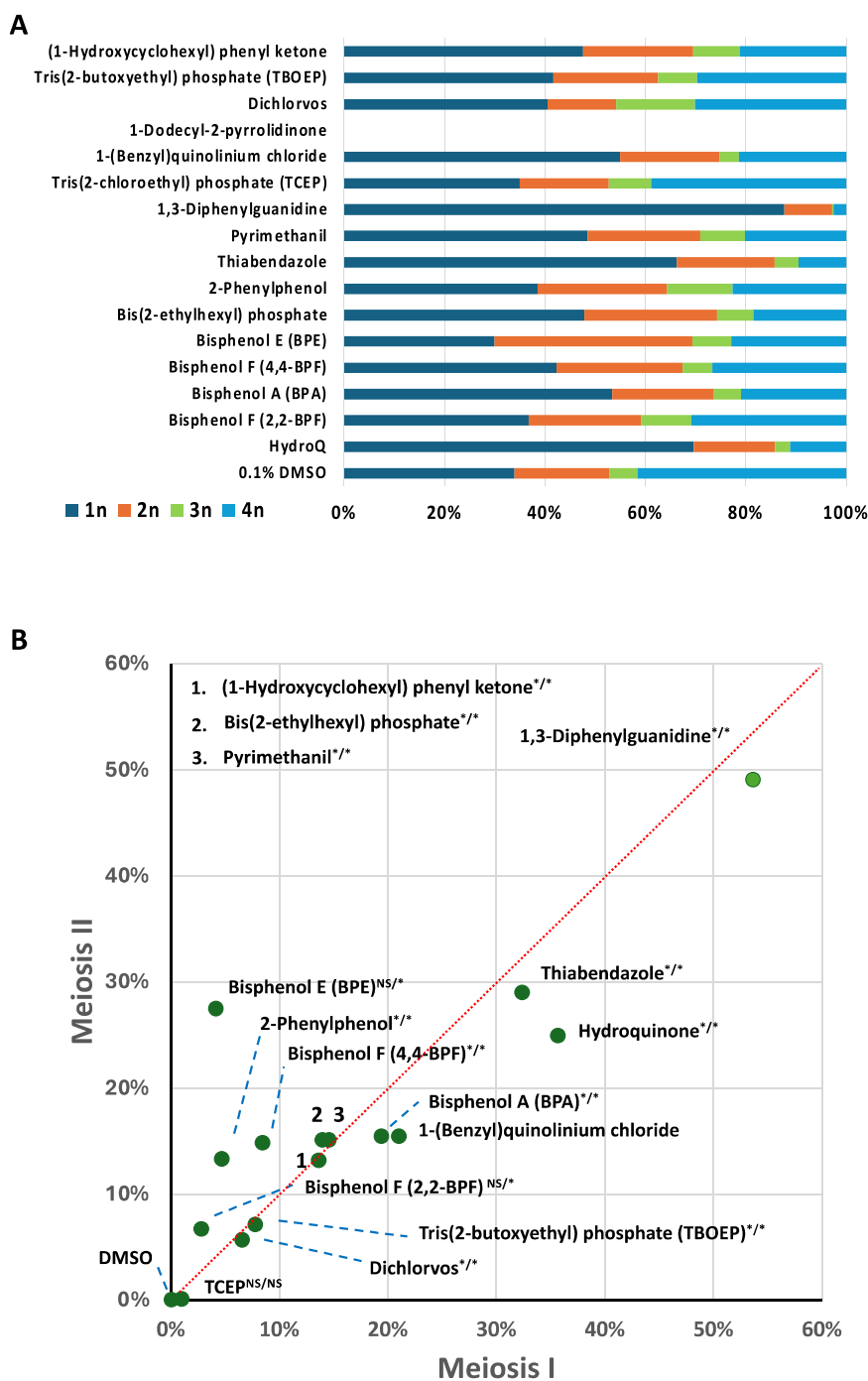
Fig. 8. Evaluation of a chemical's effect on meiotic progression A) Meiotic progression as measured by the number of DAPI stained nuclei (n) after exposure at 100 μM. B) A chemical's effect on meiosis I vs. meiosis II was evaluated by comparing the percentage of nuclei >1 n, indicating the percentage of cells that have exited meiosis I, and the percentage >2 n, indicating exit from meiosis II. These values were subtracted from 0.1 % DMSO only exposure and plotted (data found in Table S9). Significance was evaluated using a test of proportions and indicated by an asterisk (*). NS – nonsignificant. (meiosisI/meiosisII). Red dotted line indicates equal effect in meiosis I and II.

reproduction may be warranted. Our study has revealed seven chemicals: BPA, endosulfan, fenarimol, tetraconazole, thiabendazole, triflumizole and triclosan that exhibit common reproductive toxicity between yeast, nematodes and mammals (Fig. 7D). For two of these chemicals, BPA and triclosan, human studies have been reported which show an association between higher urinary levels of these chemicals and lower antral follicle counts suggestive of reduced fecundity due to chemical exposure [68–70].

As shown in this study, having a rapid assay for reproductive toxicity allows quick evaluation of the relative toxicity of potential analogs and

the effects of compound mixtures. In yeast, we show that the relative toxicities of some well-known BPA analogs are as follows: BPAF > BPF ~ BPA > BPS (Fig. 4A). Except for BPF which was not assayed, a similar ranking was found in mammalian testis and spermatogonial culture [12, 52]. In zebrafish, hatching delay and mortality in embryos were compared for the same BPA analogs [71] and a similar ranking of toxicities was observed: BPAF > BPA > BPF > BPS. However, in *C. elegans* it was observed that BPS is equally if not more toxic than BPA for reproductive and developmental toxicity [72]. In other species including mammals, although BPA, BPF and BPS were compared within single

studies, reproductive outcomes relating closely to gametogenesis were not evaluated (reviewed in McDonough et al. (2021) [73]).

BPA is considered a weak endocrine disruptor and many of its reproductive effects have been attributed to its role as an endocrine mimic. Although BPA has structural features compatible with binding to estrogen receptors, studies suggest that at least a part of BPA's activity may be distinct from estradiol [74–77]. Consistent with this notion, yeast does not have an endocrine system yet shows reproductive defects at the same stage of meiosis as seen in worms and mammals upon exposure to BPA. One potential avenue by which BPA may affect cells is via BPA's inhibition of microtubules [78–80]. Interestingly, thiabendazole another meiosis-specific chemical is another microtubule inhibitor [81] which exhibits slightly greater effects in meiosis I as seen for BPA (Fig. 8**B**).

Though there is a significant association shown in a number of chemicals between yeast and mammals, there are limitations in using yeast to fully capture reproductive toxicity related to mammalian gametogenesis. It is expected that there are meiotic proteins that are specific to yeast and not common to other organisms, as well as the reverse case, where there are human specific proteins that are not found in other organisms. Furthermore, the yeast system is not useful in elucidating any effect of chemicals on animal hormonal systems such as the endocrine system, which is important in signaling timely progression of the various steps of gametogenesis in mammalian systems. In yeast, toxic metabolites derived from non-toxic precursors in animals may be missed due to lack of chemical conversion in yeast that occurs in other organisms such as in the liver; however, metabolites, once known, can be directly tested in the yeast system. More human relevant metabolites of chemicals can be generated either by rat S9 liver fractions [82] or through heterologous expression of cytochrome P450s [83,84] which constitute the basis of metabolic processing in the liver. Another area that yeast is not useful are in sex-specific difference in gametogenesis. Although yeast exhibit MATa and MATalpha mating types, it is not equivalent to female vs male germline specification. Yeast is also more like male gametogenesis, which is continuous once its initiates, which is unlike female gametogenesis that pauses before the meiosis I division and also at the meiosis II division, thus chemicals that affect human female control of meiotic progression would be missed in yeast. Lastly, the accessibility of chemicals in yeast and human germ cells may be different for certain types of chemicals so that something toxic to the human germline may not be toxic to yeast due to the inability to enter into the yeast cell thus doses will likely be different to see an effect. Given the limitations of a single organism, combining information from many different species amenable to rapid assessment will be the most informative to human gametogenesis since commonality of reproductive toxicity across diverse species would suggest that the chemical hits a conserved process and thus will be more likely to be relevant to a wide range of organisms including humans.

5. Conclusion

In conclusion, this study illustrates advantages and disadvantages of using yeast to evaluate a chemical's impact on a complex biological process such as gametogenesis which is difficult to assess in mammals given that, at present, there is no mammalian *in vitro* culture that fully captures meiosis. The principal advantages are the ability to rapidly screen large numbers of chemicals at different doses, the ability to easily test many combinations of chemicals to assess synergistic effects, and the availability of quick and easy follow-up assays to narrow down the stage of meiosis affected by each chemical. The principal disadvantage is that this assay focuses strictly on gametogenesis and cannot detect chemicals with endocrine or other reproductive affects. A key conclusion is that our yeast based assay is in fact able to detect many reproductive toxicants with shared effects in humans, mammals, and C. elegans, indicating that the system will allow a rapid exploration of the vast number of currently untested environmental and industrial compounds that have to date gone untested due to the high cost of meiotic toxicology testing in mice. We were able to detect a number of new potential reproductive toxicants and categorize them into those that affect meiosis I versus meiosis II, which has implications for their mechanism of action as well as for how the timing of exposure might differentially affect reproductive health. This study not only offers insights on the impact of these chemicals on yeast gametogenesis, potentially opening a path to a chemical genetic approach that would synergize with the current extensive genetic analysis of yeast meiosis, but also provides a strategy for the prioritization of these chemicals for further study of reproductive toxicity in rodent models and in humans.

CRediT authorship contribution statement

Matthew de Cruz: Investigation. **Rafael Verduzco:** Investigation. **Beth Rockmill:** Writing – review & editing. **Patrick Allard:** Writing – review & editing. **Ravinder Kumar:** Investigation. **Tracey J. Woodruff:** Writing – review & editing, Funding acquisition. **Ashwini Oke:** Writing – review & editing, Software, Investigation, Formal analysis. **Juleen Lam:** Investigation. **Joshua F. Robinson:** Writing – review & editing. **Jennifer Fung:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dimitri P. Abrahamsson:** Investigation. **Julia Varshavsky:** Investigation. **Anura Shodhan:** Investigation. **Xavier Woodruff-Madeira:** Investigation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Jennifer Fung reports financial support was provided by National Institute of Health. Tracey Woodruff, Patrick Allard, Juleen Lam reports was provided by National Institutes of Health. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

We have deposited our raw data in Dryad and will be released upon publication

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.reprotox.2024.108630.

REFERENCES

[1] M.G. Hull, C.M. Glazener, N.J. Kelly, D.I. Conway, P.A. Foster, R.A. Hinton, et al., Population study of causes, treatment, and outcome of infertility, BMJ 291 (6510) (1985 Dec 14) 1693–1697.

[2] T. Hassold, P. Hunt, To err (meiotically) is human: the genesis of human aneuploidy, Nat. Rev. Genet 2 (4) (2001 Apr) 280–291.

[3] S.I. Nagaoka, T.J. Hassold, P.A. Hunt, Human aneuploidy: mechanisms and new insights into an age-old problem, Nat. Rev. Genet 13 (7) (2012 Jul) 493–504.

[4] A. Jorgensen, T. Svingen, H. Miles, T. Chetty, J.B. Stukenborg, R.T. Mitchell, Environmental Impacts on Male Reproductive Development: Lessons from Experimental Models, Horm. Res Paediatr. [Internet] (2021). ⟨https://www.karger.com/Article/FullText/519964⟩. Oct 4 [cited 2022 May 7]; Available from.

[5] R.G. Lea, A.S. Byers, R.N. Sumner, S.M. Rhind, Z. Zhang, S.L. Freeman, et al., Environmental chemicals impact dog semen quality in vitro and may be associated with a temporal decline in sperm motility and increased cryptorchidism, Sci. Rep. 6 (1) (2016 Aug 25) 31281.

[6] N.E. Skakkebæk, R. Lindahl-Jacobsen, H. Levine, A.M. Andersson, N. Jørgensen, K. M. Main, et al., Environmental factors in declining human fertility, Nat. Rev. Endocrinol. 18 (3) (2022 Mar) 139–157.

[7] G.C. Di Renzo, J.A. Conry, J. Blake, M.S. DeFrancesco, N. DeNicola, J.N. Martin, et al., International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals, Int. J. Gynecol. Obstet. 131 (3) (2015 Dec) 219–225.

[8] D. Panagopoulos Abrahamsson, A. Wang, T. Jiang, M. Wang, A. Siddharth, R. Morello-Frosch, et al., A Comprehensive Non-targeted Analysis Study of the Prenatal Exposome, Environ. Sci. Technol. 55 (15) (2021 Aug 3) 10542–10557.

[9] A. Wang, D.P. Abrahamsson, T. Jiang, M. Wang, R. Morello-Frosch, J.S. Park, et al., Suspect Screening, Prioritization, and Confirmation of Environmental Chemicals in Maternal-Newborn Pairs from San Francisco, Environ. Sci. Technol. 55 (8) (2021 Apr 20) 5037–5049.

[10] P. Allard, N.C. Kleinstreuer, T.B. Knudsen, M.P. Colaiácovo, A C. elegans screening platform for the rapid assessment of chemical disruption of germline function, Environ. Health Perspect. 121 (6) (2013 Jun) 717–724.

[11] Shin N., Cuenca L., Karthikraj R., Kannan K., Colaiácovo M.P. Assessing effects of germline exposure to environmental toxicants by high-throughput screening in C. elegans. Copenhaver GP, editor. PLoS Genet. 2019 Feb 14;15(2):e1007975.

[12] L. Yin, H. Wei, S. Liang, X. Yu, From the cover: an animal-free in vitro three-dimensional testicular cell coculture model for evaluating male reproductive toxicants, Toxicol. Sci. 159 (2) (2017 Oct 1) 307–326.

[13] Q. Zhou, M. Wang, Y. Yuan, X. Wang, R. Fu, H. Wan, et al., Complete meiosis from embryonic stem cell-derived germ cells in vitro, Cell Stem Cell 18 (3) (2016 Mar) 330–340.

[14] C.A. Easley, J.M. Bradner, A. Moser, C.A. Rickman, Z.T. McEachin, M.M. Merritt, et al., Assessing reproductive toxicity of two environmental toxicants with a novel in vitro human spermatogenic model, Stem Cell Res. 14 (3) (2015 May) 347–355.

[15] L. Yin, J.S. Siracusa, E. Measel, X. Guan, C. Edenfield, S. Liang, et al., High-content image-based single-cell phenotypic analysis for the testicular toxicity prediction induced by bisphenol a and its analogs Bisphenol S, Bisphenol AF, and Tetrabromobisphenol A in A Three-dimensional Testicular Cell Co-culture Model, Toxicol. Sci. 173 (2) (2020 Feb 1) 313–335.

[16] T.A. Peterson, D. Park, M.G. Kann, A protein domain-centric approach for the comparative analysis of human and yeast phenotypically relevant mutations, BMC Genom. 14 (S3) (2013 May) S5.

[17] G.S. Roeder, Sex and the single cell: meiosis in yeast, Proc. Natl. Acad. Sci. USA 92 (23) (1995 Nov 7) 10450–10456.

[18] B. Rockmill, G.S. Roeder, Telomere-mediated chromosome pairing during meiosis in budding yeast, Genes Dev. 12 (16) (1998 Aug 15) 2574–2586.

[19] Y. Djoumbou Feunang, R. Eisner, C. Knox, L. Chepelev, J. Hastings, G. Owen, et al., ClassyFire: automated chemical classification with a comprehensive, computable taxonomy, J. Chemin.-. 8 (1) (2016 Dec) 61.

[20] D.F. Tardiff, M.L. Tucci, K.A. Caldwell, G.A. Caldwell, S. Lindquist, Different 8-hydroxyquinolines protect models of TDP-43 protein, α-synuclein, and polyglutamine proteotoxicity through distinct mechanisms, J. Biol. Chem. 287 (6) (2012 Feb) 4107–4120.

[21] D.F. Tardiff, N.T. Jui, V. Khurana, M.A. Tambe, M.L. Thompson, C.Y. Chung, et al., Yeast Reveal a "Druggable" Rsp5/Nedd4 Network that Ameliorates α-Synuclein Toxicity in Neurons, Science 342 (6161) (2013 Nov 22) 979–983.

[22] C. Cornet, S. Calzolari, R. Miñana-Prieto, S. Dyballa, E. van Doornmalen, H. Rutjes, et al., ZeGlobalTox: an innovative approach to address organ drug toxicity using zebrafish, IJMS 18 (4) (2017 Apr 19) 864.

[23] D.W. Ferreira, P. Allard, Models of germ cell development and their application for toxicity studies: models of Germ Cell Development, Environ. Mol. Mutagen 56 (8) (2015 Oct) 637–649.

[24] P. McGrath, C.Q. Li, Zebrafish: a predictive model for assessing drug-induced toxicity, Drug Discov. Today 13 (9–10) (2008 May) 394–401.

[25] J. Varshavsky, J. Lam, C. Cooper, P. Allard, J.C. Fung, A. Oke, et al., Analyzing high-throughput assay data to advance the rapid screening of environmental chemicals for human reproductive toxicity, Reprod. Toxicol. (Submitt.) (2024 Mar 29).

[26] Ritz C., Baty F., Streibig J.C., Gerhard D. Dose-Response Analysis Using R. Xia Y, editor. PLoS ONE. 2015 Dec 30;10(12):e0146021.

[27] Keeney S., editor. Chromosome spreading and immunofluorescence methods in Saccharomyes cerevisiae. In: Meiosis [Internet]. Totowa, NJ: Humana Press; 2009 [cited 2022 May 7]. p. 3–13. (Methods in Molecular Biology; vol. 558). Available from: http://link.springer.com/10.1007/978-1-60761-103-5.

[28] A.J. MacQueen, G.S. Roeder, Fpr3 and Zip3 Ensure that Initiation of Meiotic Recombination Precedes Chromosome Synapsis in Budding Yeast, Curr. Biol. 19 (18) (2009) 1519–1526.

[29] Guthrie, Christine, Fink, Gerald Guide to yeast genetics and molecular biology. 863 p. (Methods Enzymol; vol. 194).

[30] D.D. Perkins, Biochemica Mutants in the Smut Fungus Ustilago maydis, Genetics 34 (5) (1949 Sep 1) 607–626.

[31] T.C. Chou, P. Talalay, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors, Adv. Enzym. Regul. 22 (1984 Jan) 27–55.

[32] Vizeacoumar F.J., Freywald A., editors. Chemical-Genetic Interactions as a Means to Characterize Drug Synergy. In: Mapping Genetic Interactions [Internet]. New York, NY: Springer US; 2021 [cited 2022 May 7]. p. 243–63. (Methods in Molecular Biology; vol. 2381). Available from: https://link.springer.com/10.1007/978-1-0716-1740-3.

[33] S. Loewe, The problem of synergism and antagonism of combined drugs, Arzneimittelforsch 3 (6) (1953) 285–290.

[34] T. Delaveau, A. Delahodde, E. Carvajal, J. Subik, C. Jacq, PDR3, a new yeast regulatory gene, is homologous toPDR1 and controls the multidrug resistance phenomenon, Molec Gen. Genet 244 (5) (1994 Sep) 501–511.

[35] B. Wolska-Mitaszko, T. Jakubowicz, T. Kucharzewska, E. Gąsior, An efficient technique for the isolation of yeast spores and the preparation of spheroplast lysates active in protein synthesis, Anal. Biochem. 116 (2) (1981 Sep) 241–247.

[36] S. Keeney, C.N. Giroux, N. Kleckner, Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family, Cell 88 (3) (1997 Feb 7) 375–384.

[37] Petra Ross-Macdonald, G.Shirleen Roeder, Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch repair, Cell 79 (6) (2016) 1069–1080. Dec 16.

[38] B. Rockmill, P. Lefrançois, K. Voelkel-Meiman, A. Oke, G.S. Roeder, J.C. Fung, High throughput sequencing reveals alterations in the recombination signatures with diminishing Spo11 activity, in: M. Lichten (Ed.), PLoS Genet, 9, 2013 Oct 31 e1003932.

[39] S.Y. Chen, T. Tsubouchi, B. Rockmill, J.S. Sandler, D.R. Richards, G. Vader, et al., Global analysis of the meiotic crossover landscape, Dev. Cell 15 (3) (2008 Sep) 401–415.

[40] J.H. Zhang, T.D.Y. Chung, K.R. Oldenburg, A simple statistical parameter for use in evaluation and validation of high throughput screening assays, SLAS Discov. 4 (2) (1999 Apr) 67–73.

[41] R. Koszul, K.P. Kim, M. Prentiss, N. Kleckner, S. Kameoka, Meiotic chromosomes move by linkage to dynamic actin cables with transduction of force through the nuclear envelope, Cell 133 (7) (2008) 1188–1201.

[42] M. Susiarjo, T.J. Hassold, E. Freeman, P.A. Hunt, Bisphenol A exposure in utero disrupts early oogenesis in the mouse, PLoS Genet 3 (1) (2007) e5.

[43] Vrooman L.A., Oatley J.M., Griswold J.E., Hassold T.J., Hunt P.A. Estrogenic Exposure Alters the Spermatogonial Stem Cells in the Developing Testis, Permanently Reducing Crossover Levels in the Adult. Hawley RS, editor. PLoS Genet. 2015 Jan 23;11(1):e1004949.

[44] R.R. Gerona, J. Pan, A.R. Zota, J.M. Schwartz, M. Friesen, J.A. Taylor, et al., Direct measurement of Bisphenol A (BPA), BPA glucuronide and BPA sulfate in a diverse and low-income population of pregnant women reveals high exposure, with potential implications for previous exposure estimates: a cross-sectional study, Environ. Health 15 (1) (2016 Dec) 50.

[45] Z. Li, W. Mao, L. Yao, N. Zhao, Y. Zhang, M. Zhao, et al., First report on occurrence of bisphenol A isomers in human serum and whole blood, J. Hazard. Mater. 424 (2022 Feb) 127549.

[46] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons, Human exposure to bisphenol A (BPA), Reprod. Toxicol. 24 (2) (2007 Aug) 139–177.

[47] C.J. Catenza, A. Farooq, N.S. Shubear, K.K. Donkor, A targeted review on fate, occurrence, risk and health implications of bisphenol analogues, Chemosphere 268 (2021 Apr) 129273.

[48] P. Allard, M.P. Colaiácovo, Bisphenol A impairs the double-strand break repair machinery in the germline and causes chromosome abnormalities, Proc. Natl. Acad. Sci. USA 107 (47) (2010 Nov 23) 20405–20410.

[49] M. Sym, G. Shirleen, Zipl-inducedchangesin synaptonemalcomplexstructure and l lycomplex assembly, J. Cell Biol. 128 (1995) 12.

[50] Pelch K.E., Wignall J.A., Goldstone A.E., Ross P.K., Blain R.B., Shapiro A.J., et al. NTP Research Report on Biological Activity of Bisphenol A (BPA) Structural Analogues and Functional Alternatives: Research Report 4. Research Triangle Park (NC): National Toxicology Program; 2017.

[51] K. Pelch, J.A. Wignall, A.E. Goldstone, P.K. Ross, R.B. Blain, A.J. Shapiro, et al., A scoping review of the health and toxicological activity of bisphenol A (BPA) structural analogues and functional alternatives, Toxicology 424 (2019 Aug) 152235.

[52] S. Liang, L. Yin, K. Shengyang Yu, M.C. Hofmann, X. Yu, High-content analysis provides mechanistic insights into the testicular toxicity of bisphenol a and selected analogues in mouse spermatogonial cells, Toxicol. Sci. 155 (1) (2017 Jan) 43–60.

[53] L. Mínguez-Alarcón, C. Messerlian, A. Bellavia, A.J. Gaskins, Y.H. Chiu, J.B. Ford, et al., Urinary concentrations of bisphenol A, parabens and phthalate metabolite mixtures in relation to reproductive success among women undergoing in vitro fertilization, Environ. Int. 126 (2019 May) 355–362.

[54] Abrahamsson, Dimitri, Robinson, Joshua F., Lam, Juleen, Varshavsky, Julia, Fung, Jennifer C., Allard, Patrick, et al. Identifying chemical categories for testing in in vivo / in vitro systems for reproductive and developmental effects. Zenodo [Internet]. 2021 Dec 6; Available from: https://zenodo.org/record/5760170#.YndHGZLMKxR.

[55] A. Wang, R.R. Gerona, J.M. Schwartz, T. Lin, M. Sirota, R. Morello-Frosch, et al., A Suspect screening method for characterizing multiple chemical exposures among a demographically diverse population of pregnant women in San Francisco, Environ. Health Perspect. 126 (7) (2018 Jul) 077009.

[56] K. Narayana, N. Prashanthi, A. Nayanatara, H.H.C. Kumar, K. Abhilash, K.L. Bairy, Neonatal methyl parathion exposure affects the growth and functions of the male reproductive system in the adult rat, Folia Morphol. 65 (1) (2006) 8.

[57] M. Uzunhisarcikli, Y. Kalender, K. Dirican, S. Kalender, A. Ogutcu, F. Buyukkomurcu, Acute, subacute and subchronic administration of methyl parathion-induced testicular damage in male rats and protective role of vitamins C and E, Pestic. Biochem. Physiol. 87 (2) (2007 Feb) 115–122.

[58] E. Fabjan, E. Hulzebos, W. Mennes, A.H. Piersma, A category approach for reproductive effects of phthalates, Crit. Rev. Toxicol. 36 (9) (2006 Jan) 695–726.

[59] Foster PMD, Mylchreest E., Gaido K.W., Sar M. Effects of phthalate esters on the developing reproductive tract of male rats.:5.

[60] Gulati, D.K., Bairnes, L.H., Chapin, R.E., Heindel, J. Final report on the reproductive toxicity of tris(2-chloroethyl)phosphate reproduction and fertility assessment in Swiss CD-1 mice when administered via gavage. National Toxicology Program NIEHS; 1991.

[61] A.J. Williams, C.M. Grulke, J. Edwards, A.D. McEachran, K. Mansouri, N.C. Baker, et al., The CompTox Chemistry Dashboard: a community data resource for environmental chemistry, J. Chemin.-. 9 (1) (2017 Dec) 61.

[62] Y. Low, T. Uehara, Y. Minowa, H. Yamada, Y. Ohno, T. Urushidani, et al., Predicting Drug-Induced Hepatotoxicity Using QSAR and Toxicogenomics Approaches, Chem. Res Toxicol. 24 (8) (2011 Aug 15) 1251–1262.

[63] G. Gini, QSAR: What Else? Methods Mol. Biol. 1800 (2018) 79–105.

[64] B.D. Gute, G.D. Grunwald, D. Mills, S.C. Basak, Molecular similarity based estimation of properties: a comparison of structure spaces and property spaces, SAR QSAR Environ. Res. 11 (5–6) (2001 Feb 1) 363–382.

[65] E.W. Steyerberg, M.J.C. Eijkemans, F.E. Harrell, J.D.F. Habbema, Prognostic modeling with logistic regression analysis: in search of a sensible strategy in small data sets, Med Decis. Mak. 21 (1) (2001 Feb) 45–56.

[66] E. Vittinghoff, C.E. McCulloch, Relaxing the Rule of Ten Events per Variable in Logistic and Cox Regression, Am. J. Epidemiol. 165 (6) (2007 Jan 12) 710–718.

[67] S. Watford, L. Ly Pham, J. Wignall, R. Shin, M.T. Martin, K.P. Friedman, ToxRefDB version 2.0: Improved utility for predictive and retrospective toxicology analyses, Reprod. Toxicol. 89 (2019 Oct) 145–158.

[68] J. Jurewicz, B. Wielgomas, M. Radwan, A. Karwacka, A. Klimowska, E. Dziewirska, et al., Triclosan exposure and ovarian reserve, Reprod. Toxicol. 89 (2019 Oct) 168–172.

[69] I. Souter, K.W. Smith, I. Dimitriadis, S. Ehrlich, P.L. Williams, A.M. Calafat, et al., The association of bisphenol-A urinary concentrations with antral follicle counts and other measures of ovarian reserve in women undergoing infertility treatments, Reprod. Toxicol. 42 (2013 Dec) 224–231.

[70] W. Zhu, W. Zhou, X. Huo, S. Zhao, Y. Gan, B. Wang, et al., Triclosan and Female Reproductive Health: A Preconceptional Cohort Study, Epidemiology 30 (2019 Jul) S24–S31.

[71] J. Moreman, O. Lee, M. Trznadel, A. David, T. Kudoh, C.R. Tyler, Acute toxicity, teratogenic, and estrogenic effects of bisphenol a and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish embryo-larvae, Environ. Sci. Technol. 51 (21) (2017 Nov 7) 12796–12805.

[72] Y. Chen, L. Shu, Z. Qiu, D.Y. Lee, S.J. Settle, S. Que Hee, et al., Exposure to the BPA-Substitute Bisphenol S Causes Unique Alterations of Germline Function, in: P. E. Cohen (Ed.), PLoS Genet, 12, 2016 Jul 29 e1006223.

[73] C.M. McDonough, H.S. Xu, T.L. Guo, Toxicity of bisphenol analogues on the reproductive, nervous, and immune systems, and their relationships to gut microbiome and metabolism: insights from a multi-species comparison, Crit. Rev. Toxicol. 51 (4) (2021 Apr 21) 283–300.

[74] Y. Chen, B. Panter, A. Hussain, K. Gibbs, D. Ferreira, P. Allard, BPA interferes with StAR-mediated mitochondrial cholesterol transport to induce germline dysfunctions, Reprod. Toxicol. 90 (2019 Dec) 24–32.

[75] J.C. Gould, L.S. Leonard, S.C. Maness, B.L. Wagner, K. Conner, T. Zacharewski, et al., Bisphenol A interacts with the estrogen receptor h in a distinct manner from estradiol, Mol. Cell. Endocrinol. 12 (1998).

[76] J. Peretz, Z.R. Craig, J.A. Flaws, Bisphenol A inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic pathway. Biol Reprod 87 (3) (2012 Sep) 63.

[77] A. Ziv-Gal, Z.R. Craig, W. Wang, J.A. Flaws, Bisphenol A inhibits cultured mouse ovarian follicle growth partially via the aryl hydrocarbon receptor signaling pathway, Reprod. Toxicol. 42 (2013 Dec) 58–67.

[78] O. George, B.K. Bryant, R. Chinnasamy, C. Corona, J.B. Arterburn, C.B. Shuster, Bisphenol A directly targets tubulin to disrupt spindle organization in embryonic and somatic cells, ACS Chem. Biol. 3 (3) (2008 Mar 20) 167–179.

[79] I.D.S. Adamakis, E. Panteris, E.P. Eleftheriou, Bisphenol A disrupts microtubules and induces multipolar spindles in dividing root tip cells of the gymnosperm Abies cephalonica, Chemosphere 149 (2016 Apr) 202–210.

[80] L. Lehmann, M. Metzler, Bisphenol A and its methylated congeners inhibit growth and interfere with microtubules in human fibroblasts in vitro, Chem. Biol. Inter. 147 (3) (2004 Apr 15) 273–285.

[81] K.E. Sawin, P. Nurse, Regulation of cell polarity by microtubules in fission yeast, J. Cell Biol. 142 (2) (1998 Jul 27) 457–471.

[82] M. Ooka, C. Lynch, M. Xia, Application of in vitro metabolism activation in high-throughput screening, Int J. Mol. Sci. 21 (21) (2020 Oct 31).

[83] L. Jiang, L. Huang, J. Cai, Z. Xu, J. Lian, Functional expression of eukaryotic cytochrome P450s in yeast, Biotechnol. Bioeng. 118 (3) (2021 Mar) 1050–1065.

[84] J.J. Stegeman, L. Behrendt, B.R. Woodin, A. Kubota, B. Lemaire, D. Pompon, et al., Functional characterization of zebrafish cytochrome P450 1 family proteins expressed in yeast, Biochim Biophys. Acta 1850 (11) (2015 Nov) 2340–2352.