

Supporting Information

Appendix II.

A Framework for Identifying Chemicals with Structural Features Associated with Potential to Act as Developmental or Reproductive Toxicants

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Application of the decision tree to example chemicals

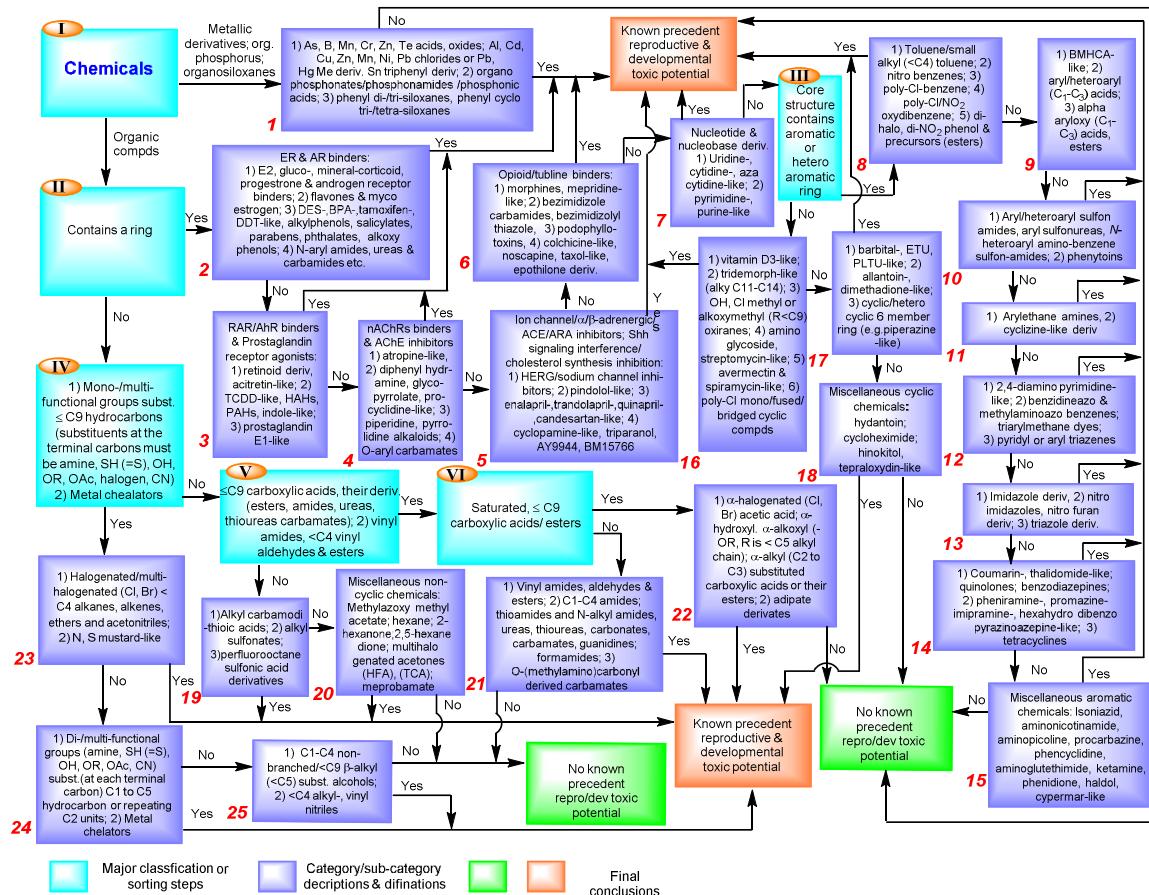


Fig. S1. Overall process of the decision tree for screening developmental/reproductive toxicity

The Overall process of the decision tree for screening developmental/reproductive toxicity is shown in the Figure S1. In applying the decision tree, it is important to note that while there is detailed structural information for the chemicals used to derive each group, the structures shown are intended to be used as guidelines to demonstrate the types of chemicals associated with DART activity. They are not intended to be rigid rules to which a chemical being assessed must completely conform. We used six chemicals (Figure S2) to demonstrate the procedure of the decision tree.

Example 1: ethylene glycol methyl ether (CAS#109-80-4) is identified as a chemical with features in common with known DART toxicants via the following steps of the decision tree: Step I; Organic compound, to II; No (*does not contain a cyclic ring*), to IV; Yes (*contains terminal methoxy groups and < C9 chain length*) to 23; No (*No halogenated <C4 ether*) to 24; Yes (*methoxy group at each terminal carbon*) to known precedent for DART.

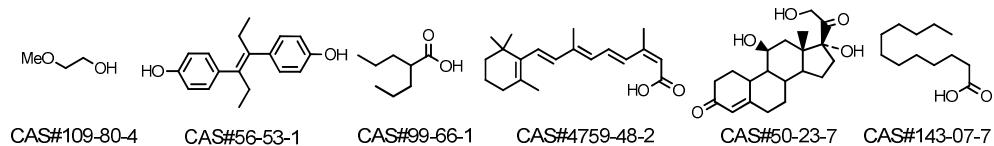
Example 2: dihydrodiethylstilbestrol (CAS#56-53-1) is identified as a chemical with features in common with known DART toxicants via the following steps of the decision tree: Step I; Organic Compound, to II; Yes (*contains a cyclic ring*), to 3; Yes (*belongs to active estrogen receptor (ER) binder (DES-like derivative)*), to known precedent for DART.

Example 3: 2-propylpentanoic acid (CAS#99-66-1) is identified as a chemical with features in common with known DART toxicants via the following steps of the decision tree: Step I; Organic compound, to II; No (*does not contain a cyclic ring*), to IV; No (*contains a carboxylic acid substituent*), to V, Yes (< C9 carboxylic acid) , to VI; Yes (*belongs to saturated < C9 carboxylic acids/esters*), to 22; Yes (*belongs to alpha-alkyl (C2 to C3) substituted carboxylic acids/esters*), to known precedent for DART.

Example 4: (2Z,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenoic acid (a retinoid derivative) (CAS#4579-48-2) is identified as a chemical with features in common with known DART toxicants via the following steps of the decision tree: Step I; Organic Compound, to II; Yes (*contains a cyclic ring*), to 2; No (*is not an ER & AR binder*), to 3; Yes (*is a retinoid derivative and retinoic acid receptor (RAR) binder*), to known precedent for DART.

Example 5: cortisol (CAS# 50-23-7) is identified as a chemical with features in common with known DART toxicants via the following steps of the decision tree: Step I; Organic Compound, to II; Yes (*contains a cyclic ring*), to 2; Yes, (*belongs to: active ER & AR binders: corticosteroid-like derivatives*), to known precedent for DART.

Example 6: dodecanoic acid (CAS#143-07-7) is identified as a chemical with features in common with no known DART toxicants via the following steps of the decision tree: Step I; Organic Compound, to II; No (*does not contain a cyclic ring*), to IV; No (> C9 hydrocarbon) to V; No (> C9 carboxylic acid) , to 19; No (*not an alkyl carbamodi-thioic acids or an alkyl sulfonate*), to 20; No (*does not belong to this group of miscellaneous compounds*), to no known precedent for DART.



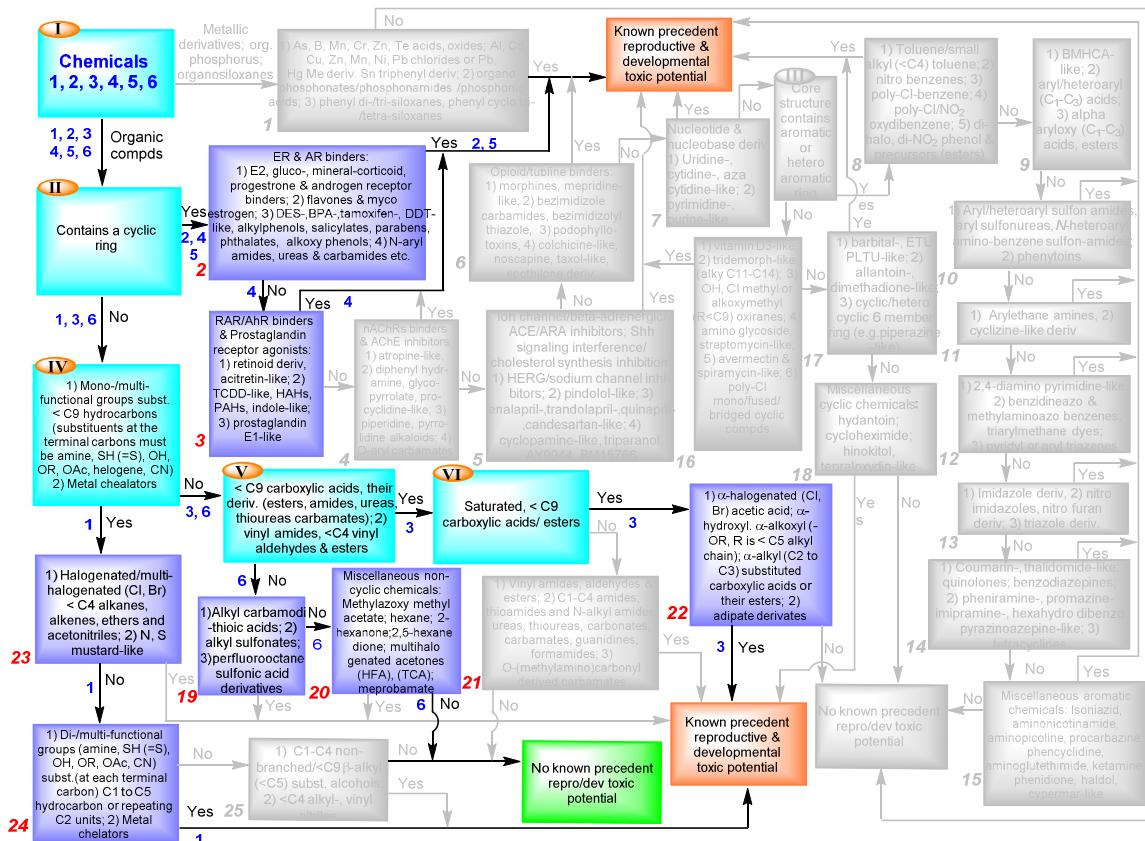


Fig. S2. The 6 examples of chemical structure and flow diagram illustration of these chemicals going through out the decision tree.

Category 1: Inorganics and derivatives: metals, metallic derivatives, organophosphorus and organosiloxane compounds

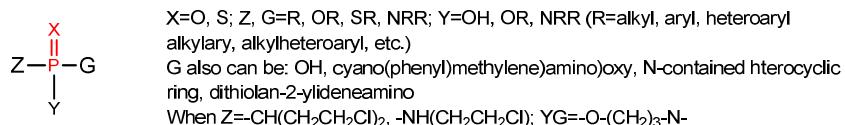
1a. Metals and metallic derivatives.

Comprehensive coverage of the vast literature on the developmental and reproductive toxicity of the metals and organo-metallics is beyond the scope of this paper. Suffice it to say that, metallics and organo-metallics should be carefully evaluated for their potential DART toxicity in the context of what is known about related materials. Many metals and their oxides, as well as their organic derivatives, are of special concern for DART effects as reviewed by Golub.¹ In addition, a number of members of this category are well known embryo/fetal toxicants in mammals and are also listed by the State of California² as developmental and/or reproductive toxicants including arsenic, cadmium, hexavalent chromium, lead, mercury and vanadium. Other metals, such as aluminum, chromium, cobalt, gallium, lithium, manganese, nickel and tin can be embryotoxic and teratogenic at high doses. After reviewing the metallic constituent of these materials it is suggested that if the metal itself does not raise a concern the organic portion of the chemical should be evaluated in subsequent steps of the decision tree.

1b. Organophosphorus compounds.

It has clearly been shown that some organic phosphate/phosphonate/phosphoramidate and phosphoryl/phosphorothioly triaziridine derivatives, exemplified by (1b) in Figure S3, are DART toxicants. All of these chemicals have a characteristic phosphoryl bond (P=O) or thiophosphoryl bond (P=S) with varying combinations of oxygen, carbon, sulfur, or nitrogen attached. In this sub-category, organophosphates represent a large class of chemicals which generate DART toxicity primarily by the

inhibition of acetylcholinesterase (AChE) enzyme. These chemicals have been summarized by Gupta and Suresh, respectively.^{3, 4} The other chemicals in this group exhibit a range of effects, with some limited to embryotoxic effects and others showing teratogenic and/or male reproductive effects. For example, cyclophosphamide (CAS# 6055-19-2) is both a teratogen and a reproductive toxicant for both males and females, and represents the high potency end of this category, while etidronic acid (CAS# 2809-21-4) anchors the lower end of the potency spectrum (very weakly embryotoxic, clearly a very different hazard profile than the previous chemicals). It is important to note that cyclophosphamide requires metabolic activation, with phosphoramide mustard the likely toxic agent⁵ (Appendix I).



1b

Fig. S3. The structural features of organophosphorus compounds

1c. Organosiloxane compounds: phenyl di-/tri-siloxanes and phenyl cyclotri-/tetra-siloxanes.

Organosiloxanes such as (1c-1) have been found to show reproductive and developmental toxicity effects. A larger series of phenyl di-/tri-siloxanes (1c-2), (1c-3) and phenyl cyclotri-/tetra-siloxanes (1c-4), as shown in Figure S4, have been tested in the uterotrophic assay (estrogenicity) and/or for effects on male reproductive organs/testosterone levels. The available data support that the primary mode of action for these chemicals is interaction with the estrogen receptor. The general core structural requirement, Ph-Si(MeR₁)-O-Si(MeR₂R₃) in (1c-2, 1c-3 and 1c-4), appears to be important for DART activity. Further, the substituent groups associated with activity in the literature are enumerated under the structures in Figure S4. In addition to the organosiloxanes, some organosilanes such as aryl, alkyl substituted silanes also show DART activity.

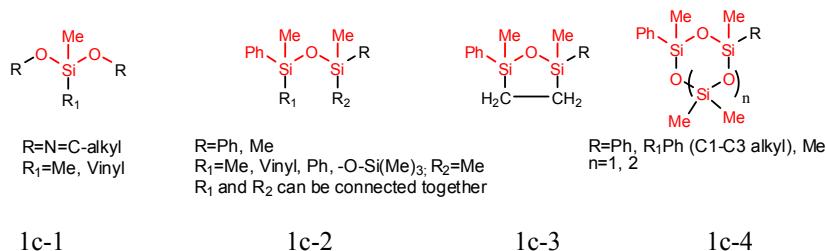


Fig. S4. The structural features of phenyl di-/tri-siloxanes and phenyl cyclotri-/tetra-siloxanes

Category 2: Estrogen receptor (ER) and androgen receptor (AR) binding compounds

2a Steroid nucleus derived ER and AR binders.

The fused tetracyclic (A, B, C and D rings) steroid nucleus (2a) in Figure S5 provides the carbon framework for different groups of mammalian hormones exemplified by estrogens, corticosteroids, mineralcorticoids, progestagens and androgens. Each has its own receptor, but there is a great deal of overlapping binding of these receptors by various ligands. For example, there is the overlap between glucocorticoid and mineralcorticoid receptor binders, and the cross reactivity between chemicals that bind the progesterone receptor and chemicals that bind to the testosterone receptor. There is more limited cross reactivity between androgen/progesterone ligands and the glucocorticoid receptors.⁶ For the purposes of this document, it is not practical to clearly separate the expected relative specificity of chemicals for the glucocorticoid versus mineralcorticoid, and androgen versus the progesterone receptor. Although the

decision tree rules do not clearly separate these classes, we have separated them for purposes of discussion by the receptor target they are primarily associated with in the literature.

2a-1. Estradiol-like compounds.

17- β -estradiol (E2, CAS# 50-28-2) and its analogs (Figure S5, 2a-1) are prototypical ligands for the ER. Estradiol has a fused tetracyclic backbone with hydroxyl groups at each end of the molecule (Figure S5). These are considered to be the most important structural features for ER binding. The general structural requirement and SAR of estradiol-like analogs are relatively straightforward. It is well accepted that the fused tetracyclic framework is essential to fit into the ER binding pocket. Substituents (R and R₂) are normally hydroxyl groups which serve as hydrogen bond donors to interact with the receptor, thus, enhancing ER binding capability. Substituents associated with activity are enumerated in 2a-1, Figure S5. None of the substituents on the fused tetracyclic backbone can be sterically hindered. The details of SARs for this group of estrogen receptor (ER) binding compounds have been discussed in the recent literature.⁷

2a-2. Glucocorticoid and mineralcorticoid receptor binders.

The general structural features of glucocorticoid and mineralcorticoid receptor binders are shown in (2a-2) (Figure S5). These chemicals constitute more complex steroids since the nonaromatic A ring increases the number of chiral centers. These compounds contain a double bond between carbon atoms C-4 and C-5 and a carbonyl group at position C-3 on the A ring, however, a small number of compounds considered contain hydrogen or a hydroxyl group at this C-3 position. The bond between C-1 and C-2 may be saturated or unsaturated. The B-ring may contain hydrogen or fluorine at position C-6, and the C-ring may contain hydrogen, a hydroxyl group or a carbonyl group for R₆ at position C-11. For the D-ring, the substituent R₂ must consist of a hydroxyacetyl or chloroacetyl moiety and R₃ may be hydrogen, a hydroxyl or a carbonyl group. R₄ may be hydrogen or a methyl group, and R₅ may be a methyl or an aldehyde group. The spironolactones are included here and contain γ -butyrolactone at C-17 occupying lactone ring position 5. When R₃ and R₄ are hydroxyl groups, they may form a ketal/acetal.

In experimental animal systems, glucocorticoids are teratogenic.⁷ Human and experimental animal studies consistently support a role for excess glucocorticoid exposure in both reduced fetal growth and body weight. Gestational exposure to exogenous glucocorticoids has been proposed to potentially have long lasting consequences for the cardiovascular system, metabolism, and neurological development and function.⁸ There are also many structurally varied synthetic glucocorticoid receptor agonists.⁹ It is anticipated that the wide variety of synthetic glucocorticoids will have similar effects on DART, based on their binding to glucocorticoid receptors although they have not been tested.

On the other hand, chemicals binding primarily the mineralcorticoid receptor show a different DART profile. For example, spironolactone (CAS# 52-01-7) is an antagonist of the mineralcorticoid receptor²⁹ blocking aldosterone (CAS# 52-39-1) binding. However, the toxic effects appear to be mediated by its anti-androgen effects that are based on interaction with the testosterone receptor.¹⁰

2a-3 and 2a-4. Progestones and androgens, anti-androgens.

The progestins, as a group, have varied effects depending on their relative affinity for the progesterone, androgen and glucocorticoid receptors. The effects of some of these chemicals are also suggestive of modulation of the physiological effects of estrogen, changes in steroid hormone metabolism and effects on gonadotropins. In general, these chemicals have adverse fertility effects in both males and females, and may have adverse developmental effects based on their interaction with other steroid hormone metabolism/receptors. They do not appear to cause structural defects by other modes of action. Similar to the corticosteroids, the progestins (2a-3) and androgens (2a-4) contain a wide variety of substituents associated with activities. Subgroup (2a-3) contains a double bond between C-4 and C-5, while the C-1, C-2 and C-6, C-7 bonds are either saturated or unsaturated. A carbonyl group at C-3 is another key structural feature. R₁ at C-6 may be hydrogen, chlorine or a methyl group, R₂ at position C-17 may be an acetyl group while the R₃ position may be a hydrogen atom or a hydroxyl, acetate or propionate group.

Analogs (2a-4) are characterized by a carbonyl group at the C-3 position, an unsaturated C-4,C-5 bond, and a saturated C-1,C-2 bond. The C-9,C-10 and C-11,C-12 bonds may be either saturated or unsaturated. Additional structural features include a hydroxyl group in the C-17 beta position (R_2) while the alpha position (R_3) may contain a hydrogen atom, or a variety of small chain groups including C1-C3 alkyl, ethyne, allyl or acetonitrile.

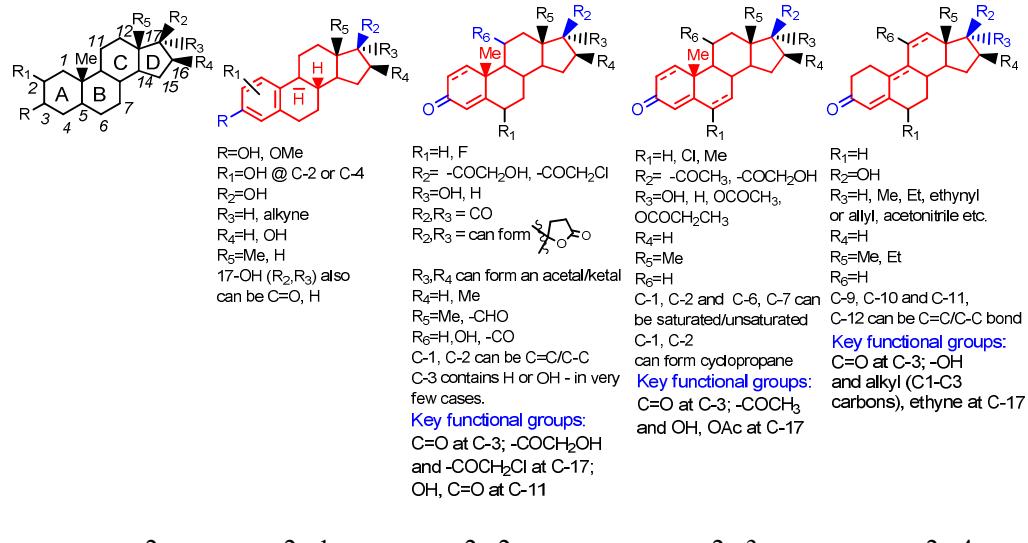


Fig. S5. The scope of structural features of steroid nucleus derived estrogen receptor (ER) and androgen receptor (AR) binders

Overall, the critical functional groups of steroid nucleus derived ER and AR binders for DART activity are the following: both the C-3 and C-17 hydroxyl groups (or other hydrogen bond donors) for estradiol-like compounds, the C-3 carbonyl group, the C-11 carbonyl/hydroxyl and C-17 hydroxyl/hydroxylacetyl groups for glucocorticoid and mineralcorticoid-like chemicals, the C-3 carbonyl group and C-17 hydroxyl/acetyl groups for progestins, and the C-3 carbonyl group and C-17 hydroxyl/alkyl/alkyne groups for androgen-like compounds.

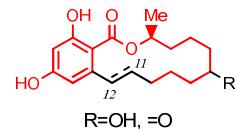
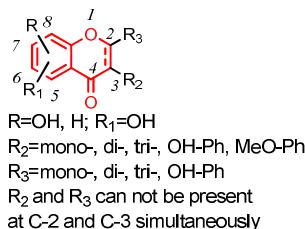
Based on receptor binding models of 17- β -estradiol (E2) and its analogs, structural features and their relationship to activity are summarized as follows: 1) hydrogen bond accepting substituents at C-3 and C-17 are required; 2) very polar substituents are unfavorable at almost all other positions; 3) small lipophilic substituents are allowed in many positions and often favorable; 4) large substituents are allowed underneath the steroid D-ring, suggesting the presence of a sizeable pocket in these receptors.^{6,11} However, for the purposes of identifying a precedent for DART activity, any chemical with a steroid nucleus should be closely scrutinized.

2b. Non-steroid nucleus derived estrogen receptor (ER) and androgen receptor (AR) binders

2b-1. Flavone and mycoestrogen related derivatives

Some phytoestrogens, such as flavonoids (e.g. flavone, isoflavone (2b-1-1), and mycoestrogens (2b-1-2)) shown in Figure S6, exhibit ER binding activity. Flavonoids (2b-1-1) are the largest class of phytoestrogens with the basic construction of a benzene ring directly connected to C-2 or C-3 of chromone (unsaturated C-2, C-3 bond) or 4-chromanone (saturated C-2, C-3 bond). Hydroxyl substituents may be present on the phenyl ring as R and R₁ groups bound to C-5, C-6 or C-7. Substituent R₂ may be a phenyl ring with one or more hydroxyl or methoxyl substituents and R₃ may be phenol, hydroxyphenol or dihydroxyphenol. Simultaneous substitution at C-2 and C-3 cannot occur.

Mycoestrogens (2b-1-2) are chemicals with a resorcinol ring fused with a macro-cyclic ester on which the R group can be a hydroxyl or carbonyl group, and the carbon-carbon bond between C-11 and C-12 may be saturated or unsaturated. These chemicals activate the ER with an efficacy comparable to that of 17- β -estradiol, the principal endogenous ER ligand. They exert a variety of DART effects in animals. Additional detail on substituents associated with activity for flavonoids and mycoestrogens is shown in 2b-1-1 and 2b-1-2 below. The SARs for these two groups of ER binding compounds have been discussed in the recent literature.



2b-1-1

2b-1-2

Fig. S6. The scope of structural features of flavonoids (e.g. flavone, isoflavone) and mycoestrogens derivatives.

2b-2. Other non-steroidal estrogen receptor (ER) binding compounds.

Non-steroidal estrogen receptor (ER) binding compounds (e.g. tamoxifen, (CAS# 10540-29-1), bisphenol-A and M (BPA, CAS# 80-05-7, BPM, CAS# 13595-25-0) as well as diethylstilbestrol (DES, CAS# 56-53-1) analogs may cause reproductive abnormalities and decrease fertility. We have reviewed several sub-categories of these chemicals, exemplified by tamoxifen, BPA, DES derivatives, DDTs, alkylphenols, parabens, and phthalates in the dataset. Chemicals in this group can be associated with endocrine disruptor effects.

The representative structural features for the tamoxifen (2b-2-1), BPA, DES (2b-2-2) and DDTs (2b-2-3) sub-category are shown in Figure S7. For the triphenylethylene derivatives (2b-2-1), the R group can be hydrogen or a hydroxyl group, R₁ can be hydrogen or a methoxyl group, R₂ can be chlorine or an alkyl group of 1 to 3 carbons (C1-C3), and R₃ can be a hydrogen atom or an N,N-dimethylaminoethoxy group.

In the case of (2b-2-2), the R group must be a hydrogen bond donor such as a hydroxyl or an amine group. The central atom X determines the functionality of the R₁ and R₂ groups and the chain length n. When X is oxygen, sulfur or a sulfur dioxide group, n=1, and R₁ and R₂ are absent. When X is a carbon atom, the chain length can be 1 or 2. For n=1, R₁ can be hydrogen or a small C1-C4 alkyl group, and R₂ can be a methyl group or the two may form an isobenzofuranone ring. For n=2, R₁ and R₂ are on different carbon atoms and can be hydrogen, a methyl group or an ethyl group.

Organochlorine pesticides such as DDT-like derivatives include structural elements shown in 2b-2-3. For these compounds, substituent R of the aromatic ring can be a hydroxyl group, a chlorine atom or a methoxy group. X and Y are carbon atoms joined by a saturated or unsaturated bond. The R₁ substituent is present only when this bond is saturated and may be a hydrogen or chlorine atom. In addition to the core structure, the chlorine substituents at the methane position of diphenylmethane and at the 4-position of both benzene rings are important for receptor binding.

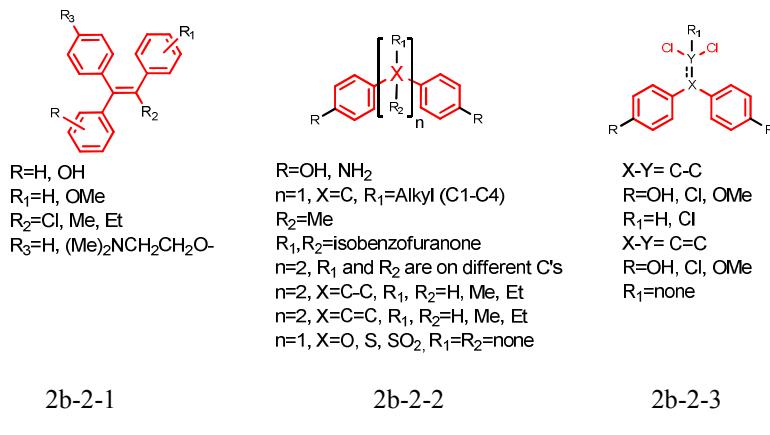


Fig. S7. The scope of structural features of BPA, DES, tamoxifen derivatives (2b-2-2) and DDT-like (2b-2-3).

2b-3. 4-alkylphenol-like derivatives.

Unlike E2 and phytoestrogens, containing two potential hydrogen bond donors at opposite ends of the molecule which can interact with the ER, 4-alkylphenol and 4-alkylaniline derivatives have the ability to form only one hydrogen bond. Even though they have a weaker interaction with the ER, chemicals in this group have also been reported to induce estrogenic responses and display DART effects. The substituent groups associated with activity are enumerated in (2b-3-1) in Figure S8. In these chemicals, the alkyl substituent provides a balance between the hydrophobic and steric properties of the molecule to permit it to fit into the ER binding pocket. Chemicals containing alkyl groups with 7 to 12 carbons (branched and unbranched) show relatively strong ER binding and DART effects. In addition, if the alkyl group is an unsaturated C3 carbon chain, as shown in (2b-3-2), these chemicals may act through different mechanisms to cause DART effects (e.g. genotoxicity).

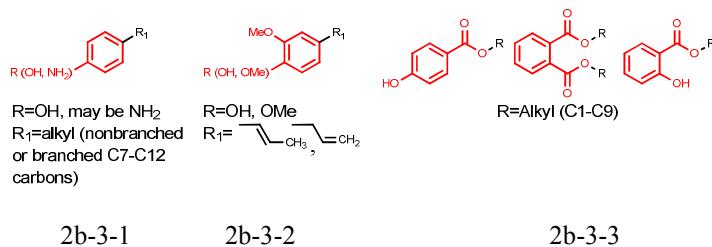


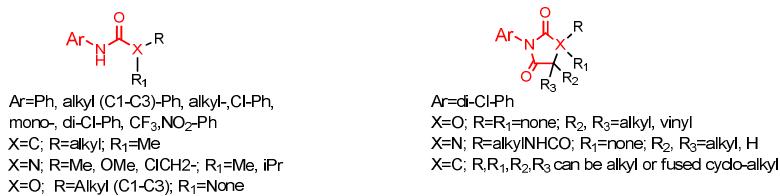
Fig. S8. The scope of structural features of alkyl phenols.

The rules and boundaries for other groups of synthetic estrogen receptor (ER) binding compounds, such as parabens, phthalates and salicylates which are shown in (2b-3-3), respectively, where the R group is an alkyl chain of one to nine carbons, are not discussed in the present paper given the number of excellent and recent works on this topic.

2b-4. N-aryl substituted urea, carbamide and amide derived androgen receptor (AR) binders.

Most androgenic chemicals activate AR-mediated transcription in mammalian cells through receptor-mediated mechanisms. For example, in the sub-category of *N*-aryl substituted ureas, carbamides and amides, many are AR binders which display developmental toxicity potential. The general core structural requirement of Ph-N-CO-X in (2b-4-1) and (2b-4-2) as shown in Figure S9, appears to be important for AR binding. The substituents associated with activity are further enumerated below. SAR analysis indicates that electron-withdrawing groups on the benzene ring, such as F, Cl, NO₂, or CN favor AR binding.^{12,13} In some cases, the substituents on X in (2b-4-1) could be fused with the NH to form five membered

heterocyclic ring moieties (2b-4-2), exemplified by imidazolidine-2,4-dione, oxazolidine-2,4-dione, and pyrrolidine-2,5-dione derivatives. For these cyclic compounds, X can be nitrogen, oxygen or carbon, and substituents (R, R₁, R₂ and R₃) are listed in Figure S9 in structure (2b-4-2). These N-aryl substituted heterocyclic ring derivatives, such as vinclozolin (CAS# 50471-44-8), iprodione (CAS# 36734-19-7) and proyamidone (CAS# 32809-16-8), have a range of developmental and reproductive effects linked to activity as an anti-androgen.^{14,15} Another androgen antagonist is prochloraz (CAS# 67747-09-5), which appears to have a distinct pattern of toxicity. Prochloraz had been reported to have multiple effects on the development of male rodents, and *in vitro* data show not only anti-androgen but also anti-estrogen effects as well as interaction with Ah receptors and inhibition of aromatase.^{16,17}



2b-4-1

2b-4-2

Fig. S9. The general structural features of *N*-aryl substituted ureas, carbamides and amides like chemicals.

Category 3: Retinoic acid receptor (RAR), aryl hydrocarbon receptor (AhR) binders and prostaglandin receptor agonists

3a. RAR binders.

3a-1. Nonaromatic RAR binders: Retinoic acid related chemicals.

Retinoic acid, retinol, and retinaldehyde play an important role in normal development, but both retinoid deficiency and retinoid excess are teratogenic. These actions are mediated by binding to two distinct classes of nuclear receptors, RAR and RXR. In addition, a huge number of synthetic retinoids have been produced and many of these have been tested for developmental toxicity. As shown in (3a-1-1 and 3a-1-2) in Figure S10, these compounds contain a multi-methyl substituted cyclohexene ring, namely a 2,6,6-trimethyl-1-cyclohexen-1-yl group, with two polyene diterpenes as the core framework, which may be the key moiety of binding the receptor. SARs for retinoids have been extensively developed,^{18,19, 20,21,22} and the results indicate that the polar (or hydrophilic) terminus on one side and ring structure terminus on the opposite side are necessary. In addition, the polyene diterpene side chain must contain more than three conjugated double bonds in order to be a strong teratogen. It is likely that the side chain plays an important role in the teratogenic activity for these chemicals. For example, chemicals containing non-conjugated or short conjugated polyene (less than 3 units) side chains or polyene side chains de-conjugated from the ring structure terminus can decrease and abolish the teratogenic activity. Furthermore, as illustrated by core structure (3a-1-1), the different functional groups on the polyene diterpenes side chain are either the metabolic precursors (alcohol and aldehyde), or derivatives (ester) of the carboxylic acid R group. However, the aminoethanol derived amide is inactive. In addition, the cyclohexenone-1-yl (3a-1-2) derivatives and cyclopentene-1-yl (3a-1-3), in Figure S10, derivatives are also developmental toxicants. Despite intensive study, the complexity of the effects of the retinoids prevents a full understanding of their effects. Their actions vary by developmental time, tissue, dose and they interact with multiple receptors with overlapping functionality. While the retinoids are most well known for their adverse effects on the skeletal system, effects on many other systems have been reported, including defects in the heart, central nervous system (CNS), eyes and genitourinary tract.^{23,24} Given the complexity of the interactions, attempting to develop specific rules for the developmental toxicity of the retinoids is outside of the scope of this paper. We have tried to cast a broad net with the design of our category rules such that any chemical in the broad group with RAR- and RXR-binding activity will be flagged.

3a-2. Aromatic RAR ligands: Acitretin-like derivatives.

Aromatic retinoids containing a substituted benzene ring and a conjugated polyene side chain or alkene connected aromatic ring side chain bind RARs and are teratogenic.²⁵ Most of these chemicals also contain a polar functional group such as a carboxylic acid, an ester, an alkyl sulfonester or a hydroxymethyl group at the end of a conjugated alkene side chain or at the para position of the aromatic ring. In this sub-category, the substitution patterns on the benzene ring are divided into two sub-groups. The first sub-group includes the acitretin-like chemicals with general structural features shown in (3a-2-1) in Figure S10 where the substituent groups associated with activity are further enumerated. For this sub-group, the side chain is generally a conjugated polyene with a carboxylic acid, ester or hydroxymethyl polar group. Insertion of a sulfur atom between the substituted benzene ring and alkene sidechain destroys the conjugating system and generates an inactive compound. This indicates that the conjugating system may be one of the crucial factors for binding the receptor. The second sub-group includes the chemicals with a 1,4-gem-di-methylcyclohexane fused benzene ring. These compounds have either a conjugated polyene side chain with a carboxylic acid or ester terminus (3a-2-2), or an alkene connected aromatic ring side chain with a carboxylic acid, ester, or alkyl sulfon (3a-2-3) substituent. When the aromatic ring on the side chain is naphthalene, the substituent is directly connected to the 1,4-gem-di-methylcyclohexane fused benzene ring (3a-2-4). These compounds with a 1,4-gem-di-methylcyclohexane fused benzene ring show profound teratogenic toxicity because the gem-dimethyl substitutions on the ring prevent microsomal oxidation, so that elimination of these retinoids occurs more slowly than for polyene retinoids. Interestingly, replacement of the 4-gem-di-methyl group on the cyclohexane ring with sulfur or of the polar group on the aromatic ring with a methyl group in (3a-2-3) preserves teratogenic toxicity.

Based on these results, the overall core structural requirements for DART effects of acitretin-like derivatives is that they must contain a multi-alkylated phenyl ring which is lipophilic and polar groups which are hydrophilic connected by a conjugated polyene (>3 units) chain.

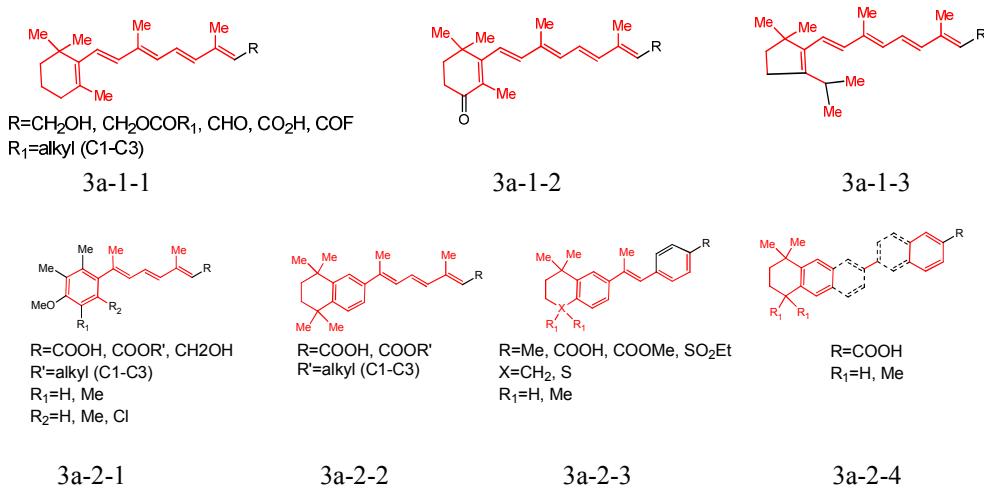


Fig. S10. The general structural features of acitretin- and retinoic acid-like derivatives.

3b AhR binders.

3b-1. Tetrachlorodibenzodioxin (TCDD)-like compounds.

Dibenzo-dioxins or furans with multi-halogen substituents (3b-1) in Figure S11 bind to the AhR. The AhR regulates the expression of metabolic enzymes in response to ligand binding, and the mechanism of AhR signaling shares many similarities with that of ER signaling.²⁶ As shown in (3b-1), X can be oxygen or none, and the substituents R can be bromine or chlorine exemplified by TCDD (X=O) and TCDF (X=none). The number of halogens (Br or Cl) ranges from 3 to 6. These chemicals have coplanar structural features and are the ligands for AhRs which elicit the AhR-dependent gene expression. Receptor binding is linked to altered endocrine homeostasis and DART effects of these chemicals. SAR studies have revealed that the ability of PCDDs and PCDFs, as well as other halogenated aromatic hydrocarbons (HAHs, e.g.

PCBs), to bind to the AhR not only correlates well with their ability to induce gene expression but also their ability to produce toxicity.²⁷

3b-2. HAH-like derivatives.

For the poly-halogenated biphenyls (3b-2) in Figure S11, the halogens are primarily bromine and chlorine for chemicals with test data. The co-planar molecules, similar to the TCDD-like chemicals as described above (3b-1), tend to share common biological effects related to their interaction with the AhR receptor, although they may also exhibit other modes of action. It is important to note that when the relative orientation of rings is not co-planar, (e.g. both aryl rings have ortho substituents) Ah receptor binding is less likely. For a sub-group of (3b-2), the toxicology of the polybrominated subset is primarily defined based on testing of two commercial mixtures, FireMaster® BP-6 and FireMaster® FF-1. Studies show that these mixtures are highly potent thyroid toxicants. Neuro-developmental effects have been reported. The role of indirect effects related to thyroid toxicity and other potential modes of action is not completely understood. Commercial PBB mixtures have been reported to have reproductive effects in both male and female experimental animals including fetotoxicity and teratogenicity. These mixtures contain both co-planar and non-coplanar constituents. While there are some mechanistic studies that evaluate single congeners and evaluate the relationship of structure to receptor binding, in general, these are not directly linked to toxicology data. Suffice it to say that modes of action are likely multiple and involve both dioxin-like AhR binding as well as other modes of action. An untested polybrominated biphenyl would be appropriately considered as both a potential reproductive and developmental toxicant.

The toxicology of the polychlorinated subset the PCBs (3b-2) is primarily based on testing of technical grade mixtures, including Aroclor 1016, Aroclor 1221, Aroclor 1232, Aroclor 1242, and Aroclor 1248.²⁸ These PCB mixtures are female reproductive toxicants. Evidence for effects on the male reproductive system is more limited. PCBs show high dose developmental toxicity. These chemicals are highly potent thyroid toxicants. Neurodevelopmental effects have been reported. Commercial PCB mixtures contain both co-planar and non-coplanar congeners. As with the PBBs, the co-planar congeners are linked to interaction with the AhR. The evidence for ER mediated effects is more developed for the PCBs than for the PBBs. PCBs which interact with the AhR tend to be anti-estrogens, while other PCBs have been shown to be weakly estrogenic. Untested PCBs would appropriately be considered as potential DART toxicants. Despite the fact that poly-halogenated oxydibenzene (described in 8d) share some structural similarity with PCBs or PBBs, they do not readily form co-planar structures and are not anticipated to share the same mode of action as the AhR interaction.²⁹

3b-3. Polycyclic aromatic hydrocarbons (PAHs).

In addition to the aromatic ring fused dioxin, furan and poly-halogenated biphenyls, PAHs represent a large class of AhR agonists and have been connected to developmental toxicity in the early life-stages of many species by their ability to bind to the AhR. These chemicals are produced as byproducts of fuel burning potent atmospheric pollutants, and occur almost always in mixtures which are impossible to cover in detail here. Some PAHs are considered to be teratogenic in addition to being carcinogenic and mutagenic. The core structural feature, represented by (3b-3) consist of 3 to 5 fused aromatic rings such as benzo[a]anthracene, and benzo[a]pyrene. Mechanistic studies indicated that several teratogenic PAHs showed high-affinity binding to the AhRs which might be the primary mediator of teratogenesis.

The binding activity of several of these chemicals to the AhR has demonstrated that this receptor is highly permissive and interacts with different classes of chemicals. In addition to TCDD, HAHs and PAHs, AhR agonist activity is also observed among the indole-related derivatives.

3b-4. Indole-related chemicals.

Based on the effects of indole-3-carbinol (CAS#700-06-1) which causes reproductive effects in both male and female offspring linked to activation of AhR,³⁰ the indole-related chemicals (3b-4) in Figure S11 are hypothesized to have DART potential despite the lack of direct test data. The substituents such as R and

R_1 can't be clearly defined based on the available data. However, the other as yet untested chemicals in this group and di-, poly-indole that have the potential to bind AhR may also share similar DART effects.

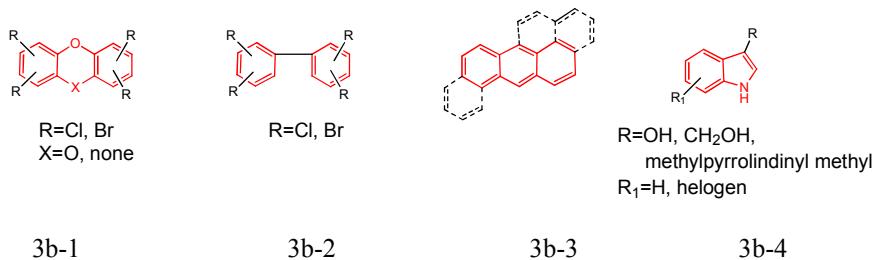


Fig. S11. The general of structural features of TCDD-, HAH-, PAHs- and indole-related derivatives.

3c. Prostaglandin receptor agonists.

Prostaglandins act via prostaglandin receptors. Although prostaglandins are endogenous hormones with essential signaling functions during development, exogenously administered prostaglandins should be considered to be potential developmental toxicants. Prostaglandins exert a range of developmental toxicities, some related to vasoconstriction. Effects range from abortifacient effects, frank malformations and more subtle developmental toxicity as illustrated by examples in the Appendix 1. Representative chemicals include prostaglandin E1 (PGE1, CAS# 745-65-3), PGE2 (CAS# 363-24-6), PGF2 α (CAS# 551-11-1) and misoprostol (CAS# 59122-46-2). Other structurally related chemicals/metabolites are 13,14-dihydro-PGE2 (CAS# 323-22-4), PGH2 (CAS# 42935-17-1), PGD2 (CAS# 41598-07-6) and thromboxane A2. The core structural features are illustrated in (3c) and (3c-1), Figure S12 along with substituent groups associated with activity.

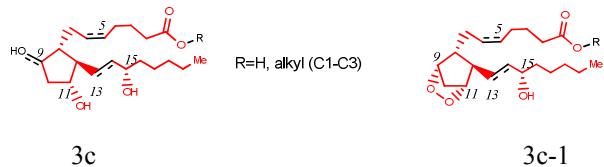


Fig. S12. The core structural features of prostaglandin-like derivatives.

Category 4: Nicotinic acetyl choline receptor (nAChR) binders and acetyl cholinesterase (AChE) inhibitors

4a-1. Atropine-like derivatives.

Atropine is a naturally occurring tropane alkaloid and its structural closely related chemicals are anticholinergics. They are competitive antagonists for the muscarinic acetylcholine receptor (mAChR). The common structural feature is the *N*-methyl azabicyclo[3.2.1]octyl or its quaternary amine salt alcohol (3-tropanol) derived ester which is shown in (4a-1), Figure S13 along with substituent groups associated with activity. In general, most of these chemicals are weak or non-teratogenic in experimental animals, but are fetotoxic at maternally toxic dosages. The *N,N*-dimethyl pyrrodinol and *N,N*-dialkylamino ethanol derived esters (4a-1-1), and their corresponding quaternary amine salts are also included in this sub-category.

4a-2. Diphenyl hydramine-, procyclidine-like derivatives.

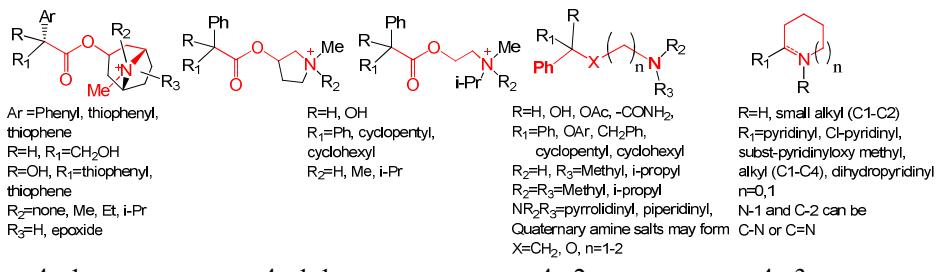
Several diphenhydramine and procyclidine related derivatives have also shown binding activity for nAChR. Substituents associated with activity are shown in (4a-2) in Figure S13. These chemicals contain phenethyl-alkyl-amine or benzyloxy-alkyl-amine as a core structural requirement. Comparison with (4a-1) and (4a-1-1), the key structural difference of these chemicals is that they lack of an ester moiety.

4a-3. Piperidine and pyrrolidine alkaloid derivatives.

Piperidine and pyrrolidine alkaloids (4a-3) in Figure S13 and their enantiomers have shown ability to activate nAChRs and cause teratogenic effects. The core structural features for these chemicals are shown in 4a-3 along with substituent groups associated with activity. All of the enantiomers and racemic compounds resulting from these structures appear to be active. However, no DART activity has been reported for structures containing an R₁ group equal to hydrogen, an alkyl (<C3) chain or a hydroxyethyl group. In addition, some chemicals with a C=N bond between N-1 and C-2 also show DART activity.³¹

4b-1 O-aryl carbamates.

O-aryl carbamates reversibly inactivate acetylcholinesterase by direct carbamylation, which results in the accumulation of the neurotransmitter acetylcholine. A considerable number of reproductive and developmental toxicity studies have been carried out with different carbamates in various animal species. From these studies it has been concluded that carbamates are capable of affecting the reproductive system of both females and males, and also affect the development of the conceptus¹³. The general core structural feature for these chemicals is shown in (4b-1) in Figure S13, where the aryl group (Ar) can be a substituted phenyl, naphthalenyl (4b-1-1), benzofuran, 1,3-benzodioxole (4b-1-2) or pyrimidinyl (4b-1-3) group. In the case of (4b-1-1), the substituent R can be methyl, dimethyl or trimethyl at any position on the phenyl ring. R also can be 3-[(1S)-1-(dimethylamino)ethyl], 2-[(ethylthio)methyl], 2-(1-methylpropyl), 2-(1-methylethyl), 3-methyl-5-(1-methylethyl), 3,5-dimethyl-4-(methylthio), 4-(dimethylamino)-3-methyl, 2-(1-methylethoxy) and naphthalenyl. R₁ may be hydrogen or a small alkyl (C1-C3) group and R₂ may be a small alkyl (C1-C3) group. In the case of (4b-1-2), Ar can be 2,3-dihydro-2,2-dimethyl-7-benzofuran or 2,2-dimethyl-1,3-Benzodioxole. R₁ can be hydrogen, N-[(dibutylamino)thio], or ethyl 3-(isopropyl(thio)amino)propanoate and R₂ is small alkyl (C1-C3). The Ar group can also be a substituted pyrimidinyl as shown in (4b-1-3). Similar to the O-Aryl carbamates, the N-[2-(4-phenoxyphenoxy)ethyl derived carbamate (4b-2), which has different structural features, appears to have DART effects at relatively high dose levels by inhibition of the AchE.

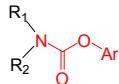


4a-1

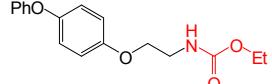
4a-1-1

4a-2

4a-3



4b-1



4b-2

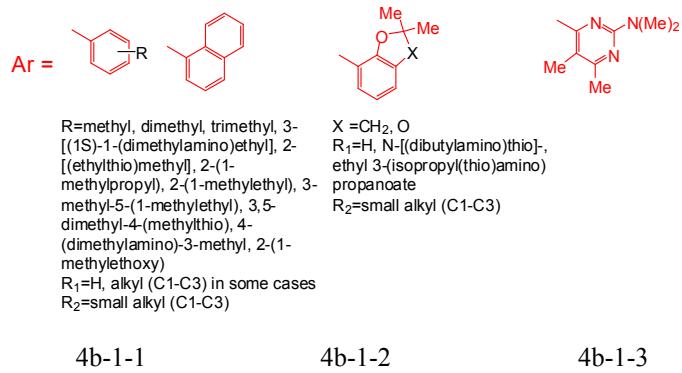


Fig. S13. The scope of structural features of nAChR binders and AChE inhibitors.

Category 5: Ion channel opener/inhibitor, beta-adrenergic inhibitors, ACE/ARA inhibitors and Shh signaling interference/Cholesterol synthesis inhibition

5a. Aromatic alkyl/aryloxy alkyl amines derived Class III antiarrhythmics with primary action on the IKr channel and sodium channel inhibitors (amiloride-like derivatives).

Class III antiarrhythmic drugs, that block the rapid component of the delayed rectifying potassium current (IKr) of the heart, cause malformations in animal models. These drugs appear to share a common mechanism for developmental toxicity related to fetal hypoxia.³² The core structural requirements for IKr blockers is represented by (5a-1) in Figure S14 where the substituent R on the phenyl ring can be sulfonamide, nitrile, fluorine, 1-(benzofuran-3-yl)ethanone, or may form a quinazolinone ring with the phenyl group. If X is CH, R₁ can be a hydroxyl group, R₂ can be hydrogen or a methyl group, R₃ is a C1-C7 alkyl chain and n can be 1-3. If X is O, the R₁ group is absent, R₂ can be a methyl or an ethyl group, R₃ can be a C1-C3 alkyl chain, a methylsulfonylphenylethyl, a propylsulfinylpropyl, or a benzoxadiazol-5-ylethyl group, and n can be 1-3 carbons. Some chemicals in this category, which have the core structure of (5a-2) that inhibit sodium channels (e.g. amiloride-like derivatives), are also shown to have DART potential. For these structures, R and R₁ can be hydrogen or C1-C3 alkyl groups.

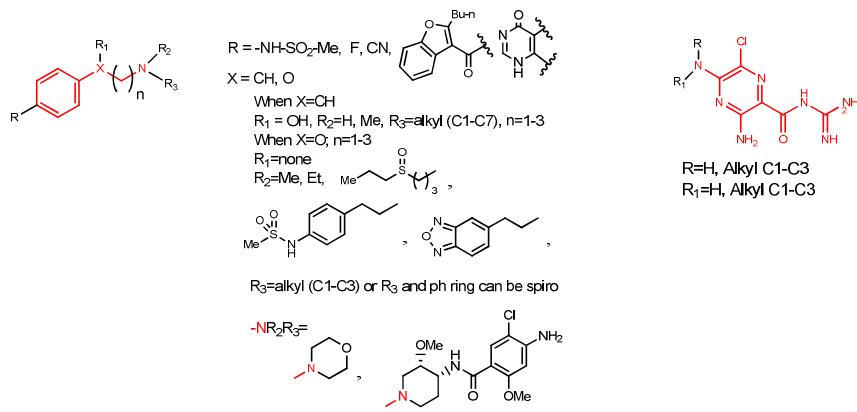


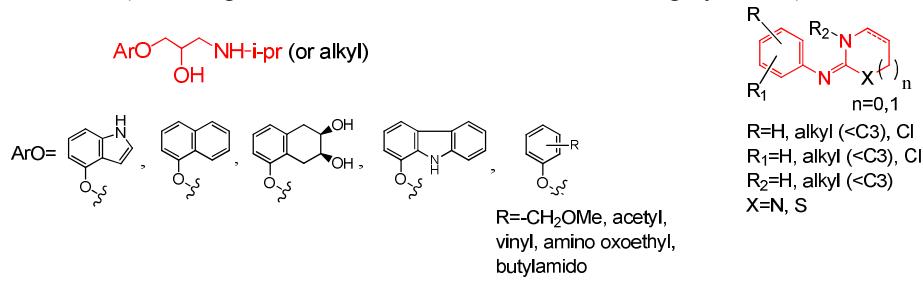
Fig. S14. The scope of structural features of aromatic alkyl/aryloxy alkyl amines derived Class III antiarrhythmics.

5b. Aryloxy propanamine-like derivatives act as beta-adrenergic inhibitors.

The aryloxy propanamine-like derivatives, such as pindolol (CAS# 13523-86-9), inhibit the beta-adrenergic receptor and exhibit a wide range of embryotoxic potency, and induce a very similar pattern of

abnormalities. The core structural feature for this sub-category is an aryloxy moiety and *N*-isopropyl amine group separated by a 2-hydroxyl substituted three-carbon alkyl chain, as shown in (5b-1), Figure S15. Aryloxy groups in (5b-1) vary and may include the following: indolyloxy, naphthyoxy, 6,7-dihydroxyhexahydronaphthalene-1-yloxy, methoxyethylphenoxy, vinylphenoxy, amino oxoethylphenoxy and butylamido phenoxy. The *N*-alkyl group also may include a small alkyl (C1-C4) or *N,N*-dialkyl group.

In addition, some of the alpha-2-adrenergic agonists, for example, the aromatic ring connected imidazolidinylidene or thiazinanylidene/thiazolylidene like chemicals (5b-2) also showed DART potential (not enough chemicals with data to form a sub-category to date).



5b-1

5b-2

Fig. S15. The scope of structural features of aryloxy propanamine (or pindolol)-like derivatives and alpha-2-adrenergic agonists.

5c. Chemicals interacting with Angiotensin Converting Enzyme System or Angiotensin Receptor (ACE/ARA inhibitors).

As reviewed by Serreau et.Al.,³³ angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor type I antagonists (ARAs) have known effects on fetal development. Angiotensin II is critical to normal nephrogenesis. Renal defects were observed in angiotensin enzyme and angiotensinogen null mice. Angiotensin 1 receptors mediate the effects of angiotensin II. ARAs selectively compete with angiotensin at the receptor level, while ACE inhibitors inhibit the metabolism of angiotensin I to angiotensin II. Therefore, the observed fetal toxic effects are thought to be related to the reduction of angiotensin II production and/or effect. Relative to the human fetus, most animal species are resistant to the fetal toxicity of this group of drugs because the kidney and the renin-angiotensin system in the experimental animal models do not develop until closer to term, when the fetus is relatively more mature and, therefore, less vulnerable.²⁷ ACE inhibitors may cause severe renal dysfunction with oligohydramnios and neonatal anuria, skull ossification defects, reduced fetal growth, stillbirth and dysmorphic features. ARAs are newer drugs and less well studied than the ACE inhibitors. Similar to ACE inhibitors, animal studies have shown that intrauterine exposure to ARAs decrease fetal body weight, induce renal dysfunction with histological changes in the kidneys and may cause fetal death. The effects of the ARAs appear to be less severe than those of the ACE inhibitors, but it is not clear if that reflects a difference in potency or a reporting bias, given that much of the information on adverse effects of the ACE inhibitors comes from human observations and they have had a much longer history in the market. Shepard³⁴ concludes that the rat is not a good animal model to study the effects of this group of chemicals, but that there is a pattern of effects seen in humans which includes oligohydramnios, fetal renal failure and decreased calcification of the skull. These agents do not appear to be associated with reproductive toxicity.⁷

The core structural features of captopril-like ACE inhibitors and candesartan-like ARAs are indicated in (5c-1), (5c-2) and (5c-3) along with the substituents important for activity in Figure S16. In the case of (5c-1), the R group may be absent or may be fused cyclohexane, fused benzene or fused di-methoxybenzene. R₁ can be a methyl or an alkyl (C2-C5) amine group. R₂ can be mercaptomethyl, alpha-amino phenylbutanoic acid, alpha-amino pentanoic acid and their alkyl esters. The ring can be five (n=0) or six (n=1) membered. Interestingly, the benzazepine acetic acid derivatives shown in (5c-2) showed similar ACE inhibition and DART effects to those exhibited by 5c-1 which may be due to similar peptide

backbones shown in red in (5c-1-1) and (5c-2-1) respectively. However, the tetrazolylbisphenylmethyl group may be important for inhibition by ARAs in the case of (5c-3) where R can be a heterocyclic or a non-cyclic group such as 7-carboxylic acid-2-ethoxyl benzimidazole, 2-butyl 1,3-diazaspiro[4,4]-non-len-4-one, 2-butyl-4-Cl-5-methanol imidazole and N-1-oxopentyl-L-valine.

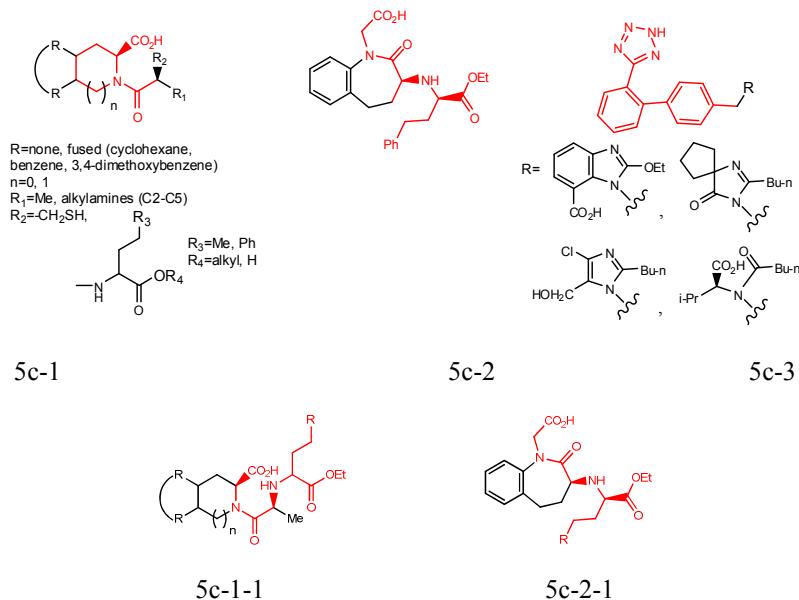
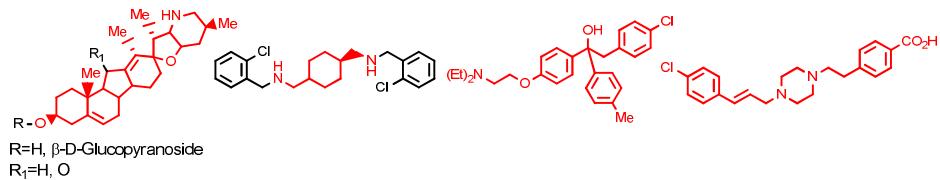


Fig. S16. The core structural features of captopril-like ACE inhibitor and candesartan-like ARA inhibitors.

5d. Sonic hedgehog signaling interference/Cholesterol synthesis inhibition.

As reviewed by Incardona and Roelink,³⁵ holoprosencephaly, or an undivided forebrain, is a complex brain malformation associated with *Sonic hedgehog* (Shh) mutations and with exposure to certain chemicals that affect cholesterol metabolism and/or hedgehog pathway signaling. The archetypical xenobiotics associated with this phenotype are cyclopamine (CAS# 4449-51-8) and jervine (CAS# 469-59-0), and a glycoside derivative, cycloposine (CAS# 23185-94-6). The core structural features of these chemicals are shown in (5d-1) in Figure S17. R can be hydrogen or β -D-Glucopyranoside, and R₁ can be hydrogen or a carbonyl group. Cholesterol is potentially important for both biogenesis of Shh and in signal transduction. Cyclopamine and related alkaloids appear to have an effect on Shh signal transduction that is unrelated to cholesterol synthesis or transport. A more detailed review of the mode of action of the cyclopamine alkaloids is provided by Sakata and Chen.³⁶ These authors contend that the most recent G protein-coupled receptor (GPCR)-like protein, smoothened (Smo), is the target mediating cyclopamine teratogenesis.

Inhibitors of cholesterol synthesis at downstream steps show similar effects and include AY-9944 (CAS# 366-93-8) and BM 15766 (CAS# 86621-92-3) that inhibit 7-dehydrocholesterol reductase, which catalyzes the last step of cholesterol synthesis, and triparanol (CAS# 78-41-1) that inhibits D2, 4-dehydrocholesterol reductase. The structures of these chemicals are shown in (5d-2), (5d-3) and (5d-4), respectively in Figure S17. They cause holoprosencephalic brain anomalies, anomalies of the limbs and male genitalia,³⁷ and inhibition of cholesterol synthesis, subsequently impacting signaling through the hedgehog pathway which appears to be central to the mode of action.³⁸



5d-1

5d-2

5d-3

5d-4

Fig. S17. The scope of structural features of cyclopamine-like derivatives and structure of AY9944, triparanol and BM 15766.

Category 6: Opioid receptor binders and tubulin receptor interactors

6a. Opioid receptor binders: Morphine-, and meperidine-like derivatives.

Chemicals with a morphine-like core structure (6a-1) are shown in Figure S18. In general, most of these chemicals are opioid receptor binders and do not appear to be significant teratogens, and signs of fetotoxicity are generally limited to high doses. However, these chemicals have been shown to exert effects on neurobehavioral function following *in utero*/perinatal exposure, and may also exert effects via the endocrine system that impact reproductive organs. It was observed that a variety of substituents such as OH, OR and carbonyl functional groups on this core structure will result in inhibition of the opioid receptor. As illustrated by structure (6a-1), eliminating the double bond between the C-7 and C-8 carbons, replacing OR₁ with hydrogen or a carbonyl group, and adding an extra cyclic ring (e.g. 6a-2) will result in similar DART activity, regardless of whether R₂ is a methyl, allyl or methyl cyclobutyl group. However, as the R₂ group on the N atom changes from methyl to allyl or methyl cyclobutyl, the resulting chemicals produce decreased DART effects in animals, but show respiratory depression and behavioral effects. These conclusions are based on examination of the effects of the chemicals enumerated in Appendix 1. Interestingly, chemicals (6a-3 to 6a-6), which were generated by removing one or two cyclic rings from (6a-1), showed mixed opioid antagonist/agonist activity. However, these chemicals indicated no clear evidence of fetotoxicity and no evidence of teratogenicity.

In addition to the morphine derivatives, other opioid analgesic drugs, such as meperidine-like chemicals (6a-7), also interact with the opioid receptor and exhibit DART effects. As shown in (6a-7), the core structural feature of these chemicals is a gem-phenyl, carboxylate substituted cyclic amine.

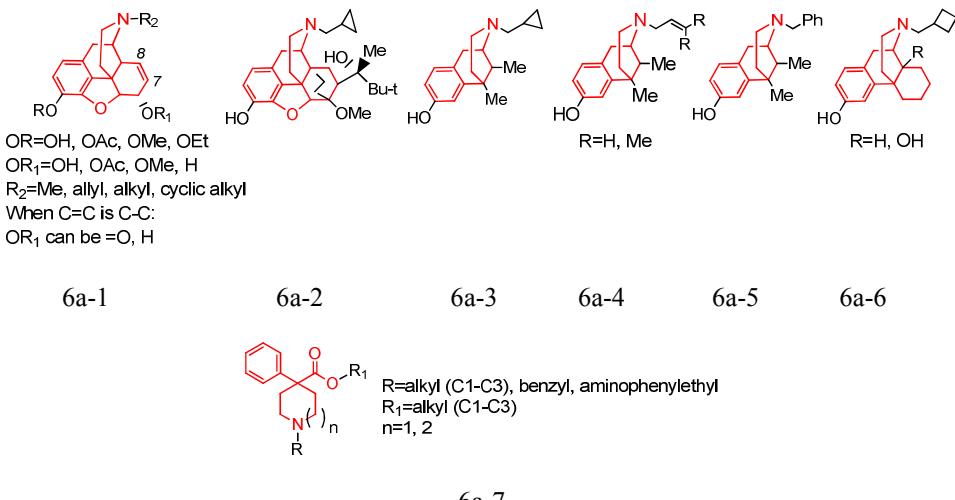


Fig. S18. The scope of structural features of morphine-, meperidine-like derivatives.

6b. Tubulin interactors.

6b-1. Benzimidazole-like derivatives.

The benzimidazole fungicides and veterinary drugs form one of the classes of mitotic poisons that act on tubulin and disrupt cell division. There are data on many of these chemicals which suggest that they bind to a site that is similar to that for colchicine (CAS# 64-86-8).³⁹ Their common mode of action would suggest the potential for a shared toxicological profile between the benzimidazoles and other structurally diverse mitotic spindle poisons. As indicated in (6b-1) in Figure S19, the core structural alerts for the majority of collected compounds (enumerated in Appendix 1) are the benzimidazole carbamates where substituents R on the benzimidazole ring can be hydrogen, or a phenylthio-, propylthio-, phenylsulfinyl-, benzoyl-, fluorobenzoyl-, thiophene-2-carbonyl- and thiazol-4-yl group. However, limited data indicate that replacement of the carbamate group with a thiazol-4-yl group or alkylthio group on the benzimidazole ring (e.g. 6b-1-1) and the metabolic precursors of 6b-1 shown in (6b-1-2) showed DART activity. The R₁ group in these structures is an alkyl group of 1 to 3 carbons.

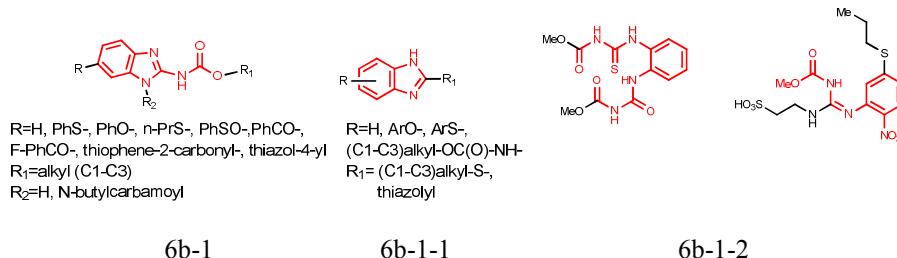


Fig. S19. The scope of structural features of benzimidazole-like derivatives.

6b-2. Podophyllotoxin derivatives.

Podophyllotoxin (CAS# 518-28-5) is a well known plant-derived toxicant reported to bind to the same site as colchicine in eukaryotic cells. Depending on structural substitutions, some podophyllotoxin related chemicals show little inhibition of tubulin polymerization, but instead inhibit topoisomerase II, and consequently disrupt the normal cell cycle. Podophyllotoxin is reported to have high dose fetotoxic effects in experimental animals, but not to be teratogenic⁷ and is reported to exhibit testicular toxicity.⁴⁰ The representative core structure (6b-2) is shown in Figure S20, where substituent R is a hydroxyl or methoxyl group and R₂ is a methoxyl group. Examination of structure and activity of these chemicals indicates that chemicals with a hydroxyl or amine R₁ group are likely to better inhibit tubulin polymerization than topoisomerase II. In contrast, chemicals with an R₁ group of ethylene glycol, amino ethanol or ethylidene-beta-d-glucopyranosyloxy better inhibit topoisomerase II than tubulin polymerization.

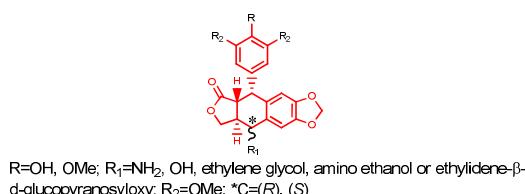


Fig. S20. The scope of structural features of podophyllotoxin-like derivatives.

6b-3. Other tubulin interactors.

Antimitotic chemicals that interact with tubulin cut across diverse structural classes and have also been defined based on their specific binding sites, whether or not they are microtubule destabilizing or microtubule polymerization inhibiting chemicals. This mechanism of action is reviewed by Jordan and Wilson.⁴¹ These authors note that historically the microtubule-destabilizing agent classification has included: the *Vinca* alkaloids (vinblastine (CAS# 865-21-4), vincristine (CAS# 57-22-7), vinorelbine (CAS# 71486-22-1), vindesine (CAS# 53643-48-4), vinflunine (CAS# 162652-95-1)), cryptophycin (CAS# 124689-65-2), halichondrin (CAS# 147427-91-6), estramustine (CAS# 2998-57-4), colchicine and combretastatin (CAS# 82855-09-2), noscapine (CAS# 128-62-1), maytansine (CAS# 35846-53-8), rhizoxin (CAS# 90996-54-6), spongistatin (CAS# 149715-96-8), podophyllotoxin, steganacin (CAS# 41451-68-7) and curacin (CAS# 20585-97-1). The representative chemicals such as vinblastine, colchicine and noscapine are shown in (6b-3-1), (6b-3-2) and (6b-3-3), respectively of Figure S21. In (6b-3-1), R may be an amine or an alkoxyl group, R₁ may be hydrogen, a hydroxyl, or a methoxyl group, R₂ may be hydrogen, a methyl or formyl group, R₃ may be a hydroxyl or an OAc group, and R₄ may be an alkyl, hydroxyl, or an OAc group. In (6b-3-2), R may be hydrogen or an acetyl group and in (6b-3-3) R may be hydrogen or a methyl group. Unless otherwise noted, we failed to locate relevant toxicology information on these agents and did not verify the literature concerning interaction with tubulin. However, the list does serve the purpose of showing that tubulin interaction is an effect that is shown by a wide variety of natural toxins with potential DART properties.

The second main group historically classified as the microtubule-stabilizing agents are the ones that stimulate microtubule polymerization⁴¹ and prevent cancer cells from dividing by interfering with tubulin polymerization. These chemicals are exemplified by taxane related derivatives (6b-3-4) and epothilone related derivatives (6b-3-5) in Figure S21. As shown in (6b-3-4), the R group on the eight membered ring of the taxane core structure is hydroxyl or acetoxyl, and the R₁ group is tert-butoxy, phenyl, alkyl or aryl. The core structural feature of epothilones is indicated in (6b-3-5) where the R group can be a methyl, ethyl, or hydroxymethyl group, R₁ can be hydrogen or a methyl group, R₂ can be hydrogen with a double bond between C-13 and C14 or the corresponding epoxide ring. Other chemicals in this sub-category include discodermolide (CAS# 127943-53-7), the eleutherobins, sarcodictyins, laulimalide (CAS# 115268-43-4), rhazinilam (CAS # 36193-36-9), and polyisoprenyl benzophenones (phytochemicals).

Recent publications indicated that the classification of microtubulin stabilizing and destabilizing agents is overly simplistic and explain that drugs that increase or decrease microtubule polymerization at high concentrations suppress microtubule dynamics at lower concentrations. High dose colchicine is reported to be embryotoxic and teratogenic in experimental animals.⁷ We failed to locate data on the DART toxicity of structures closely related to colchicine. However, given the pivotal role of tubulin binding and subsequent events in the mode of action of other related agents that bind to the colchicine site on tubulin, we infer that agents that bind the colchicine site on tubulin and have similar antimitotic effects *in vitro* are likely to share the DART effects of the colchicines. This conclusion has been supported by recent experimental work which has shown that 2-methoxyestradiol binds to the colchicine binding site and has antimitotic effects.⁴²

Furthermore, Nguyen et al.⁴³ have used docking models in an attempt to define a common pharmacophore for the diverse structures that have modes of action similar to colchicines. However, the diverse structures discussed in their paper are not readily adaptable to inclusion in a single group for the purposes of this decision tree, and hence these chemicals are split between several decision tree groups.

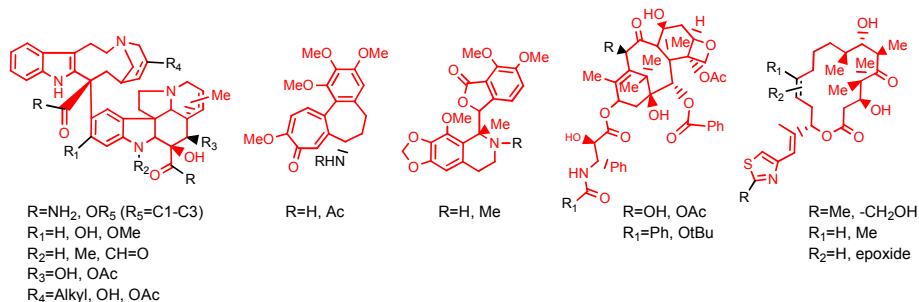


Fig. S21. The scope of structural features of colchicine, noscapine, taxol and epothilones derivatives.

Category 7: Nucleotide and nucleobase derivatives

7a. Uridine and cytidine-like derivatives.

Uridine and cytidine-like derivatives are related in structure to the nucleosides, which are involved in the biosynthesis of RNA and DNA. As embryogenesis involves extensive cellular proliferation, any interference with this process is potentially teratogenic. Uridine and cytidine did not show DART effects at biologically relevant concentrations. However, lack of or excessive exposure to these nucleosides may disrupt DNA synthesis leading to developmental toxic outcomes. Therefore, agents that interfere with nucleotide pool balance are likely to have developmental toxicity potential. The basic core construction of these chemicals consists of uracil or cytosine with a ribose/deoxyribose ring directly connected by a β -N-1-glycosidic bond. The structural scope of the chemicals (Appendix 1) exemplified by the general core structure (7a) in Figure S22 is described as follows: the hydrogen at the C-5 position of the uracil (CAS# 66-22-8), cytosine (CAS# 71-30-7), uridine (CAS# 58-96-8) and cytidine (CAS# 65-46-3) (where Z=C in 7a) can be replaced by a halogen or a methyl group. The methylene group (at C-5) in the heterocyclic ring of cytosine can also be replaced with nitrogen, to form the structurally related analogs of azacytidine (CAS# 320-67-2) (Z=N). These structural changes in the nucleosides result in developmental toxicity. Elimination of the 2' (R₁=H) hydroxyl group on the ribose ring of cytosine or uridine generates deoxycytidine (CAS# 951-77-9) and deoxyuridine (CAS# 951-78-0) which are not developmental or reproductive toxicants. This is also true for the nucleosides consisting of adenosine (CAS# 73-24-5) or guanosine (CAS# 118-00-3) derivatives such as deoxyadenosine (CAS# 958-09-8), and deoxyguanosine (CAS# 961-07-9) which are structurally related chemicals. However, halogenated (where Z=C in 7a) deoxycytidine and deoxyuridine as well as corresponding 5-Aza-2'-deoxycytidine at the C-5 position retain developmental toxic activity.



7a

Fig. S22. The core structural features of uridine and cytidine-like derivatives.

7b. Purine and pyrimidine-like derivatives.

Purine related chemicals exemplified by adenine (CAS# 73-24-5), and guanine (CAS# 73-40-5) which are two of the four bases found in deoxyribonucleotides and ribonucleotides, the respective building blocks of DNA and RNA, are not DART toxicants at biologically relevant concentrations. However, at high concentrations, both adenine and guanine are toxic to preimplantation embryos.⁴⁴ The core structural features of these chemicals are represented by (7b-1) and (7b-2) in Figure S23. In (7b-1), R, R₁, and R₂ can be hydrogen or a methyl group while R₃ is absent. However, R₃ can be 2-hydroxyethoxymethyl or a ribofuranose ring while R₂ is absent. X can be an NH group or an O atom and Y can be O or S. Z typically is a CH group and in a few cases it is a N atom. In (7b-2), R can be hydrogen or a ribofuranose group and X can be an NH group or S atom. The chemicals used to define these rules and their associated toxicity data are collated in the Appendix 1. Similar to purines, pyrimidine derivatives such as fluorouracil (CAS# 51-21-8), and propylthiouracil (CAS# 51-52-5) are also identified as DART toxicants. Based on limited data, chemicals with core structural features described in (7b-3) (Figure S23) may display similar DART effects.

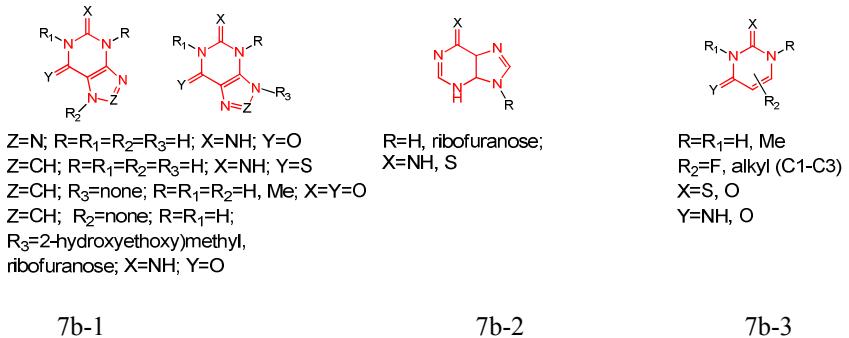


Fig. S23. The core structural features of purine and pyrimidine-like derivatives

Category 8: Aromatic compounds with alkyl, multi-halogen and nitro groups

Chemicals within this category include the following five sub-categories: 8a. toluene and small alkyl toluene derivatives; 8b. NO_2 -alkyl/ NO_2 -benzene derivatives; 8c. polyhalogenated benzene derivatives; 8d. polyhalogenated-, NO_2 /halogenated-oxydibenzene; 8e. dihalogen-, dinitro-phenol and their ester derivatives. The general core structures of these chemicals include the toluene, oxydibenzene and phenol ring with alkyl, halogen and/or nitro substituents as shown in (8a to 8e) in Figure S24.

For sub-category 8a, toluene and a single alkyl chain substituent (< 5 carbon atoms) present on toluene are included. The alkyl substituents can be at *ortho*, *para* or *meta*-positions. For sub-category 8b, the majority of chemicals are mono-, di-, tri-nitrobenzene or nitrotoluene with *ortho*, *para* or *meta* relative substituent placement. Members of 8a and 8b without nitro substituents appear to be primarily developmental toxicants, while addition of a nitro group may be associated with a distinctive pattern of male reproductive toxicity. Sub-category 8c includes multi-chlorinated benzene derivatives containing from 2 to 6 chlorine atoms. Other possible substituents include methyl or nitrile groups. The members of this class included here are primarily developmental toxicants (Appendix 1). Sub-category 8d includes multi-substituted oxydibenzene with halogen or halogen/nitro substituents. Because these chemicals normally do not readily form co-planar structures, they are not anticipated to bind to the AhR, (see section 3b-2), nor do they have a mode of action dependent on the AhR interaction.²⁹

Within 8d, the polybrominated oxydibenzenes show some evidence of developmental toxicity (primarily at maternally toxic doses) and little evidence of reproductive toxicity. There is some evidence of the potential for developmental neurotoxicity, and some members of this class have thyroid effects in rodents. In addition, *in vitro* data suggest the potential for estrogenic effects, particularly of hydroxylated derivatives/metabolites.²⁹ It should be noted that test data are primarily limited to evaluation of commercial mixtures, and that little *in vivo* data are available on the lower brominated oxydibenzenes. Nevertheless, the existing data are sufficient to place these chemicals in a sub-category of potential concern for DART effects. The polychlorinated oxydibenzenes (polychlorinated diphenylethers, PCDE) lacking a nitro group, show toxicity that is distinct from the polychlorinated nitro diphenyl ethers. Some of the PCDEs result in significant prenatal litter loss (e.g. 2,2',4,4',5,6 CDE, 2,3',4'6 CDE, 2,2',4,5,6'CDE, and 2,2',4,4',5,5'CDE). Other PCDEs show less pronounced effects on prenatal mortality, with effects on post natal survival and weight gain (Appendix 1).

A distinct sub-group within the 8d sub-category includes those that contain the NO_2 group. Nitrofen (2,4-dichlorophenyl 4'-nitrophenyl ether; CAS# 1836-75-5) has been the reference chemical studied in this sub-group. Nitrofen causes a wide range of developmental defects in rodents as well as a potent peri-natal lethality, resulting in the loss of entire litters. The toxicity of nitrofen has been associated with effects on the thyroid.⁴⁵ There have been a number of studies investigating the effects of chemicals structurally related to nitrofen (Appendix 1). The structural feature of these chemicals include the presence of a NO_2 substituent para to the ether group on one of the two aromatic rings with the other ring containing one to three halogen substituents that may be F, Cl or Br. As the number of chlorine substituents is fixed, the position of chlorine on the phenyl ring plays an important role for the developmental toxicity of the PCDE.

Generally, *ortho*-Cl substitution favors occurrence of prenatal mortality, while *para*-Cl substitution favors postnatal syndrome.⁴⁶ The structure activity relationships of the PCDEs is complex such that a new, untested PCDE should be considered to potentially exhibit developmental toxicity in the absence of additional data.

Sub-category 8e includes halogen and nitro substituted phenols and their ester derivatives. These chemicals are primarily reported to show developmental toxicity, although it is not clear they have all been adequately tested for reproductive toxicity (Appendix 1). The alert core structural features of the majority of these compounds include 2,6-halogen or 2,4-nitro phenols and derived esters. The other substituents on the phenol ring can be hydrogen, alkyl, nitrile or amine with further details described in (8e) in Figure S24.

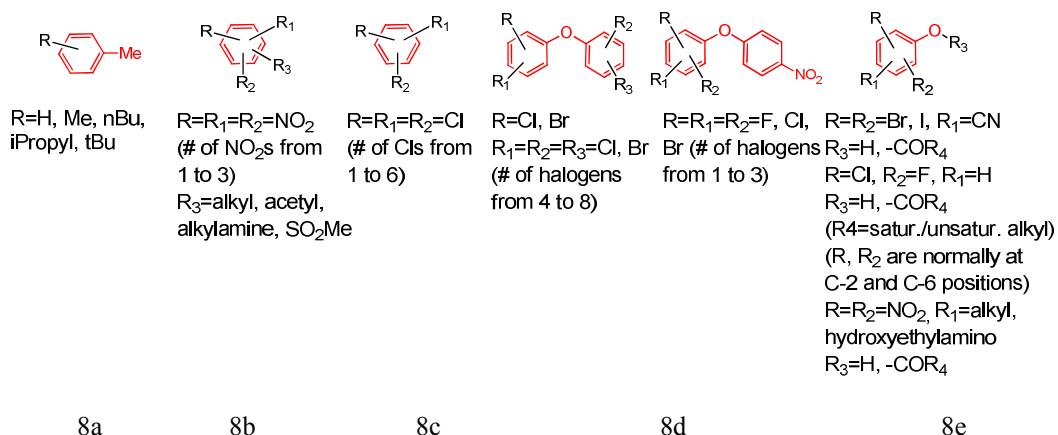


Fig. S24. The structural scope of alkyl substituted benzene, alkyl/NO₂-substituted benzene, poly-halogenated benzene, oxydibenzene, poly-halogenated, poly-NO₂ phenol and their esters.

Category 9: Aromatic compounds contain alkyl chain with alcohol, aldehyde, acid functional groups; poly-Cl aryloxy derived acids and esters

Aromatic chemicals with aliphatic carbon side chains (C1-C4) containing alcohol, aldehyde, carboxylic acid, and ester functional groups as represented by the core structures of p-t-butylidihydrocinnamaldehyde (BMHCA-like) (9a), aryl/heteroaryl substituted alkyl (C1-C3) acids (9b), and alpha aryloxy substituted acetic acid (9c), are shown in Figure S25.

9a. The *p*-tert-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA)-like chemicals.

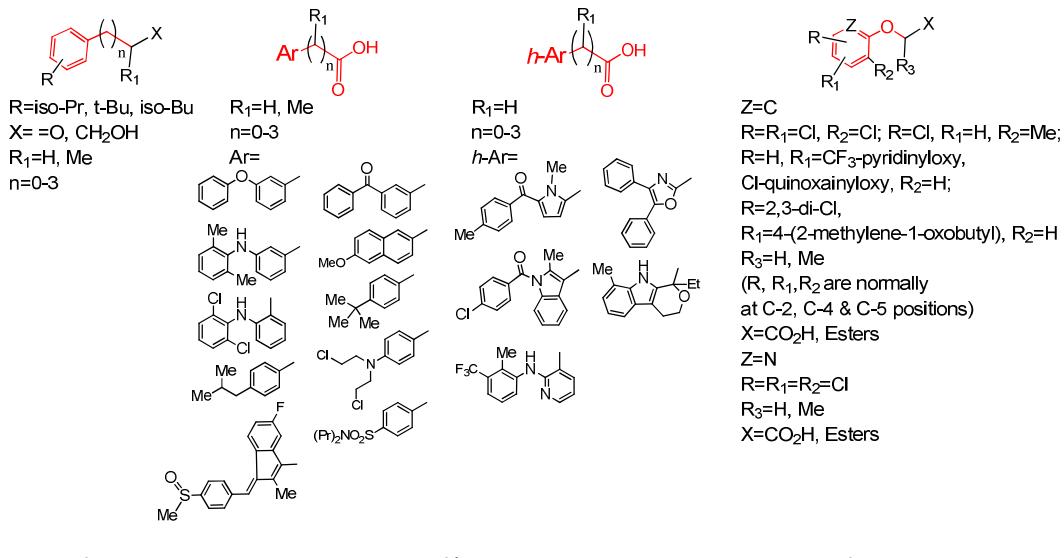
Sub-category 9a: The *p*-*tert*-butyl-alpha-methylhydrocinnamic aldehyde (BMHCA)-like chemicals (9a) induces male reproductive toxicity in animals (Appendix 1). Although the structure activity relationships for this group are not completely characterized, the chemicals and their biological data show that the substituents R on the phenyl ring at the para position must be sterically hindered alkyl groups such as *tert*-butyl, *iso*-butyl or *iso*-propyl. The functional group X is normally an aldehyde or an alcohol in a few cases. Analogs where replacement of the *p*-*tert*-butyl group (e.g. in 3-(4-*t*-butylphenyl)-2-isobutyraldehyde (CAS# 80-54-6)) with a small alkyl (e.g. 2-methyl-3-*p*-tolylpropanal (CAS# 41496-43-9) or alkoxy group (e.g. 3-(4-methoxy phenyl)-2-methylpropionaldehyde (CAS# 5462-06-6)) did not induce testicular toxicity in animals. This indicates that the substituent on the aromatic ring is one of the major requirements for this toxic effect. One non-conforming example is that 2-phenylpropionaldehyde (CAS# 93-53-8) showed similar male reproductive toxicity to the BMHCA-like chemicals, but lacks a sterically hindered alkyl group at the para position of the phenyl ring.

9b. Aryl/heteroaryl substituted alkyl (C1-C3) acids.

Sub-category 9b: This group includes the aromatic substituted small alkyl/heteroaryl (C1-C3) acids (9b) that act as non-steroidal anti-inflammatory drugs (NSAIDS). The core structural features of this sub-category support that the aryl/heteroaryl substituted alkyl (C1-C3) acid moiety plays an important role in anti-inflammatory activity. These drugs (and likely others operating by this pharmacological mechanism) appear to share a similar range of DART effects (Appendix 1). These include perinatal developmental effects such as failure to close the ductus arteriosus, high dose developmental toxicity in experimental animals including pre-implantation loss, delay of parturition, and for some, a distinctive spectrum of cardiac abnormalities. Some have also been associated with ovarian toxicity. These agents act primarily through the inhibition of prostaglandin synthesis by altering the activity of cyclooxygenase (COX) enzymes. Other NSAID drugs which are not related to this sub-category structurally (e.g. phenylbutazone (CAS# 50-33-9) like derivatives), also showed developmental toxicity at high doses. NSAIDs late in pregnancy raise the concern for development of fetal ductus arteriosus, a disorder that occurs as a result of inhibition of the prostaglandin synthesis. As a consequence of an increased right ventricular pressure, there is a risk for the development of neonatal pulmonary artery hypertension.⁴⁷ NSAIDs may play a role in female infertility. Prostaglandin inhibition appears to increase the incidence of luteinized unruptured follicle syndrome. Another potential reproductive effect of NSAIDs is a reduction in seminal prostaglandins, however, a direct link to male infertility has not been made.⁷ Data are inadequate to clearly determine if differences in reported effects across agents are due to differences in the study design, or true chemical specific differences. For example, naproxen (CAS# 22204-53-1) was not teratogenic in experimental animals in older studies. However, more recently, cleft palate and fetal death were observed among the offspring of pregnant mice, and in rats, and increased incidence of heart defects has been reported (Appendix 1). It is important to note that, some acids in this sub-category may have a very different pattern of effects than that of a corresponding aldehyde in the BMHCA sub-category, even though they share similar structural features and the acid can be also considered as an oxidative metabolite of aldehyde. For example, ibuprofen (CAS# 15687-27-1, 9b, R=isobutyl, R₁=methyl and X=CO₂H) is a weak developmental toxicant and has some effects on female reproduction, in contrast to the BMHCA like chemicals 3-(4-isobutylphenyl)-2-methylpropanal (CAS# 6658-48-6, 9a, R=isobutyl, R₁=methyl and X=CHO), which are primarily male reproductive toxicants. The compound chlorambucil (CAS# 305-03-3) is included in this sub-category because it contains a C4 carboxylic acid side chain connected to a (bis(2-chloroethyl)amino)phenyl group Ar group and also is a potential alkylating agent that has shown developmental toxicity.

9c. Alpha aryloxy substituted acetic acid.

Sub-category 9c: The third sub-category of related structures is the alpha aryloxy or heteroaryloxy substituted C2-C3- aliphatic acid derivatives (9c). The structural features of this sub-category require attachment of the aryloxy or heteroaryloxy group to the alpha position of the aliphatic acids. The aryloxy or heteroaryloxy groups of the majority of these chemicals are 2,4-di/2,4,5-tri-Cl phenoxy (Z=C) and pyridinyloxy (Z=N) moieties. Details of substituents associated with activity are enumerated in (9c). Examples of these chemicals include herbicides, which are weak DART toxicants when tested as purified materials (Appendix I). Although some chemicals in this group were initially thought to be potent reproductive toxicants, it was later determined that the test material had been contaminated with dioxins.⁴⁸



9a

9b

9c

Fig. S25. General core structures of BMHCAs (9a), aryl/heteroaryl substituted alkyl (C1-C3) acids (9b) and alpha aryloxy substituted acetic acids (9c).

As described above, we deliberately grouped the corresponding alcohol-, aldehyde-, acid- and ester-derivatives in the same sub-category, since it is possible that the functional groups of these chemicals might be metabolized to each other via oxidation, reduction or hydrolysis pathways.

Category 10: Aromatic compounds with sulfonamide and urea moieties, phenytoins

We group these chemicals into two sub-categories based on core structures which include the following: *a.* aryl heteroaryl substituted sulfonamides (10a-1, 10a-2, 10a-3), and ureas (10a-4); and *b.* phenytoins (10b).

10a. Aryl, heteroaryl substituted sulfonamides and ureas.

The general core structures of 10a are shown in Figure S26. These chemicals contain a variety of aromatic or heteroaromatic sulfonyl moieties.

For the non-*N*-substituted sulfonamides (10a-1), substituent R on the phenyl ring may be one of the following: a methyl, 2-(furanylmethyl)amino, 1-hydroxy-3-oxo-1-isoindolinyl, or a sulfamoyl group. R₁ can be hydrogen or chlorine, and R₂ can be hydrogen, chlorine, or a carboxylic acid. Within this sub-category, many of the structures studied are drugs with relatively weak DART effects which also appear to be species-specific (Appendix 1). Furosemide (CAS# 54-31-9) does not appear to be directly toxic to the developing fetus, however, developmental toxicity may result indirectly from acid/base imbalance, and additionally some peri-natal toxicity has been reported.⁷ Other members of this class inhibit carbonic anhydrase, and these chemicals appear to show species-specific effects on developmental toxicity, although the mechanism is not clearly established. The thiazide diuretics do not appear to be developmental toxicants during gestation, but have been associated with neonatal toxicity.

For the heteroaryl sulfonamides (10a-2), the variety of chemicals with heteroaryl group such as substituted 1,3,4-thiadiazolyl, ethoxy-benzothiazolyl, dihydro-1,1-dioxide benzothiadiazinyl, 1,1-dioxide benzothiadiazinyl or acetamide-1,3,4-thiadiazol-2(3H)-ylidenyl related groups are associated with the DART activity.

For the *N*-substituted sulfonamides (10a-3), most heteroaryl groups (e.g. pyrimidinyl, pyridazinyl, isoxazolyl, thiazolyl or pyridinyl group) and some small alkyl, alkoxy groups (e.g. methyl, methoxy) on the

nitrogen are associated with the DART activity. However, this sub-group is limited to the 4-amino (or amino precursor) substituted phenylsulfonamide derivatives, which are the sulfa anti-infectives. These chemicals show a variety of DART effects including male infertility, teratogenicity in some species and fetal toxicity. In addition, there are concerns for displacement of bilirubin from protein binding sites by this sub-category of chemicals, and hence are generally avoided prior to delivery (Appendix 1).

Similar to the aryl, heteroaryl substituted sulfonamides, the arylsulfonureas (10a-4) are also merged as a sub-category which showed DART activity. As shown, the aryl group can be an amino, methyl chloro or acetyl-substituted phenyl ring, and the R group can be an alkyl chain (C1-C4 carbons, e.g. propyl, n-butyl) or an aliphatic cyclic ring (5 to 7 membered ring). The R₁ group can be alkyl (C1-C4 carbons), halogen, acetyl, Ar-AcNHalkyl, or an amine and is normally at the para-position relative to the sulfonamide group. R₂ can be hydrogen or an amine group. Many of these chemicals are teratogenic in rodents (Appendix 1). However, glycyclamide (CAS# 664-95-9) has been reported to not be teratogenic in rats, in a study comparing it to carbutamide (CAS# 339-43-5) which is teratogenic.⁴⁹ No studies addressing the teratogenicity of acetohexamide (CAS# 968-81-0) were found, although some of the databases appear to cite data from glycyclamide for this chemical.

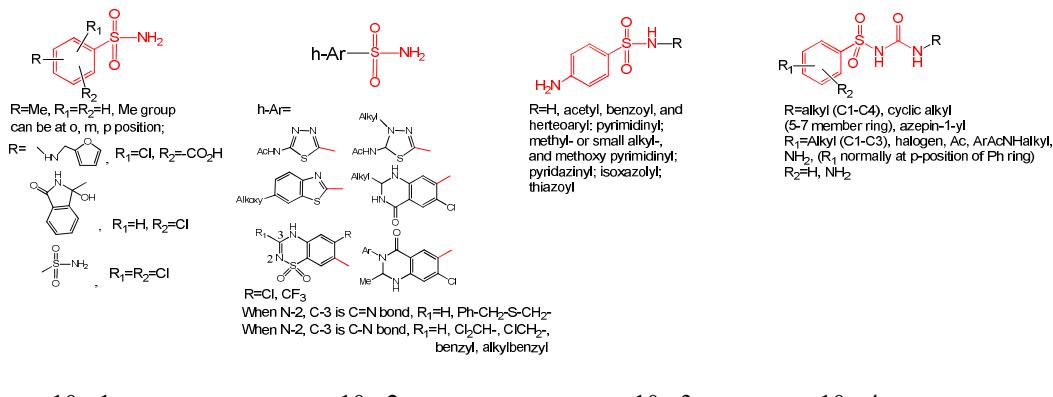
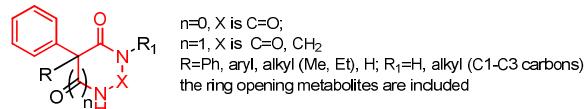


Fig. S26. The scope of structural features of aryl, heteroaryl substituted sulfonamides and ureas.

10b. Phenytoins.

Diphenylhydantoin (CAS# 57-41-0) is an anticonvulsant drug in clinical use. More than 9 diphenylhydantoin related derivatives are suspected of being teratogenic. In this sub-category, the core structural feature (10b) is the hydantoin or pyrimidinedione ring where the substituent R can be a phenyl, aryl, hydrogen or a small alkyl (C1-C3) group, and R₁ on one of the nitrogen atoms can be hydrogen or a small alkyl group (C1-C3). The ring size can be expanded from five (n=0) to six (n=1), where X can be a methylene or carbonyl group, producing compounds shown to exhibit teratogenic activity. Furthermore, the ring opening product of phenylhydantoin, phenylacetylurea (63-98-9) is also a teratogenic toxicant. (Figure S27).

Diphenylhydantoin produces a spectrum of teratogenic effects in rodents. The mechanism for these effects is debated with both reactive oxygen and effects on the retinoic acid system hypothesized.⁶ The primary phenytoin metabolite, hydroxyphenytoin (CAS# 2784-27-2) is reported to not be teratogenic, which is consistent with phenytoin teratogenicity being potentiated by inhibition of metabolism.⁵⁰ Brown et al.⁵¹ report on a series of structurally related chemicals similar to diphenylhydantoin, most of which were teratogenic in rodents (Appendix 1). There are some reports that phenobarbital (CAS# 50-06-6) can adversely impact male reproductive function.⁷ No studies of reproductive toxicity were located for other members of the sub-category. In addition, some members of the sub-category have been suggested to be folic acid antagonists.⁷



10b

Fig. S27. The scope of structural feature of diphenylhydantoin derivatives.

Category 11: Aromatic compounds (non-fused ring system) with aliphatic amine moieties

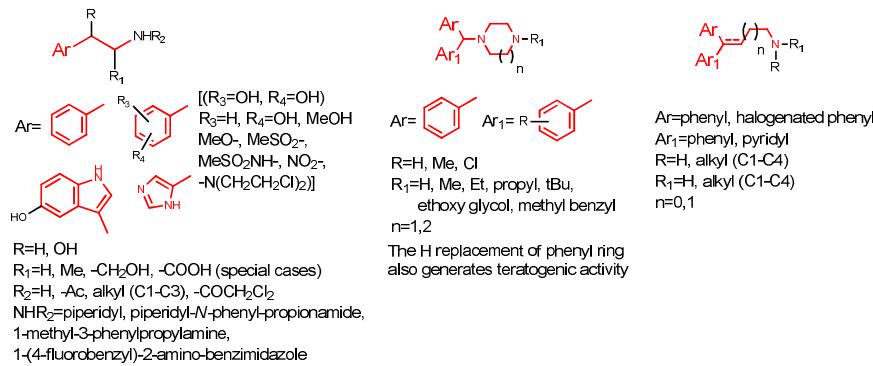
The aromatic chemicals containing an aliphatic amine moiety include two different sub-categories, and are exemplified by arylethanamine (11a) and cyclizine-like derivatives (11b) in Figure S28.

11a. Arylethanamine-like derivatives.

The generic core structural feature for arylethanamine derivatives associated with DART activity is the separation of the aromatic ring and amine group by two carbons as shown in (11a). Our dataset contains more than 15 of these chemicals (Appendix 1). These chemicals include a variety of aryl/heteroaryl groups including phenyl, hydroxyphenyl, nitrophenyl, sulfonyl-phenyl, di-hydroxy phenyl, imidazol-2-yl, or indol-2-yl. The substituents R may be hydrogen or a hydroxyl group, and R₁ may be hydrogen, a small alkyl (Me, Et) or a hydroxymethyl group. Also, a single case is noted where R₁ is a carboxylic acid. R₂ is hydrogen, a small alkyl (C1-C3), an acetyl or a chloroacetyl group. The arylethylamine structural backbones may be critical for DART activity for this sub-group of chemicals. This is supported by a fact that almost all of these collected chemicals in the data set, containing arylethylamine structural features, despite different substituents either on the aromatic ring or on the alkyl side chain, show DART activity. However, the sterically hindered or alkyl aromatic substituents R₂ (e.g. t-butyl, propylphenol) on the amine group of hydroxyphenethyl amines abolish the DART activity. Of the aromatic amino acids, only phenylalanine (CAS# 150-30-1) (R₁=CO₂H), showed adverse fetal effects in the presence of a metabolic defect (phenylketonuria), thought to be due to the production of phenylethylamine. Other aromatic amino acids do not appear to have significant developmental or reproductive toxicity. In general, the chemicals in this sub-category are not potent reproductive toxicants, but may exhibit teratogenic and/or fetotoxic effects at maternally toxic doses. An exception is the melphalan (CAS# 148-82-3) which is a potent alkylating agent. There is a suggestion of teratogenic effects in rodents in an older foreign language publication not reviewed in ReproRisk⁷ or by us. There are also several reports of impaired ovarian function and amenorrhea in women treated with melphalan.

11b. Cyclizine-like derivatives.

The cyclizine derivatives (benzhydrylpiperazine-like compounds) (11b) in Figure S28 are a group of drugs that are purported to exert their teratogenic effects via a common teratogenic metabolite.^{52,7} Structurally, the Ar group is a phenyl ring, and the Ar₁ group may be a phenyl ring or a methyl or chloro substituted phenyl ring. There is evidence that the teratogenic effect is maintained upon replacement of a phenyl group with hydrogen (e.g. CAS# 303-26-4 vs CAS# 23145-88-2). In addition, some of the diaryl compounds containing substituted benzene or pyridine rings connected to the alkyl amine by a saturated or unsaturated C-C bond also show DART activity.



11a

11b

Fig. S28. The scope of structural features of aromatic compounds (non-fused ring system) with aliphatic amine moieties.

Category 12: Aromatic diamine, their diazo moieties, and aromatic triazene derivatives

12a. Aromatic di-amines: 2,4-diamino pyrimidine related derivatives.

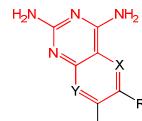
In addition to the aromatic ring substituted aliphatic amines, some aromatic di-amines and their derivatives show DART potential. Among these chemicals, we grouped more than 18 of 2,4-diamino pyrimidine (2,4-D) related derivatives as a sub-category. The core structural features of these compounds are: 2,4-diamino pyrimidine (12a-1), 2,4-diamino pteridine, 2,4-diamino quinazoline and 2,4-diamino pyridopyrimidine (12a-2) in Figure S29. The substituents associated with activity are shown below. The substituent R_1 at the C-6 position of 2,4-diamino pyrimidine is normally an alkyl group of 1 to 5 carbons and R at the C-5 position is a phenyl or a halogenated phenyl group. However, replacement of the phenyl, halogenated phenyl or di- tri-methoxybenzyl groups with nitro benzyl or an aryloxy group at the C-5 position of 2,4-diamino pyrimidine (e.g. 6-methyl-5-(4-nitrobenzyl)pyrimidine-2,4-diamine (CAS# 7331-21-7), 5-(4-chlorophenoxy)pyrimidine-2,4-diamine (CAS# 7331-20-6), 5-(4-chlorophenoxy)-6-methylpyrimidine-2,4-diamine (CAS# 7331-19-3) vs CAS#s 7331-25-1, 18588-50-6, 7761-45-7, 5355-16-8, 738-70-5 and 56518-41-3) resulted in no teratogenic activity. Many of the 2,4-diamino pyrimidine derivatives are either antimalarial drugs, analogs of these drugs, or antineoplastics and act as folate inhibitors. Published test data are for many of the antimalarial analogs. These studies are old, and in many cases we were not able to access the full text of the publications. These compounds have been associated with both teratogenic and embryotoxic effects, as well as adverse effects on sperm production (Appendix 1).

The 2,4-diamino pyrimidine core structure can also be extended to 2,4-diamino pteridine, 2,4-diamino quinazoline and 2,4-diamino pyridopyrimidine (12a-2). The core structural features of these chemicals include bi-heteroaromatic ring moieties (e.g. both X and Y are nitrogen, carbon or X is nitrogen and Y is carbon) with either alkyl, phenyl or alkylamino aromatic substituents. This group shows a range of potency and effects, from potent teratogenic and fetotoxic effects to weak fetotoxic effects only present at maternally toxic doses. Some of these chemicals have been reported to show male reproductive effects.



$R_1=H$, alkyl (C1-C5)
 $R=Ar$, alkyl-Ar, alkyl
 When $R_1=H$, $R=$ aryl methyl
 (Aryl methyl is normally di-, tri-methoxyphenylmethyl or methoxy-, halogen phenylmethyl), quinolinyl methyl

$R_1=alkyl$, $R=Ar$
 (Ar is normally phenyl, mono-, or poly-halogenated phenyl)



When $X=Y=N$
 $R_1=NH_2$, $R=phenyl$
 or when $R_1=H$, $R=$
 $\begin{array}{c} \text{CO}_2\text{H} \\ | \\ \text{Z}-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H}) \end{array}$
 $\begin{array}{c} \text{Me} \\ | \\ \text{Z}-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H}) \end{array}$
 $\begin{array}{c} \text{Et} \\ | \\ \text{Z}-\text{C}(=\text{O})-\text{NH}-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H})-\text{CH}(\text{CO}_2\text{H}) \end{array}$
 where $Z=N, C$
 When $X=Y=C$
 $R_1=H$, $R=\text{Z}-\text{C}(=\text{O})-\text{NH}-$
 When $X=C$, $Y=N$
 $R_1=H$, $R=isopropyl$

12a-1

12a-2

Fig. S29. The scope of structural features of 2,4-diamino pyrimidine related derivatives.

12b. Aromatic di-amine derived diazo dyes

Most of the compounds in this sub-category are the benzidine diazo derivatives, and methyl, dimethyl or diethylaminoazobenzene derivatives, which are represented by the general core structures (12b-1) and (12b-2), respectively in Figure S30. The benzidine diazo derivatives shown in (12b-1) generally gave variable results in teratogenicity testing, including teratogenic, fetotoxic and/or male reproductive effects. The benzidine and methyl, methoxyl and chlorine substituted benzidine as well as diaminofluorenone (which could be considered a benzidine derivative with two aromatic rings connected by a carbonyl group) and derived diazo compounds (12b-1) have been associated with DART activity. In these cases, aryl groups (Ar & Ar_1) in (12b-1) are normally sulfonic acid substituted amino naphthalene, aminonaphthalenol, diamino phenyl or hydroxybenzoic acid. The substituents R on the benzidine ring are generally hydrogen, methyl, methoxy and chlorine groups.

Furthermore, diaminodimethoxybenzene derived diazo derivatives, exemplified by (12b-2) have also shown DART activity. The dialkylaminoazobenzene derivatives (12b-3) have been subject to very little testing for DART endpoints, although some evidence of teratogenic effects following parenteral exposure has been reported. The limited data available indicate that chemicals with core structural features of (12b-3) where the substituent R is methyl or ethyl, and the R_1 group is trifluoromethyl, fluorine, chlorine, methyl, or hydrogen are potentially teratogenic.

12c. Triarylmethane dyes.

In addition to aromatic di-amine derived azo dyes, some triarylmethane dyes may show weak DART effects. As shown in (12c) in Figure S30, the triarylmethane dyes contain a triphenylmethane backbone. Since only a few members of this group have some DART data, it is difficult to define the general structural requirements for DART effects. The active chemicals normally have amine or N -substituted amine R functional groups at the para-position of at least two phenyl rings. The R_1 and R_2 groups on the nitrogen may be small alkyl groups (e.g. Me or Et). However, no DART activity has been observed for compounds containing a benzylamine R group or aryl rings (phenyl or naphthyl) with sulfonic acid or carboxylic acid substituents.

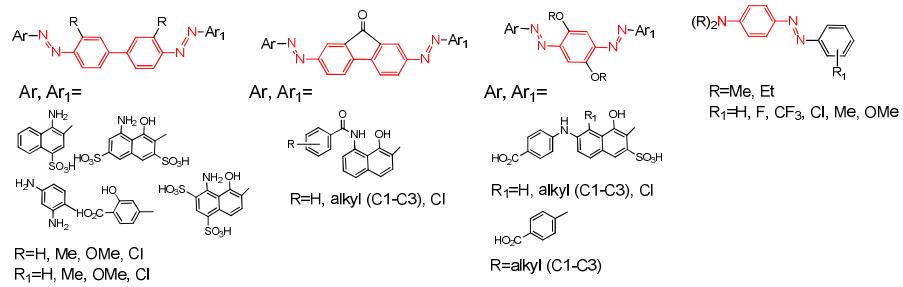


Fig. S30. The scope of structural features of 2,4-diamino pyrimidine, benzidine diazo and methyl dimethyl or diethylaminoazobenzene derivatives.

12d. Aryl triazene derivatives.

The phenyl and chlorophenyl triazenes and pyridyl, chloropyridyl and oxidepyridyl triazines are teratogenic. These chemicals are generally alkylating agents which may share the same bioactivation mechanism as the nitroso ureas (see section 3.2.21b). These triazene derivatives are represented by general structure (12d) in Figure S31 where X can be CH, N or a N=O group and Y can be CH or N and the heteroaromatic ring may be five- or six-membered. The R group can be hydrogen or chlorine. The N,N-dialkyl R₁ and R₂ groups are mainly methyl and ethyl substituents but may include one or two propyl groups. Due to the lack of testing data, the structural features of these chemicals are narrowly defined. The general core structural requirement for causing DART effects is the aryl triazine moiety. Because these chemicals are alkylating agents, they are capable of interacting with DNA via a methane (or alkane) diazonium ion (Figure S31). It could be hypothesized that cell death resulting from DNA damage could play a role in the DART effects of these chemicals.⁵³

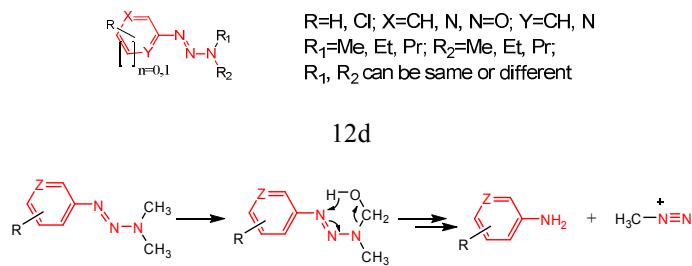


Fig. S31. The core structural features of aryl triazene derivatives and hypothesized mechanism for the formation of methane diazonium ion.

Category 13: Imidazole, nitro imidazoles derivatives, nitro-furfurylideneamino and triazole derivatives

13a. Imidazole derivatives.

Imidazole derivatives, especially the antifungal agents, represent another sub-category of chemicals exhibiting DART effects in experimental animals. The core structural features (13a-1) and (13a-2) are

shown in Figure S32. In (13a-1), the core structures are both the phenethyl imidazole (where n=1) and phenylmethyl imidazole (where n=0) with substituents associated with activity enumerated in Figure S32. In (13a-2), the core structural feature is either phenoxyethyl imidazole where X is an oxygen atom or (imidazoleyl methylene)aniline where X is a nitrogen atom with substituents associated with activity also shown.

Some chemicals in this sub-category are teratogenic/fetotoxic at high doses and also appear to have anti-androgen effects (Appendix 1). Ketoconazole (CAS# 65277-42-1) decreases testosterone synthesis, which in humans has been hypothesized to be due to inhibition of C17-20 lyase. Prochloraz (CAS# 67747-09-5) feminized the male offspring after perinatal exposure, which has been attributed to diminished fetal steroidogenesis.¹⁶ Scott et al.¹⁷ concluded that prochloraz can suppress testosterone production in the fetal and postnatal rat by inhibiting CYP17 enzyme activity. Other imidazole related chemicals such as the methythio, phenylamino substituted imidazolone derivative (CAS# 161326-34-7) also showed DART effects.

13b. Nitro imidazoles derivatives, nitro-furfurylideneamino derivatives.

Nitro-imidazole derivatives are reported as male reproductive toxicants. As shown in (13b-1) in Figure S32, the core structural requirement for the DART effects is nitro imidazole with various substituents on the carbon and nitrogen (N-1 position) associated with DART activity. Like the nitro-imidazoles, the nitro-furfurylideneamino derivatives with oxazolidinone, urea and 1,1-dioxidethiomorpholine substituents shown in (13b-2) in Figure S32 such as furazolidone (CAS# 67-45-8), nitrofurazone (CAS# 59-87-0) and nitrofurtimox (CAS# 23256-30-6) are also reproductive toxicants, causing testicular toxicity.⁷ However, they appear not to be teratogenic in experimental animals.

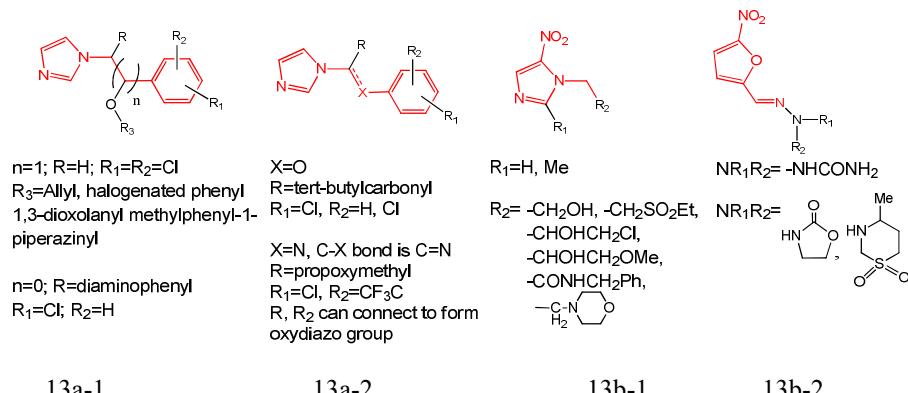


Fig. S32. The core structural features of imidazole, nitro imidazoles, nitro-furfurylideneamino derivatives

13c Triazole derivatives

Triazole-derivatives are antimycotics used in agriculture as well as in clinical and veterinary therapy. They all have a triazole ring with nitrogen atoms at the 1, 2, and 4 positions. As shown in Figure S33, these compounds can be divided into two groups. The first group includes simple triazole derivatives represented by (13c-1), where R is hydrogen or an amino group. The second group includes triazole antifungal drugs and agricultural triazole fungicides represented by (13c-2) and (13c-3). In (13c-2), the core structural requirement is attachment of the methyl triazole moiety to a tertiary or quaternary carbon with substituents listed in Figure S33. In (13c-3), the core structural feature is attachment of the triazole and substituted phenoxy rings to the C-1 position of 3,3-dimethyl-butan-2-one or butan-2-ol where R represents a carbonyl or alcohol group, respectively. The aryl (Ar) group can be a chlorine- or phenyl-substituted phenoxy group. This sub-category shows both teratogenic and fetotoxic effects, with a wide range of potency (some members only showing effects at maternally toxic doses). Effects on thyroid hormone status and testosterone synthesis have been reported.

Machera⁵⁴ reviewed the developmental toxicity of some of the triazole fungicides. Of note, the isosteric replacements of carbon by silicon in (CAS# 117546-98-2) generated the silicon related triazole fungicide flusilazole (CAS# 85509-19-9), which showed fetotoxic effects.⁴⁸ From a chemistry perspective, this is a rather natural replacement, as silicon and carbon belong to the same group of the Periodic Table (IVB), and therefore have many chemical similarities. Therefore, the carbon isosteric analog (CAS# 117546-98-2) of flusilazole may share similar DART effects and is included in this group.

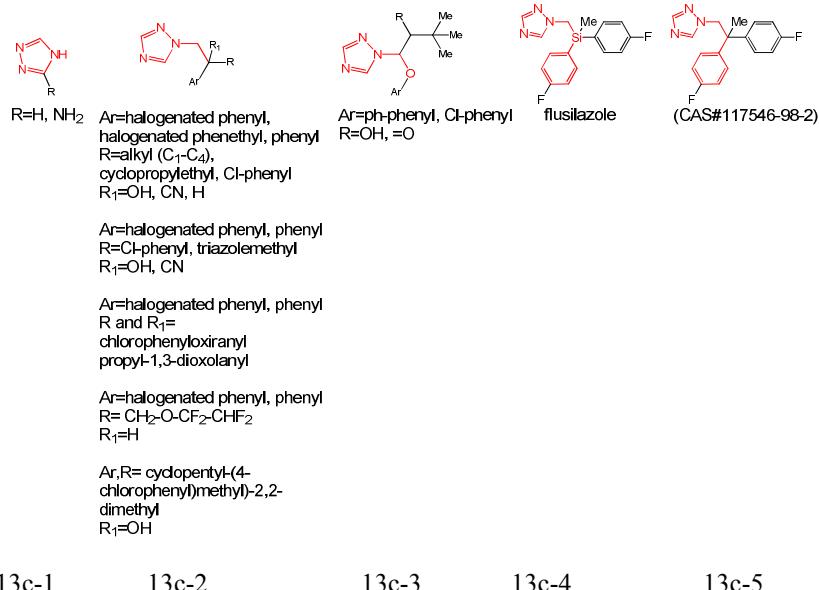


Fig. S33. The core structural features of triazole derivatives.

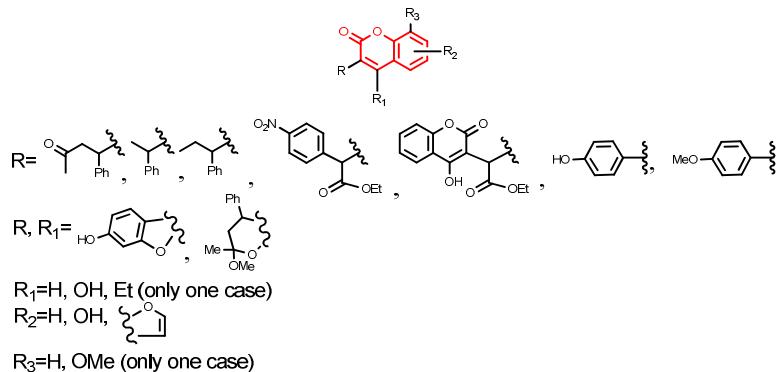
Category 14: Aromatic ring fused cyclic-, heterocyclic derivatives

A number of aromatic ring fused cyclic-, and heterocyclic derivatives were identified and divided into the following four major structurally distinct chemical sub-categories: *a* bicyclic compounds, which include aryl fused O-containing heterocycles; *b* aryl fused N-containing heterocycles; *c* tricyclic compounds, and *d* tetracyclic compounds.

14a. Bicyclic compounds with aryl fused O-containing heterocycle-coumarin-related derivatives.

A number of coumarin derivatives such as warfarin (CAS# 81-81-2), but not coumarin (CAS# 91-64-5) itself, are used as anticoagulants and show CNS teratogenic and embryotoxic effects. The representative core structure (14a) is shown in Figure S34 where R on the coumarin ring may be hydrogen or a phenyl substituted alkyl or ketone moiety, a nitrophenyl-3-oxobutyl, 2-(hydroxycoumarinyl)-1-ethoxy-1-oxoethan-2-yl, hydroxy-, or a methoxy-phenyl group. R and R₁ groups may contribute to benzofuro, or 3,4-dihydro-2-methoxy-2-methyl-4-phenyl-2H,5H-pyrano rings. The R₁ group may be hydrogen, a hydroxyl or an ethylgroup (only one case). The R₂ group may be hydrogen, a hydroxyl or a furo group, and R₃ may be hydrogen or a methoxy group (only one case). The hydroxyl substituted coumarin ring is apparently necessary for teratogenicity and substituents associated with activity are shown below. Furthermore, it is noted that the stereoisomers of these chemicals and the dimer forms of hydroxycoumarin, such as bis-hydroxycoumarinyl methane and bis-hydroxycoumarinyl acetate also show similar teratogenic activity. It has been hypothesized that disruption of vitamin K homeostasis may be important in the mode of action, since skeletal defects and fetal hemorrhage have been reported. Coumestrol (CAS# 479-13-0) is not used in

human therapeutics, but instead is a phytoestrogen encountered in the diet, and clearly has very different effects than the anti-vitamin K therapeutics, including estrogenic activity⁵⁵ and toxicity to the ovary.⁵⁶



14a

Fig. S34. The scope of structural features of coumarin derivatives.

14b. Bicyclic compounds with aryl fused N containing heterocycle.

14b-1. Thalidomide related derivatives.

Thalidomide (CAS# 50-35-1) is one of the most notorious human teratogens. Many thalidomide derivatives (Figure S35) and their metabolic products, have shown teratogenicity. For these chemicals, the core structural feature is the isoindolinedione or isoindolineone moiety (14b-1-1) and (14b-1-2). Around the isoindolinedione core structure, the chemicals can have a variety of substituents at the N-1 position including a methyl, phenyl, butyl, piperidinedione or piperidinone group. The substituents on the isoindolinedione also include amine or nitro groups. It should be noted that not all members of the group are anticipated to be potent teratogens. However, evidence suggests that the metabolites of thalidomide derivatives may be responsible for the teratogenicity. Therefore, the corresponding metabolites generated by opening of the piperidinedione/piperidinone (R₁) rings, for example, the N-1 substituted glutamic acid/esters, butyric acid, butyramide, (2S)-butanedionic acid, (2S)-pentanedioic acid and 2-acetic acid derivatives, are allocated to this sub-category. There have been a number of suggested modes of action for thalidomide, but inhibition of angiogenesis is the mode of action that has the greatest weight of evidence supporting it.⁵⁷ Appendix 1 illustrates the structures of a variety of analogs and their DART effects. SAR analysis has shown that N-1 substituents are important determinants of teratogenicity. For instance, replacement of methyl, phenyl, or 2-acetic acid R₁ groups with n-butyl, pyridinyl, or 2-acetamide groups respectively as well as replacement of the piperidoldione/piperidolone moieties with tetrahydrodioxopyran generate inactive compounds. Furthermore, the isoindoledione core structure also plays an important role where reduction of the phenyl ring or replacement of one of the carbonyl groups with a sulfonyl group removes teratogenicity. Finally, a variety of data suggest that stereospecific conformation plays a role in the developmental effects of thalidomide. For example, only the d-stereoisomeric form of thalidomide appears to be active as a teratogenic toxicant.⁵⁸ Ockenfels⁵⁹ have reported that *N*-phthaloyl-L-aspartic acid (CAS# 42406-53-1) caused fetal resorptions, as well as malformations of the surviving fetuses. Under the same conditions, *N*-phthaloyl-D-aspartic acid (CAS# 62249-41-6) had no significant effects on fetal resorption or malformation rates. The thalidomide metabolite *N*-phthalyl-L-glutamic acid (CAS# 340-90-9) was teratogenic in mice. The D-isomer (CAS# 22911-88-2) was not teratogenic. EM-12 (CAS# 26581-81-7) (sometimes designated alpha-EM-12) appears to be a very potent, stereo-specific teratogen in primates eliciting a similar spectrum of malformations as does thalidomide.⁶⁰ In contrast, beta-EM-12 (or EM-16, CAS# 26581-91-9) is reported not to be teratogenic.⁶¹ In addition, the isoindolineone derived chemicals (CAS# 14b-1-2) with structural features close to those of thalidomide such as lenalidomide (CAS# 191732-72-6) and 2-(2-oxopiperidin-3-yl)-3H-isoindol-1-one (CAS# 79458-80-3) appear to be DART toxicants.

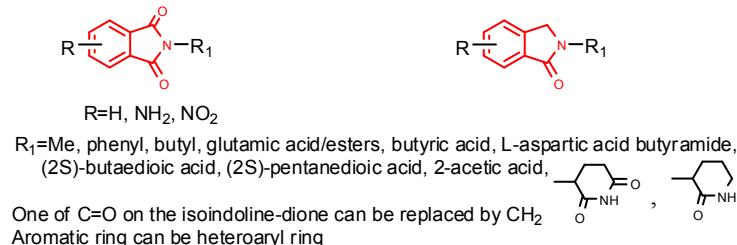


Fig. S35. The scope of structural features of thalidomide derivatives.

14b-2. Quinolones.

The synthetic broad-spectrum antibacterial drugs including quinolones (14b-2-1) and fluoroquinolones (14b-2-2) shown in Figure S36, act by inhibiting bacterial DNA gyrase with several different factors leading to teratogenic and fetotoxic concerns. The fluoroquinolones cross the human placenta⁶² and can theoretically have mutagenic and carcinogenic effects on the developing fetus. The representative core structures in (14b-2-1) and (14b-2-2) show that most quinolones have an amine containing heterocyclic ring at the C-7 position. Various substituents at the C-8 and N-1 positions, including heterocyclic rings, may generate DART activity. The chemicals listed below are associated with fetotoxic or developmental toxicities.⁶³⁻⁶⁸

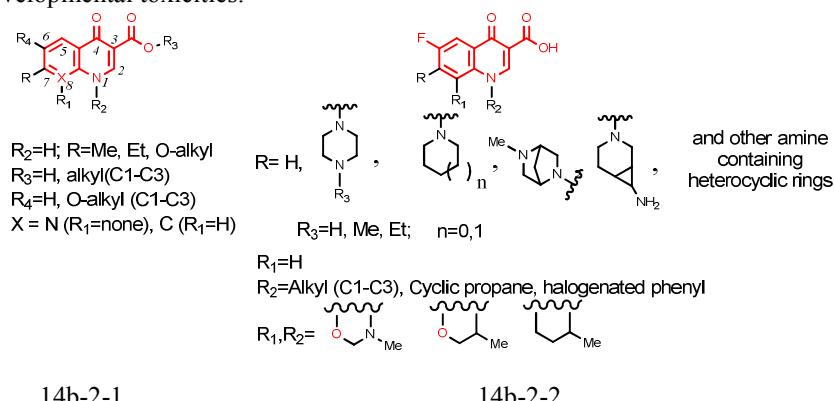


Fig. S36. The core structural features of quinolone derivatives.

14b-3. Benzodiazepines.

The antianxiety agents, benzodiazepines, may also show developmental toxicity. These chemicals had been considered to be human teratogens, based on some early data that have now been discounted.⁷ The representative core structures (14b-3-1), (14b-3-2), and (14b-3-3) are shown in Figure S37. Substituents on the two aromatic rings and N-1, C-3 positions of the heterocyclic ring associated with activity are enumerated below. SAR results indicate that structural requirements for DART activity may be flexible enough to tolerate a simple hydrogen atom or small alkyl group at the N-1 position (R₂). In addition, halogen or nitro substituents on either one or both phenyl ring (R and R₁), as well as attachment of an additional heterocyclic ring (e.g. 14b-3-2) on to the basic benzodiazepine nucleus, display developmental toxicity. Important routes for the metabolism of benzodiazepines involve the introduction of a hydroxyl group at the C-3 position in the heterocyclic ring (e.g. 14b-3-1, R₃=OH) and oxidation of the N-4 nitrogen to a nitroso group (e.g. 14b-3-3). The fact that these metabolites also show DART activity opens the possibility that it may be these metabolites which are the active species. However, as a class, these chemicals do not appear to be potent developmental toxicants, although some members of the group are reported to be teratogenic or fetotoxic in experimental animals. In addition, developmental neuro behavior

effects have been reported for some members of this sub-category,⁷ and these effects are postulated to be associated with pharmacologic activity on neurotransmitter systems.⁷

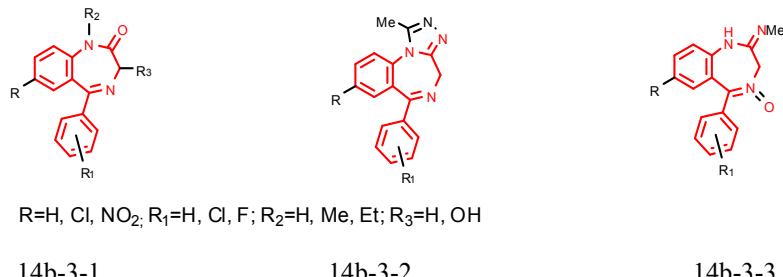


Fig. S37. The scope of structural features of benzodiazepine derivatives.

14c. Tricyclic compounds of two aryl ring fused cycles, heterocycles with alkyl amine moieties

The two aryl ring-fused cycles, heterocycles with alkyl amine moieties such as tricyclic psychomimetic drugs, represented by generic core structural features (14c-1) through (14c-6) are shown in Figure S38. In this sub-category, the two aryl groups can be fused to a saturated or unsaturated seven membered ring as in (14c-1) and (14c-2). The bond between the tricyclic ring and the alkyl amine side chain may be saturated or unsaturated. The alkyl amine group may be cyclic or noncyclic. In (14c-1), X may be carbon, oxygen or sulfur. If X is carbon, the C-X bond may be saturated or unsaturated, and if X is oxygen or sulfur, the C-X bond is saturated. The R₁ and R₂ groups may be hydrogen or a small alkyl (C1-C3) group. In (14c-2), Z may be carbon, nitrogen or sulfur. If Z is carbon or nitrogen, n is 2 and if Z is sulfur, n is 1. The R group is a small alkyl (C1-C3) group. The two aryl ring-fused heterocycles as in (14c-3) and (14c-4) which contain nitrogen or two heteroatoms (N and S) represent bioisosteres of the two aryl ring fused cycles in (14c-1) and (14c-2). For both (14c-3) and (14c-4), R groups may be hydrogen, halogen, methylsulfanyl or trifluoromethyl. The R₁ and R₂ groups may be di-methyl, methylpiperziny1, piperzinylethanol, or methylpiperdiny1. In these structures, the ethylene chain is replaced by a sulfur bridge of approximately the same size and a trigonal sp² carbon is replaced by a nitrogen atom. It was also found that phenothiazine (CAS# 92-84-2), which does not contain the alkyl amine side chain, still maintains DART effects suggesting that the two aryl ring-fused thiomorpholine moiety of (14c-4) may be essential for DART activity. Members of this group show a variety of different types of effects including teratogenicity, embryotoxicity, toxicity to the developing pancreas, behavioral effects and testicular effects. In addition, hexahydro dibenzo pyrazinoazepine and dibenzo[b,f][1,4]oxazepine derivatives (14c-5) and dibenzepine derivatives (14c-6) with the structural feature of two aryl rings fused by a nitrogen-containing seven membered ring, as well as iprindole (14c-7) which is a cyclooct[b]indole derivative are also included in this sub-category. The X in (14c-5) can be carbon or nitrogen and the R group can be a small alkyl (C1-C3) chain.

Trifluoperazine (CAS# 117-89-5), an inhibitor of calmodulin, is also thought to diminish androgen and estrogen receptor levels in the cytosol of Sertoli cells, and to inhibit a calcium and calmodulin-regulated cyclic nucleotide phosphodiesterase.⁷ Phenothiazines are associated with hyperprolactinemia which can cause menstrual irregularities and infertility in women, and galactorrhea and sexual dysfunction in both men and women.⁷

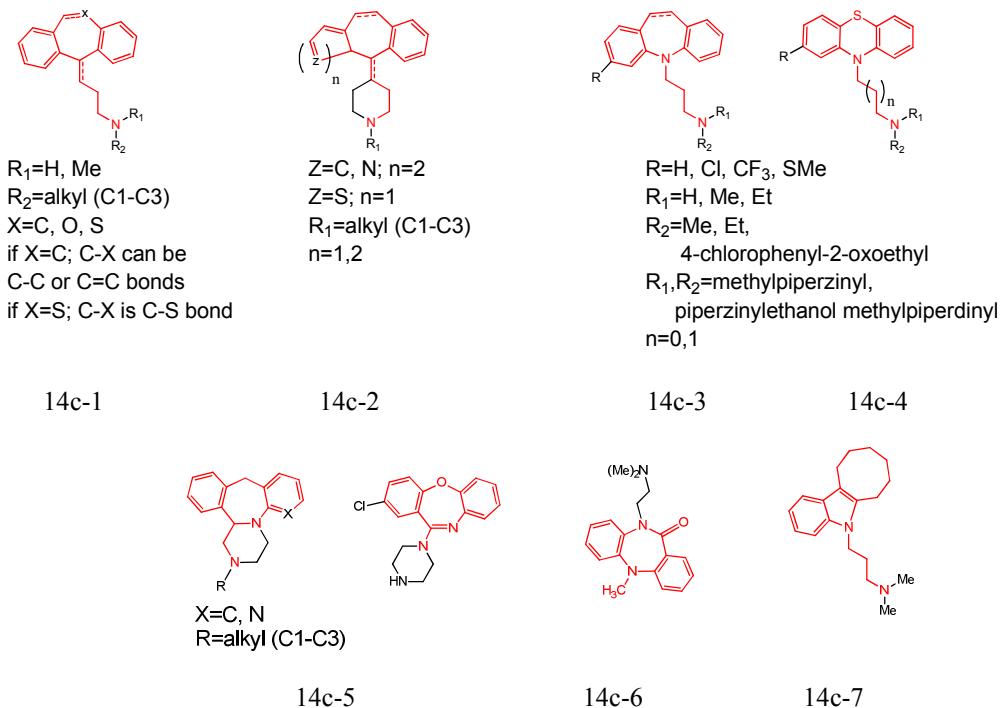
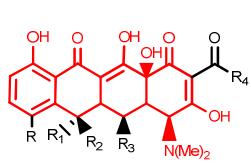


Fig. S38. The scope of structural features of Tricyclic compounds of two aryl rings fused cycles, heterocycles with alkyl amine moiety.

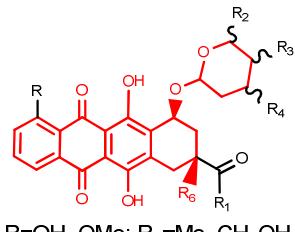
14d. Tetracyclic compounds.

Tetracycline derivatives are a group of antimicrobials that act by inhibiting protein synthesis and can be deposited in bone during development. These agents were used commonly during pregnancy, but this use was sharply curtailed after it became clear that tetracyclines can cause discoloring of the deciduous teeth when used after the 25th week of gestation and inhibition of bone growth.⁷ The general core structural requirement for DART effects is represented by tetracyclines (14d-1) and anthracyclines (14d-2) in Figure S39. The substituents on the tetracyclic ring are normally defined within the scope of the structures of these two sub-groups. Compounds with substituents listed in (14d-1) exhibit teratogenic and fetotoxic activity.

The anthracyclines (14d-2) appear to have a distinctly different pattern of toxicity than the tetracyclines. These are cancer therapeutics that act by forming complexes between topoisomerase II and DNA, preventing rejoicing of DNA strands.⁶⁹ This group of molecules is planar, and some have been shown to intercalate into DNA. The majority of anthracyclines in the dataset contain a hydroxyl R group, however, one compound has a methoxyl R group with remaining substituents listed in Figure S39. These chemicals show low dose teratogenic effects and testicular toxicity.



R=H, Cl, NMe₂; R₁=H, OH; R₂=H, Me
 R₃=OH, H; R₄=NH₂, NHR₅
 R₅=alkyl (C1-C3)



R=OH, OMe; R₁=Me, CH₂OH, CH₂OR₅ for example R₅ can be COCH₂CH₂OH
 R₂=Me, H; R₃=OH; R₄=OH, NH₂
 R₆=OH, NH₂

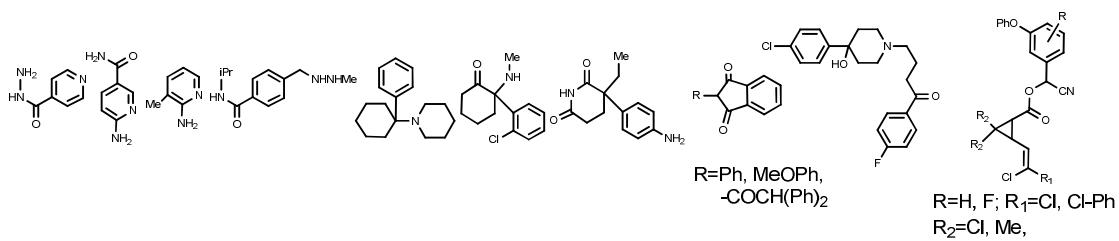
14d-1

14d-2

Fig. S39. The scope of structural features of tetracycline derivatives.

Category 15: Miscellaneous aromatic chemicals and antibiotics

The chemicals that do not belong to any of the groups mentioned above are found in miscellaneous aromatic chemicals, antibiotics and some endogenous-like chemicals. When we were unable to group four or more chemicals with DART data into a structural group, we placed them in this category. It is anticipated that, as we learn more about structure and mode of action, these chemicals will either be placed into existing groups by expanding group boundaries, or we will bring additional chemicals into the tree to form additional groups that will include these chemicals. The representative chemicals are shown in (15a-15j) in Figure S40. For example, 6-Aminonicotinamide (CAS# 329-89-5) acts as a teratogen by antagonizing niacin, which is essential for normal development.⁷⁰ We did not locate any other structures that have been shown to be teratogenic by acting as a niacin anti-metabolite. However, this raises the point that chemicals with structural similarity to essential nutrients, or endogenous chemicals, should be carefully reviewed to understand the potential for antagonism. Phencyclidine (CAS# 77-10-1) has been shown to be teratogenic in experimental animals at maternally toxic doses,⁷ and has been associated with developmental neurobehavioral changes in rodents.⁷¹ Ketamine (CAS# 6740-88-1) is reported to be fetotoxic, but not teratogenic and to cause developmental neurobehavioral effects.⁷ Synthetic pyrethroids, which are insecticides created to mimic chemical properties of naturally occurring insecticide pyrethrum, have shown fetal toxicity.



15a 15b 15c 15d 15e 15f 15g 15h 15i 15j

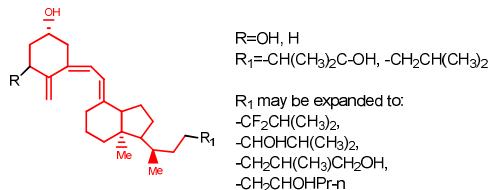
Fig. S40. Miscellaneous aromatic cyclic chemicals.

Category 16: Non-aromatic cyclic hydrocarbon ring, heterocyclic ring contain only oxygen atom and multi Cl single/fused cyclic hydrocarbons

In addition to the aromatic, heteroaromatic cyclic chemicals, a number of non-aromatic cyclic chemicals exhibit DART activity. In this category, we enumerate several sub-categories of chemicals exemplified by vitamin D3 like derivatives, tridemorph, glycidyl ether derivatives, aminoglycoside, streptomycine, macrocyclic lactone and multi Cl single/fused cyclic hydrocarbons.

16a. Vitamin D3 like derivatives.

This sub-category encompasses a group of liposoluble secosteroids, including natural and synthetic products. Vitamin D3 has multiple effects on reproductive performance and development, mediated in large part by effects on calcium regulation. The core structural feature of these vitamin D3 like derivatives is shown in (16a), Figure S41. Although limited DART data are available, the existing data indicate that the R group in (16a) can be hydrogen or a hydroxyl group, and R₁ can be a hydroxymethyl propyl or an isobutyl group with additional R₁ groups listed in the figure.



16a

Fig. S41. The core structural features of vitamin D3 derivatives.

16b. Tridemorph derivatives.

The core structural feature represented by tridemorph (CAS# 24602-86-6) is shown in (16b), Figure S42. Tridemorph shows teratogenicity, developmental toxicity and testicular toxicity.⁷² Based on the characteristics of the structures in Appendix 1, it is observed that the DART activity of these chemicals is closely related to the length of the alkyl chain on the nitrogen. Chemicals with alkyl chains ranging from 11 to 14 carbon atoms exhibit DART effects. The mechanism responsible for the DART toxicity of these tridemorphs is not known.

16c. Glycidyl ether derivatives.

Several reports established that various epoxides and glycidyl ether derivatives, represented by the general structures (16c-1) and (16c-2) in Figure S42 are DART toxicants. In the case of (16c-1), the DART toxic effects are observed if X is a hydrogen atom, a hydroxymethyl, or a chloromethyl (or other halogen) group. In (16c-2), the substituent R is normally an allyl, benzyl, butyl or other alkyl group with a chain length less than 10 carbons. Again, the mechanism responsible for the toxicity of these glycidyl ethers is not known, although the epoxide moiety is a plausible candidate for involvement given its reactivity.

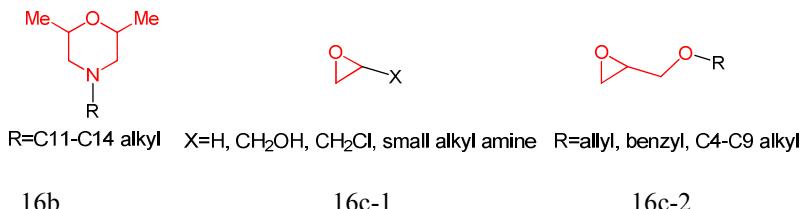


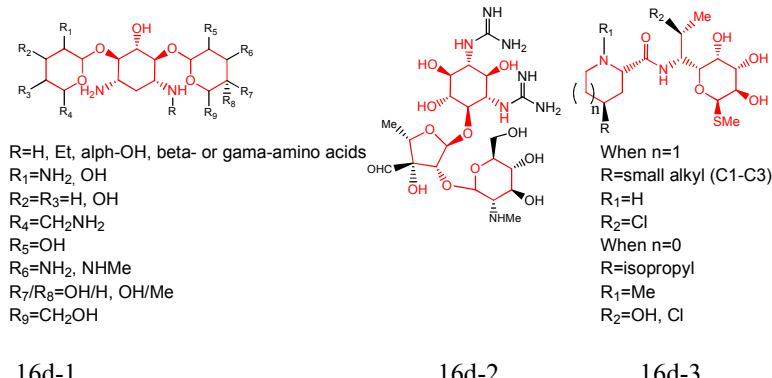
Fig. S42. The core structural features of tridemorph and glycidyl ether derivatives.

16d. Aminoglycoside antibiotics.

The large group of aminoglycoside antibiotics shows distinctive toxicity. As shown in (16d-1) in Figure S43, the core structural features of most of the aminoglycosides consist of a highly functionalized aminocyclitol, 2-deoxystreptamine (2-DOS), which connects with two monosaccharides typically at the 4 and 6 positions. The substituents (R₁-R₈) on each saccharide ring typically are hydroxyl, gem-methyl/hydroxyl, amine, hydroxymethyl, or aminomethyl groups. The number of substituents can be 2 to 4.

Generally, these chemicals are not teratogenic, however, they can produce nephrotoxicity and ototoxicity in offspring when exposure occurs during gestation.

The related antibiotic streptomycin (CAS# 57-92-1), which is shown in (16d-2), appears to share similar toxicity.⁷ Another group of lincosimide antibiotics is reported not to have teratogenic or reproductive toxicity, but is fetotoxic at high doses. The core structural feature is indicated in (16d-3). These chemicals are amides derived from substituted proline or pipecolinic acid with methyl thiolincosaminide, where R can be a small alkyl (C1-C3) group, R₁ can be hydrogen or a methyl group, R₂ can be a hydroxyl group or chlorine, and n can be 0 or 1.



16e. Macrocylic lactone antiparasitics and antibiotics.

Several classes of macrocyclic lactones antiparasitics and antibiotics such as avermectins, spiramycins and oligomycins have shown DART effects. For example, the two groups of 16-membered macrocyclic lactone derivatives, avermectin-like antiparasitics (16e-1) and spiramycin-like antibiotics (16e-2) in Figure S44, show fetotoxic toxicity. As shown in (16e-1), the core structural features of the avermectin-like derivatives consist of a 16-membered macrocyclic lactone fused with hexahydrobenzofuran and dioxaspirodecene moieties. The R and R₁ groups linked to this core structure are oligosaccharides and alkyl groups, respectively. Most of these chemicals function as chloride channel activators by binding gamma aminobutyric acid- (GABA) and glutamate-gated chloride channels and as a result, disrupting nerve signals within arthropods. The role of these effects in the MOA for DART effects in mammals is unknown.

Similar to the avermectin-like compounds, the spiramycin-like antibiotics (16e-2) which act via inhibition of protein synthesis in bacterial cells also contain a 16-membered macrocyclic lactone ring which links two or more deoxy sugars, usually cladinose and desosamine, instead of ring systems as in (16e-1).

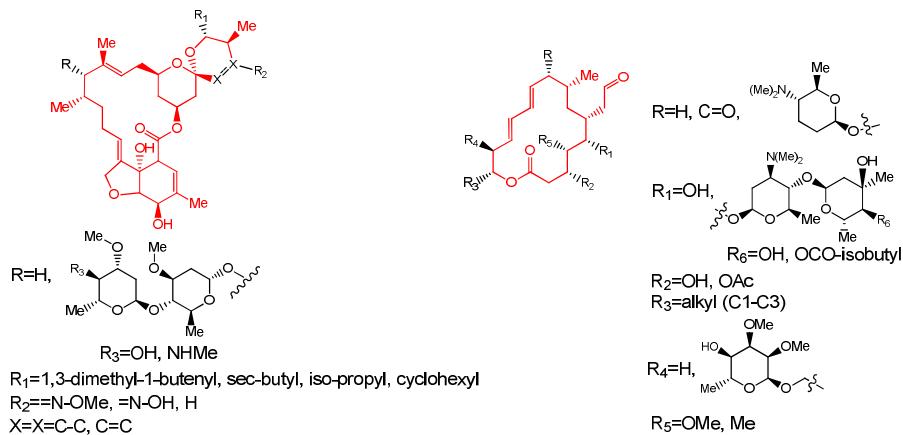


Fig. S44. The core structural features of avermectin-like antiparasitics and spiramycin-like antibiotics

16f. Polychlorinated mono- or fused/bridged-cyclic compounds.

The polychlorinated cyclic compounds are the chlorinated cyclodiene pesticides and are environmentally persistent derivatives (Figure S45). They are manufactured from hexachlorocyclopentadiene (CAS# 77-47-4) as the starting material.⁷³ The core structural features of these chemicals are indicated by (16f-1) through (16f-5). All of these chemicals contain polychlorinated single or fused/bridged cyclic rings, which can be saturated or unsaturated, with a ring size ranging from 4 to 9 carbons. One of the rings is fully substituted by chlorine, and each chemical contains at least 6 chlorine atoms. Many of these chemicals are reported to have testicular and/or developmental toxicity potential, although they do not appear to be potent teratogens. Some of these pesticides have been reported to be weakly estrogenic, but the role of estrogenicity (if any) in the reported DART effects of these chemicals is not clear.⁷⁴

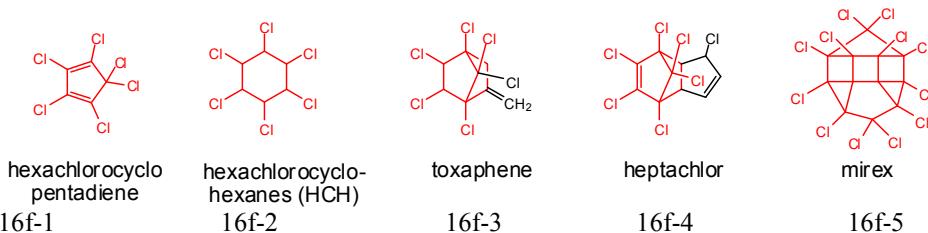


Fig. S45. The core structural features of polychlorinated cyclic compounds.

Category 17: Heterocyclic, cyclic compounds contain nitrogen, oxygen/sulfur atoms

Heterocyclic chemicals which exhibit DART potential have a broad range of structural diversity. In this dataset, we divided these chemicals into the following three sub-categories: 17a. barbital-like derivatives and ethylene thiourea (ETU), propylene thiourea (PLTU)-like derivatives; 17b. allantoin and dimethadione-like derivatives; 17c. piperazine-, dioxane-, morpholine-, tetrahydrothiopyran-like derivatives and cyclohexanamine.

17a. Barbital and ETU, PLTU-like derivatives.

In this sub-category we have included various barbiturates and ethylene thiourea-derivatives with the generic core structure of pyrimidinetrione (17a-1) or five, six membered cyclic urea/thiourea (17a-2) respectively (Figure S46).

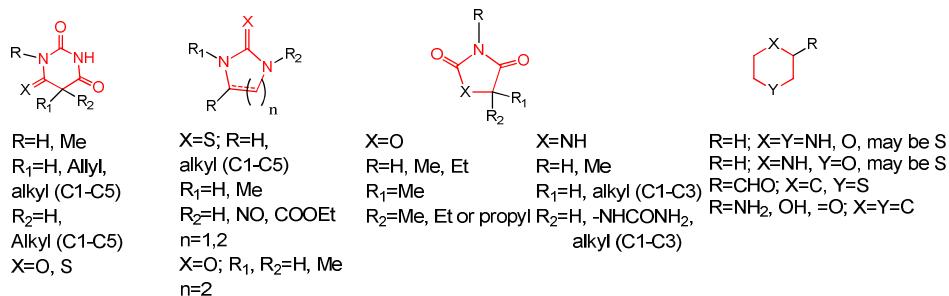
Barbiturates, as a sub-group of chemicals, are classified as developmental toxicants and as such are listed in Proposition 65 (<http://oehha.ca.gov/prop65.html>). The barbital derivatives (substituted pyrimidine derivatives in which the basic structure common to these drugs is barbituric acid (CAS# 67-52-7)) contain two more carbonyl groups. Based on the structural features (17a-1) of 8 barbital derivatives we have collected in our data set, substituents on the pyrimidine-dione/trione ring that generate activity include the following: substituent R may be hydrogen or a methyl group; R₁ may be hydrogen, an allyl or an alkyl group (C1-C5); R₂ may be hydrogen or an alkyl group (C1-C5); and X may be oxygen or sulfur. Another group of chemicals in this sub-category are ETU and PLTU-like derivatives. These chemicals are teratogenic in rats. Generally, these chemicals affect the CNS and elicit skeletal anomalies and malformations in the fetus. The core structural feature of these chemicals is ethylenethiourea (CAS# 96-45-7) (17a-2) (Figure S46) where the R group on the ring is a small alkyl (e.g. Me, Et) chain, and the nitrogen-bound substituents R₁ and R₂ are hydrogen, a methyl or a nitroso group. In addition, teratogenic activity also is preserved for carboxylic ester (COOEt) R₂ groups as well as for replacement of the saturated imidazolethione group with the unsaturated imidazolidinethione group or with the tetrahydropyrimidine-2-thione ring for which n=2.

17b. Allantoin and dimethadione-like derivatives.

The allantoin (CAS# 97-59-6) and dimethadione (CAS# 695-53-4) -like anticonvulsant derivatives, shown in (17b) in Figure S44, are reported to be fetotoxic and developmental toxicants. The core structural features of these chemicals are the oxazolidinedione (CAS# 12770-97-7) and imidazolidinedione rings for which X is O or NH, respectively. Substituents at the N-3 position and C-5 position of the oxazolidinedione and imidazolidinedione rings are generally hydrogen, or alkyl (C1-C3) groups. However, when X is NH, one of substituents at the C-5 position can be a urea group. While there are no data supporting DART toxicity for this urea derivative, (CAS# 97-59-6), it is included here because of its similar core structure.

17c. Piperazine-, dioxane-, morpholine-, tetrahydrothiopyran-like derivatives and cyclohexanamine.

Some chemicals with 6 membered cyclic or heterocyclic rings including derivatives of piperazine, dioxane, morpholine, tetrahydrothiopyran, cyclohexanamine, cyclohexanone, or cyclohexanol appear to be DART toxicants.^{75,76,77} The general structures of these chemicals are shown in (17c) in Figure S46 along with substituents associated with activity.



17a-1

17a-2

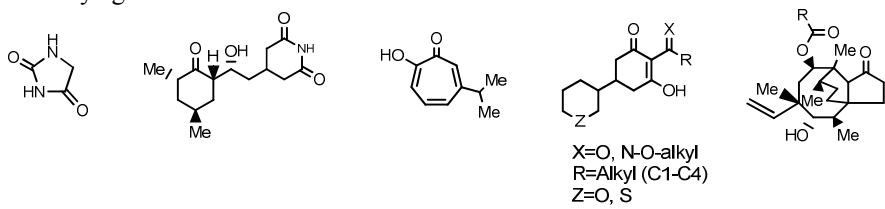
17b

17c

Fig. S46. The core structural features of barbital, ETU, PLTU-like, allantoin- and dimethadione-like derivatives as well as piperazine-, dioxane-, morpholine-, tetrahydrothiopyran-like derivatives and cyclohexanamine

Category 18: Miscellaneous cyclic chemicals

There were a number of cyclic chemicals, cyclic antibiotics and endogenous cyclic amino acids with DART effects where we were unable to find sufficient group members with data to create rules. These chemicals are exemplified by hydantoin (CAS# 461-72-3), cycloheximide (CAS# 66-81-9) and hinokitol (CAS# 499-44-5) are shown in (18a), (18b) and (18c) respectively in Figure S47, and their effects are noted in Appendix 1. Additionally, commercial herbicides described in (18d), such as tepraloxpydin, exhibit developmental and fetal toxicity. These chemicals inhibit acetyl Co-enzyme A carboxylase (ACCase) activity in the lipid biosynthetic pathway of sensitive plant species. Their MOA for DART effects in mammals is not known. The other macrocyclic antibiotics, such as mutulin derived esters (18e), also indicated the activity against bovine semen.



18a

18b

18c

18d

18e

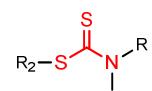
Fig. S47. Miscellaneous cyclic chemicals.

Category 19: Alkyl carbamodi-thioic acids, alkyl sulfonates and perfluorinated compounds (PFCs)

Several alkyl carbamodithioic acid derivatives are developmental and/or reproductive toxicants. The substituents on the sulfur atom of carbamodithioic acid derivatives (19a) in Figure S48 are limited to hydrogen, small alkyl (C1-C3 groups) and a dialkylthiocarbamoyl group. Substituents on the nitrogen include mono-alkyl or di-alkyl with a C1-C3 carbon chain.

Another sub-category of chemicals we have included is alkylated sulfonates. The core structural features of these chemicals are represented by (19b) in Figure S48. Similar to the carbamodithioic acid derivatives, the R₁ group of sulfonates generally is a small alkyl (C1-C3) chain, and the R group is a small alkyl (C1-C3) group or a methylene/polymethylene (C1-C4) group connected to the methane sulfonate moiety. This sub-category includes direct alkylating agents that have DART properties with particular toxicity for the bone marrow. For example, according to ReproRisk⁷ busulfan (CAS# 55-98-1) has been shown to damage mouse and human embryonic cells, probably through irreversible inhibition of DNA synthesis. Limb malformations are seen in rats treated during gestation. Busulfan is toxic to primordial germ cells and in rat models, has been used for germ cell depletion in both male and female animals. Experimental animals also show gonadal toxicity from postnatal exposure to busulfan. However, when R₁ is a hydroxyl group, such as methyl hydrogen sulfate (CAS# 75-75-2), DART effects are not reported. In general, these chemicals share a similar mechanism of action with that of methyl- or ethyl-nitrosourea and methyl- or ethyl-nitrosoguanidine (discussed in section 3.2.21b), namely, they are capable of donating either a methyl or ethyl group to nucleophiles to generate developmental⁷⁸ and testicular toxicity.⁷⁹ It is generally accepted that the genotoxic property of these alkylating agents is important to the mode of action for their reproductive and developmental toxicity.⁸⁰

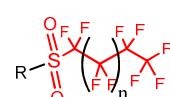
Perfluorinated compounds (PFCs) with sulfonate functional groups, were studied and showed both reproductive and developmental toxicity. The core structural features for these chemicals are indicated in (19c) in Figure S48 where R is OH, F (or another halogen), N(R₁R₂), or OR₃. R₁ and R₃ are alkyl groups with 1 – 4 carbons, and R₂ is a hydroxyethyl group. The alkyl chain length can range from 4 to 10 carbons (n=1-6), and PFCs with longer chain lengths, such as perfluorooctanesulfonate (PFOS) (C8) tend to be more toxic than those with shorter chains (C4). The crystal structure of the HAS-PFOS complex indicates that the strongly polar sulfonyl group interacts with hydrophilic residues of HAS, while the perfluorinated carbon tail interacts with adjacent hydrophobic residues of HAS.^{81,82}



R=Alkyl (C1-C3)
R₁=H, Me, Et
R₂=H, -S-CS-N(RR₁)



R=alkyl (C1-C3),
-(CH₂)_n-O-SO₂R₁ (n=1-4)
R₁=Alkyl (C1-C3)



R=OH, F (or halogen), N(R₁R₂), OR₃
(R₁, R₃=C1-C4 alkyl, R₂=CH₂CH₂OH)
n=1-6

19a

19b

19c

Fig. S48. The scope of structural features of alkyl carbamodithioic acids and methylated sulfonates and perfluorinated compounds (PFCs).

Category 20: Miscellaneous non-cyclic chemicals

Six chemicals are included in this category, since the existing data indicate that they are reproductive and/or developmental toxicants, but we were unable to put these into other groups. The structural features of these chemicals (20a-20e) are shown in Figure S49, and the individual chemical data are listed in Appendix 1.

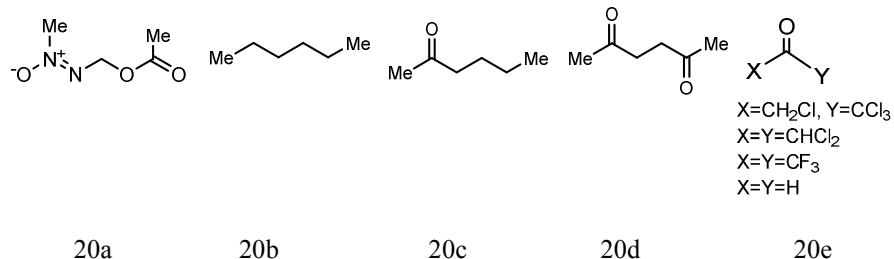
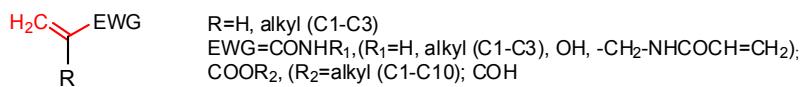


Fig. S49. Miscellaneous non-cyclic chemicals

Category 21: Vinyl amides, aldehydes, esters and alkyl amides (<C4), *N*-substituted amides, ureas, carbonates, guanidine and carbamates

21a. Vinyl amide, aldehyde and ester derivatives.

Vinyl amides, aldehydes and esters represented by the general core structure (21a) shown in Figure S50 have been reported to exhibit teratogenic toxicity. From the structural point of view, most of the chemicals in this sub-category are Michael acceptors, which have an activated alpha-beta unsaturated carbon-carbon bond, and are capable of interacting with nucleophilic sites of DNA and proteins. Based on the structural scope of compounds assessed in this study (Appendix 1) these double bonds can be unsubstituted, or substituted by small alkyl groups (C1-C3, e.g. R₁=Me or Et), and the electron withdrawing groups (EWG) attached to the double bond can be amide, *N*-alkyl amide, aldehyde, or ester groups (e.g. -CONHR₁, (R₁=H, alkyl (C1-C3), OH, -CH₂-NHCOCH=CH₂); -COOR₂, (R₂=alkyl (C1-C10); COH). Although the mechanism of causing teratogenicity by these chemicals is unknown, it was suspected to relate to the genotoxicity or mutagenicity.



21a

Fig. S50. The scope of structural features of vinyl amides, aldehydes and esters derivatives.

21b. Alkyl amide, urea, thiourea, nitroso urea, carbonate, guanidine and carbamate derivatives.

Many small alkyl amides (<C4), small *N*-alkyl amides, ureas and *N*-nitrosoureas, thioureas, carbonates, carbamates and guanidines show DART effects. The general structural requirements for these chemicals are represented by (21b-1 a-g) shown in Figure S51 with the following seven groupings and their associated substituents that have been linked to DART activity: *a*. Alkyl formamide analogs *b*. Alkyl amide or thioamide analogs *c*. Alkyl urea, nitroso urea or semicarbazide compounds. *d*. Thiourea analogs. *e*. Carbonate compounds. *f*. Carbamate analogs (also include alkyl attached two carbamate functional groups). *g*. Guanidine analogs.

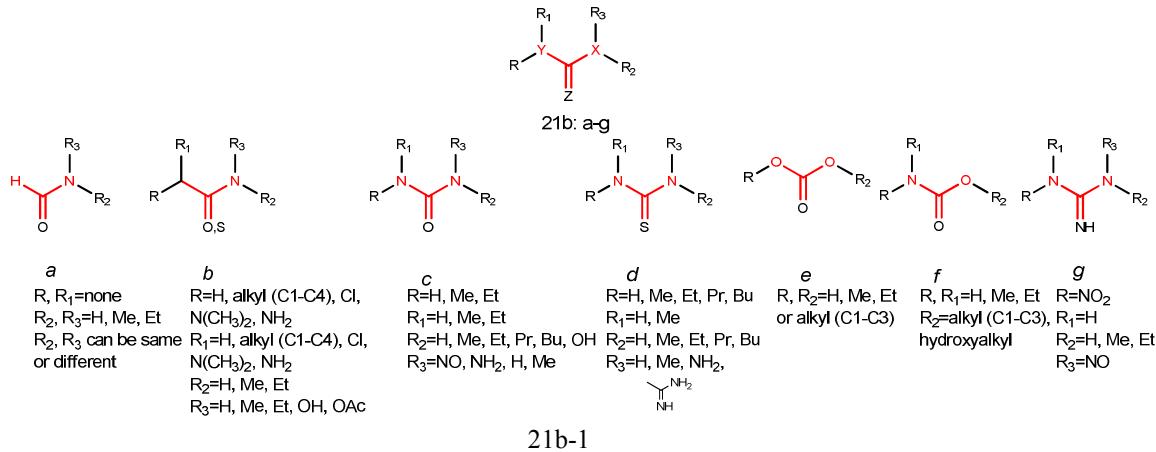


Fig. S51. The scope of structural of alkyl amide, urea and carbamate derivatives.

Most of the *N*-nitrosoureas are alkylating agents, which may share the bioactivation mechanism similar to that of aryl triazines (see section 3. 2.5d). These chemicals also generate methane (or alkane) diazonium ion (Figure S52), and induce cellular DNA damage that leads to chromosomal aberrations, mutations, tumor initiation, cell death, and also to teratogenesis. Some of these alkylating agents are also transplacental carcinogens, capable of inducing neurogenic tumors in laboratory animals.⁸³ Methylated ureas are teratogenic in laboratory animal models.

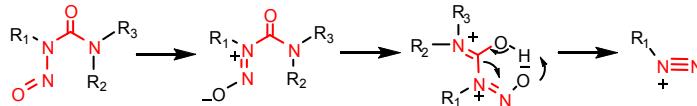
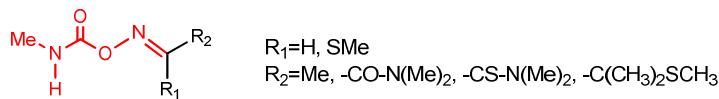


Fig.52. Hypothesized mechanism for the formation of methane (or alkane) diazonium ion of *N*-nitrosoureas

In addition to various alkyl thioureas or thiourea derivatives, semicarbazide (CAS# 57-56-7) and thiosemicarbazide (CAS# 79-19-6), which have core structural features similar to *N*-alkyl urea/thiourea, also have shown DART effects. The substituents associated with activity are illustrated in (21b-1). It is expected that small alkyl substituted derivatives in this sub-category may be DART toxins. Furthermore, some O-(methylamino)carbonyl derived carbamates, as shown in (21b-2) in Figure S53, also are DART toxicants.^{84,85,86,87} These chemicals contain a core O-(methylamino)carbonyl carbamate backbone and a methylthio group. It is reported that these chemicals share a common MOA (e.g. inhibition of AChE) with the O-aryl carbamates (see section 3.2.4b).



21b-2

Fig. S53. The core structural features of O-(methylamino)carbonyl derived carbamates

Category 22: Alpha-substituted carboxylic acids, esters and di-acid derived esters

Many aliphatic carboxylic acids and derivatives, especially those with 9 or fewer acid moiety carbon atoms, are known to have teratogenic properties. The SAR relationships have been discussed in two comprehensive reviews.^{88,89} The teratogenic properties of carboxylic acids are highly structure-dependent. Three sub-categories of alpha-substituted carboxylic acids and derivatives are described as follows: *a*. alpha-halogenated (Cl, or Br) acetic acid and esters; *b*. alpha-alkoxy acetic acid and esters; *c*. alpha-alkoxy substituted acetic acid and esters (Figure S54).

22a. Alpha-halogenated acetic acid derivatives.

The first sub-category of the alpha-substituted carboxylic acids includes halogenated acetic acid derivatives (22a). The scope of structural features include mono- to tri-halogenated acetic acids, especially the chlorinated acetic acids (where X may be a halogen (e.g. Cl, Br), and Y and Z may be a halogen or hydrogen. Several haloacetic acid derived esters are also included in this sub-category where R is a small alkyl group (C1-C3).

22b. Alpha-hydroxy and alkoxyacetic acid derivatives.

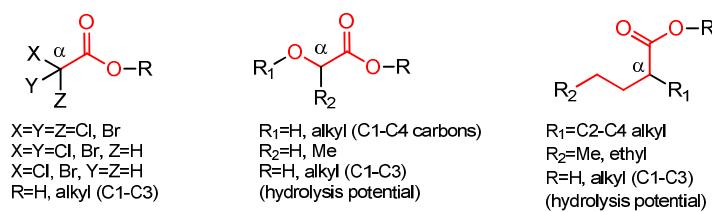
The second sub-category of alpha-substituted carboxylic acids includes alpha-hydroxy and alkoxyacetic acid derivatives with core structural features and substituents associated with activity shown in (22b). For the alkoxyacetic acids, the data indicate that the size of the alkoxy group significantly impacts DART toxic potential. Compounds with smaller alkoxy groups (e.g. OMe, or OEt) are stronger DART toxicants, and DART toxicity potential decreases with increasing alkoxy chain size. For example, in cultured rat embryos, the congeners n-propoxyacetic acid (CAS# 54497-00-6) and n-butoxyacetic acid (CAS# 2516-93-0) were markedly less embryotoxic producing only minor anomalies relative to the toxicity of methoxyacetic acid (CAS# 625-45-6) and ethoxyacetic acid (CAS# 627-03-2) at the same concentrations. In fact, n-butoxyacetic acid showed no evidence of being a developmental toxicant.⁹⁰ A minimum carbon chain size also appears to be important for DART effects, since glycolic acid (CAS# 79-14-1), which is the smallest alpha-hydroxy acetic acid, induces malformations only at high, maternally toxic doses.⁹¹ However, it has been determined that glycolate is the proximate developmental toxicant for ethylene glycol (CAS# 107-21-1).⁹²

22c. Alpha-alkylcarboxylic acid derivatives.

The third sub-category of alpha-substituted carboxylic acids includes alpha-alkylcarboxylic acid derivatives (22c). Many studies have identified alpha-alkyl substituted aliphatic acids, such as valproic acid (CAS# 99-66-1), as developmental toxicants. The alpha substitution (at C-2) which can be a carbon chain of 2 to 3 carbon atoms is required for activity. SAR studies indicate that most of these DART active chemicals have an acid moiety of 9 or fewer carbon atoms. Also, the alkyl substituents at the alpha position of the carboxylic acid generally have a significant impact on DART effects. For example, valproic acid, ethylhexanoic acid (CAS# 149-57-5) and octanoic acid (CAS# 124-07-2) are isomeric C8 organic acids with widely variable teratologic potency. Valproic acid induced a moderate to severe teratologic outcome with a potency twice that for ethylhexanoic acid. Octanoic acid was non-teratogenic, even at a very high dose.

Comparing the developmental toxicity potential of valproic acid and related chemicals, Narotsky et al.⁸⁹ determined that 2-ethylhexanoic and 2-propylhexanoic acid caused effects on rat development (including mortality, extra pre-sacral vertebrae, fused ribs, and delayed parturition), confirming the strict structural requirements for developmental toxicity of the short-chain aliphatic acid. Consistent with this conclusion, one other short-chain aliphatic acid, 2-butylhexanoic acid (CAS# 3115-28-4) is less toxic to the developing conceptus than valproic, 2-ethylhexanoic or 2-propylhexanoic acid, eliciting only an increase in the incidence of lumbar ribs in the developing rat.⁸⁹

Additionally, the small alkyl (C1-C3) esters of these acids were also grouped in this sub-category, since they have the potential to be metabolized to the acids via an ester hydrolysis pathway.



22a

22b

22c

Fig. S54. The core structural features of alpha-substituted carboxylic acids and esters.

22d. Di-carboxylic acid derivatives (adipates).

In this sub-category, we have included various esters of adipic acid. The core structural feature of these chemicals is adipic acid (CAS # 124-04-9). The ester moiety needs to fall within the carbon range of C1-C8 and can be a non-branched or branched alkyl chain, as shown in (22d) in Figure S55. All of these adipic acid esters exerted some degree of deleterious effect upon the developing embryo and fetus at high concentrations, including gross skeletal and visceral abnormalities, and decreased fetal size, and, in some cases, fetal death and resorptions. For example, dioctyl adipate (aka di(2-ethylhexyl)adipate) (CAS# 103-23-1) is a developmental toxicant.⁹³



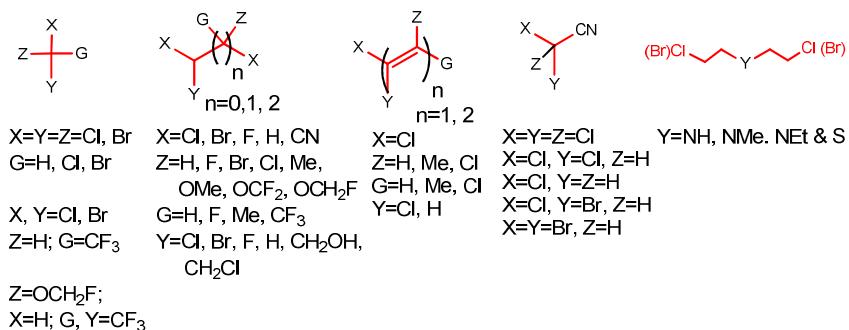
Fig. S55. The scope of structural features of adipic acid esters.

Category 23: Small- (C1-C4) halo-, multihalo-alkanes, alkyl ether/alkenes and halogenated acetonitriles as well as N, or S related mustards

The general structural features for many multi-halogenated small alkanes (23a), alkyl ethers (23b), alkenes (23c), acetonitriles (23d), as well as bis(halogenated ethyl)-amine and sulfide related mustards (23e) are shown in Figure S56 and are described below.

Three or four halogenated C1 alkanes (23a) (X=Y=Z=Cl or Br; G=H, Cl, or Br) and two to five halogenated C2-C3 alkanes (23b) show both developmental and reproductive effects. In addition to C2-C3 alkanes, the halogenated alkyl and methoxy derived ethers are also included in (23b) where Z can be OMe, OCHF₂, or OCH₂F. These chemicals show similar DART effects. The core structural features of (23c) include halogenated or poly-halogenated ethylene and their dimers. In the case of (23c), X and Y can be hydrogen or chlorine. Z and G can be hydrogen, a methyl group or chlorine. Finally, several halogenated acetonitriles (23d) show DART effects. In (23d), X, Y, and Z can each be a halogen (Cl or Br) or hydrogen forming tri- di- or mono-haloacetonitrile for which the halogen substituents may or may not be identical.

Additionally, the bis(chloroethyl)-amine and sulfide related mustards (23e), such as nitrogen mustard (CAS# 51-75-2) and mechlorethamine hydrochloride (CAS# 55-86-7) are developmental toxicants. In the case of (23e), Y is normally NH, NMe, NEt or S. These chemicals are alkylation reagents and capable of covalently binding to nucleophilic sites of various proteins and DNA.



23a

23b

23c

23d

23e

Fig. S56. The scope of structural features of multi-halogenated small alkane, alkyl ether and alkene derivatives.

Category 24: Di/multi-OH, NH₂, substituted amine, SH (=S), OR, OAc substituted (at each terminal carbon) C1 to C5 hydrocarbon chain or repeating C2 units as well as metal chelators

The general structural features and structural scope associated with DART activity of this category are represented by (24a), (24b) and (24c) in Figure S57. The key structural features include a di-substituted hydrocarbon chain with the following substituents: OH, NH₂, OAc, substituted amine, thio, sulfur, C1 to C4 alkoxy, or nitrile (-CN) at the terminal carbons or C2 repeating units. The length of the hydrocarbon chain can be 1 to 5 carbons. The chemicals in this category have somewhat ambiguous boundaries for DART potential, because some of them are not reproductive or developmental toxicants per se, however, their metabolites are the culprits. These chemicals can show a range of effects including developmental toxicity and male reproductive toxicity as well as embryonic toxicity.

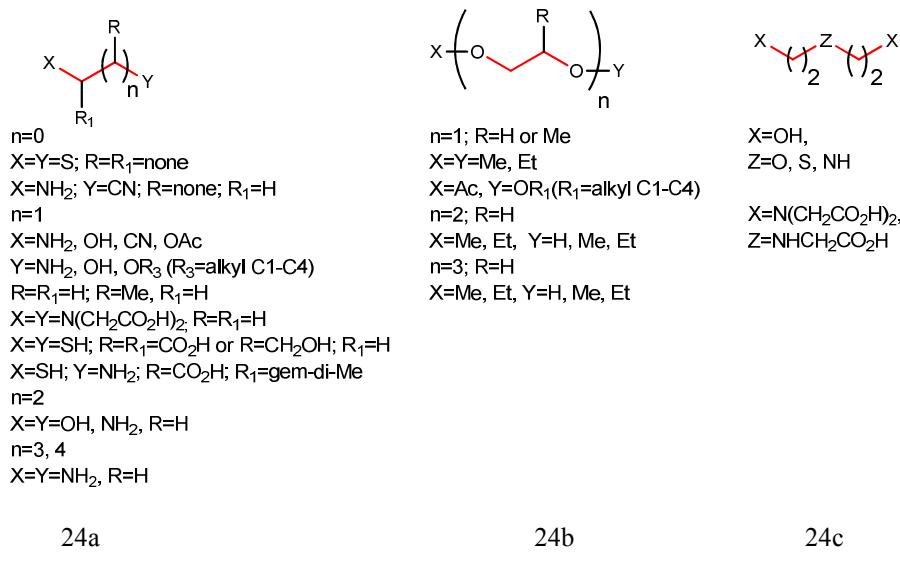


Fig. S57. The scope of structural features of di/multi-OH, NH₂, substituted amine, SH (=S), OR, acetyl substituted (at each terminal carbon) C1 to C5 hydrocarbon chain or repeating C2 units.

The mode of action for some chemicals in this category is related to bioactivation to the corresponding alkoxyacetic acid via the alcohol oxidation pathway. For example, ethylene glycol monomethyl ether (CAS# 109-86-4) is oxidized and converted into methoxyacetic acid (CAS# 625-45-6), which is particularly toxic to tissues with rapidly dividing cells and high rates of energy metabolism, such as the testes, thymus and the fetus.⁹⁴ Based on this mechanism, their similar chemical analogs, 2-(alkoxy)-1-propanols (Figure S58) are likely to have similar toxicity, since the primary alcohol moiety of these chemicals can be oxidized to the corresponding carboxylic acid. On the other hand, 1-(alkoxy)-2-propanols (Figure S58) are not expected to have the same level of DART toxicity, since they cannot be metabolized to the corresponding acids. The reproductive organs of the male seem to be particularly susceptible to the toxic effects of ethylene glycol ethers, where testicular atrophy with marked degeneration of the seminiferous tubules is the main histopathological finding.⁹⁵ Ethylene glycol monomethyl ether appears to be the most toxic, followed by ethylene glycol dimethyl ether (CAS# 110-71-4), ethylene glycol monomethyl ether acetate (CAS# 110-49-6), ethylene glycol monoethyl ether (CAS# 110-80-5), and ethylene glycol monoethyl ether acetate (CAS# 111-15-9).⁹⁶ Within the glycol ethers, it has been determined that, in general, shorter alkyl chain glycol ethers produce greater embryotoxicity than those with longer chains, and the corresponding ester produces effects equivalent to those of the ether.^{97,98}



Fig. S58. The substituent effects on developmental/reproductive toxicity of alkoxy propanols.

Several metal chelating agents exemplified by ethylenediaminetetraacetic acid (EDTA, CAS# 60-00-4), dimercaptosuccinic acid (DMSA, CAS# 304-55-2), 2,3-dimercapto-1-propanesulfonic acid (DMPS, CAS# 4076-02-2), penicillamine (DPA, CAS# 52-67-5) and 2,3-dimercaptopropanol (BAL, CAS# 59-52-9), as shown in (24d-f) in Figure S59, also fit into the structural scope of this category. These chemicals possess at least two functional groups with electron donating atoms (e.g. SH, NH₂/N(R)₂) capable of combining with a metal by donating a pair of electrons and situated in the molecule to allow the formation of a ring with the metal atom as the closing member (e.g. five member ring formation). It has been recognized that these chelating agents can effectively tie up metal ions and act to prevent the normal function of some enzymes, producing physical defects in the developing embryo. The developmental toxicity data on these chemicals are not complete and not all have been shown to be developmental toxicants. Most chelating agents have been shown to be developmentally toxic due to, at least in part, induced embryonic/fetal zinc and/or copper deficiencies, and it would be appropriate to hypothesize that chelators dosed at levels that create deficiencies of essential trace metals have the potential to cause adverse effects on development. Domingo ⁹⁹ presents a review of the available data.

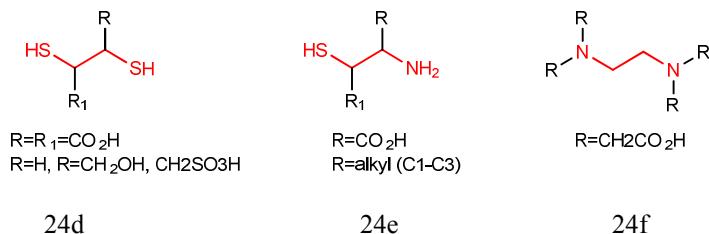


Fig. S59. The structural features of metal chelators.

Category 25: C1 to C4 non-branched/<C9 beta-alkyl (<C5) substituted alcohols and <C4 alkyl, vinyl nitriles

Some alkyl alcohol derivatives are known to be teratogenic, especially when the alkyl R group is a non-branched carbon chain of less than 5 atoms (R=C1-C4) (25a) (Figure S60). These alcohols are developmental toxicants at relatively high doses.

In this category, we have also included several beta-alkyl substituted alcohols and their acetyl derivatives in the dataset, as shown in (25b) (Figure S60), which are the precursors of alpha-alkyl substituted carboxylic acids. It is well known that the primary alcohol moiety will be oxidized readily by hepatic alcohol dehydrogenase (ALDH) to yield the corresponding alpha-alkyl substituted carboxylic acid. Therefore, it would be reasonable to suggest that the DART effects of these alcohols are related to the formation of their corresponding carboxylic acids.

In rodents, 2-ethyl-1-hexanol (CAS# 104-76-7) is a developmental toxicant at maternally toxic doses.¹⁰⁰ 2-ethyl-1-hexanol is also a metabolite of di(2-ethylhexyl) phthalate (CAS# 117-81-7), however, it has no role in the developmental toxicity of this phthalate.¹⁰¹ Although no data are available to determine the DART effects of 2-propyl hexanol (CAS# 817-46-9), its metabolite 2-propyl-hexanoic acid (CAS# 3274-28-0) is a longer-chain aliphatic acid structurally related to valproic acid, and, therefore, may possess the potential for DART effects. The existing data indicate that 2-propylhexanoic acid is a developmental toxicant.

Another well represented sub-category is the aliphatic nitriles. Several alkyl and vinyl substituted small nitriles (<C6) have been shown to produce embryonic developmental toxic effects in rats and hamsters. Figure S58 shows the core structural features of alkyl nitriles along with an enumeration of substituents associated with activity (25c) and vinyl nitriles (25d). It has also been shown that the potency of embryonic developmental toxicity can vary widely and is structure-dependent. Normally, the order of increasing potency is acetonitrile (CAS# 75-05-8) < propionitrile (CAS# 107-12-0) < n-butyronitrile (CAS# 109-74-0), methacrylonitrile (CAS# 126-98-7), allylnitrile (CAS# 109-75-1) < cis-2-pentenenitrile (CAS# 25899-50-7) < acrylonitrile (CAS# 107-13-1) < 2-chloroacrylonitrile (CAS# 920-37-6). The mechanism of generating developmental toxicity may involve maternal production of cyanide, through the CYP-mediated hydroxylation at the carbon atom alpha to the cyano group to form a cyanohydrin intermediate, which spontaneously decomposes to release cyanide (Figure S60). It has also been suggested that distinct metabolites derived from microsomal metabolism of unsaturated nitriles may also play a role.¹⁰²

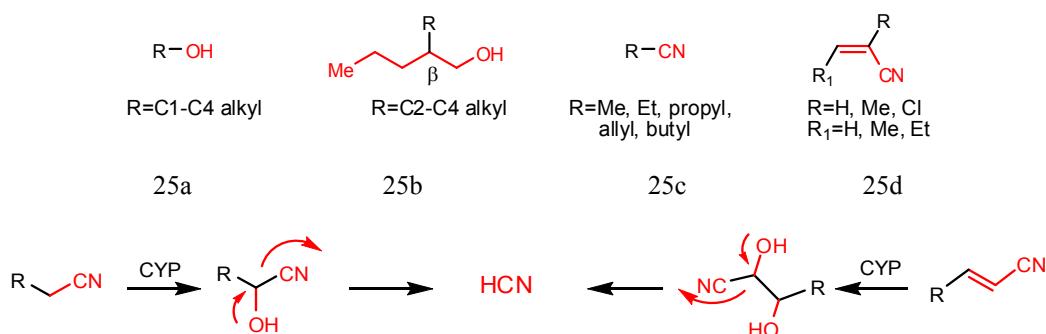


Fig. S60. The scope of alkyl alcohol and alkyl, vinyl nitrile derivatives and hypothesized mechanism for the formation of cyanide from alkyl, vinyl nitriles

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