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The Expanded Decision Tree: Ranking Toxic Potential

Contents

Phase II of the External Peer Review

4.3.8 The Finalized External Validation Database

The finalized external validation DB contains 1,241 substances, 1,132 of which have established NELs. Given the available data, only LELs could be established for the remaining 110 substances.

4.4 The External, Independent, Validation of the EDT

4.4.1 The External Validation of the EDT

The EDT was validated with the help of the finalized external validation DB and the independent (non-government, external) scientists (referred to as validation chemists in the text of this document). These validation chemists have expertise in organic chemistry, metabolism, and structure-toxicity relationships. Prior to the start of the external validation, the validation chemists received a supervised demonstration on how to run substances through the EDT. After the training, the validation chemists were provided with the pre-validation EDT and the external validation EDT DB and were tasked with 1) classifying all compounds in the external validation EDT DB according to their EDT classes using the pre-validation EDT, 2) recording the path traversed through the EDT for all compounds, and 3) providing input on the EDT questions. The input consisted of various components such as 1) whether the EDT questions were clear, and if not, providing recommendations for updating the text of the questions to ensure that there is no ambiguity in the post-validation EDT questions, 2) whether the EDT questions were appropriate/scientifically sound based on their knowledge of organic chemistry, ADME, and structure-toxicity relationships, and 3) recommendations for possible changes or further refinement of some questions to better address the toxic potentials of certain congeneric groups of compounds. Finally, the validation chemists were asked to review and provide feedback on the discussion in section "4.4.3 Justifications and Scientific Basis for the Updating the EDT" that summarizes the changes made to the EDT based on their review and feedback and our investigations.

4.4.2 Lessons Learned from the External Validation

Based on the results of the external validation, updates were made to the EDT questions. These updates were based on 1) recommendations from the validation chemists, 2) mistakes made by the validation chemists during the classification of the compounds in the external validation DB due to a lack of clarity (see Table 4 below), and 3) the additional toxicology data in the external validation EDT DB combined with the classification data. FDA notes, that even though the combined (original (pre-validation) plus the validation) EDT DB contains over 3,100 unique substances, after a thorough review of all questions, we found that for some of the sub-questions and sub-sub-questions we did not have an adequate amount of supporting data. Therefore, FDA searched all publicly available toxicological databases for additional substances to support specific congeneric groups (e.g., azetidines, isocyanates, certain natural toxins, and various types of antibiotics) for which we did not have enough data even in the combined EDT DB. These new data served as underpinning for the updates along with additional review articles and FDA internal documents on structure-toxicity relationships.

	Chemist 1	Chemist 2
% Correct	87%	91%
% Incorrect due to ambiguity of Qs[1]	6%	6%
% Incorrect	7%	3%

Table 4. Correctness rate of the classifications by the external validation chemists

The lessons learned from the EDT validation prompted either i) changing class assignments of related compounds captured at the same question, sub-question, or sub-sub-question to better reflect the toxic potentials (i.e., additional data found in the validation DB prompted us to reconsider the class assignment of certain congeneric groups of compounds for which we had limited data in the original (pre-validation) EDT DB); ii) creating new questions based on new toxicological data in the external validation DB for some congeneric groups of compounds that either did not have representative substances in the original (pre-validation) EDT DB or only had very limited number of representative substances; iii) refining existing questions for certain congeneric groups of compounds; or iv) rephrasing certain questions and/or add clarifying statements to eliminate any ambiguity in the EDT questions.

Complex compounds with multiple functional groups and/or moieties and/or skeletons with differing toxic potential should be classified based on their most toxic structural feature. A shortcoming of the pre-validation EDT that FDA discovered during the external validation stemmed from the 'linearity' of the EDT questions that prevented some compounds from being classified based on the most toxic structural feature present in the molecule. For example, certain natural and synthetic toxins FDA aimed at capturing and classifying at Q6 into classes of high, very high, or extreme toxicity (Classes IV, V, and VI, respectively) never reached Q6 if they also contained one or more N- and/or S- containing functional group that were captured and classified at Q3 normally into a class of lower toxicity concern than what the compound would have received at Q6 if it reached Q6. Therefore, FDA found it necessary to ask the user at the end of some questions to crosscheck whether the substance prompts a yes response at another question to ensure that all substances are classified based on their most toxic structural features (see section 4.4.3 for multiple examples of crosschecking).

4.4.3 Justifications and Scientific Basis for Updating the EDT

The updated, post-validation EDT is provided in section 4.5. All changes made to the text of the EDT questions as a result of the validation are in red[2] in the post-validation EDT to ensure that the reader can quickly identify all changes compared to the pre-validation EDT.

[1] Whether a mistake was due to the ambiguity of a question was determined based on feedback from the chemists.

[2] Please note that while the red color used in the EDT may not be visible to all readers, all changes are clearly described in Section 4.4.3. Moreover, the original (pre-validation) EDT is provided in the Phase I document and can be used for comparison to the post-validation EDT. Hence, being able to see the red is not necessary to understand or identify the updates made.

While the pre-validation EDT underwent some edits to reduce ambiguity of questions, and improve its granularity, read-across capability, and ability to more accurately sort a wide range of chemicals according to their relative chronic oral toxic potential, these changes were minor as

1) The order of the EDT questions did not change, the number of main questions remained the same (47), and the number of sub-questions only increased by less than 3% (from 138 to 142). While there was an increase in the number of sub-sub-questions, the main reason for this was subdividing previously existing sub-questions into multiple sub-sub-questions to increase the read-across capability of the tool. Most functional groups and moieties addressed in the new sub-sub-questions in the post-validation EDT were already included in the pre-validation EDT.

2) The pre- and post-validation TTCs remain largely constant (see section 4.6.3) indicating that the pre-validation EDT was capable of accurately sort compounds with broad structural variation based on/according to their relative chronic oral toxic potential.

3) The ability of the EDT to resolve the varying toxic potentials of chemicals was maintained through the validation, based on Figure 4 (Comparison of the NEL distributions of substances found in the various EDT Classes using the pre-validation EDT with the post-validation EDT (see section 4.6.2)).

4.4.3.1 Bismuth (Qs 1-3, 5)

FDA, based on the recommendation of one of the validation chemists, researched and reviewed the available safety data for bismuth (e.g., Bradley et al., 1989; Tillman et al., 1996; Wang et al., 2019; Pelepenko et al., 2022) to evaluate whether bismuth could be added to Qs 1, 2, and 3 as a counterion that could be disregarded at these questions and whether the classification of organobismuth compounds should be changed to a lower EDT class.

The data showed that certain bismuth preparations, albeit usually at high doses, can cause toxicity such as neurotoxicity and nephrotoxicity. FDA determined that based on the data available for bismuth ion (see Table 5 for example data) showing relatively low toxic potential, the bismuth counterion will be disregarded at Qs 1, 2, and 3. Moreover, for the same reason, FDA added bismuth ion to Q5a) as one of the ions that will be disregarded going forward.

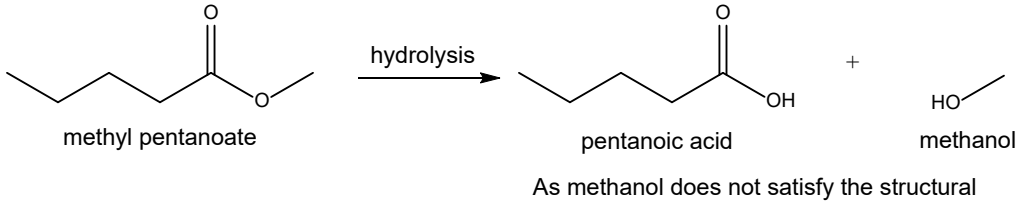
Regarding organic compounds that have covalently bound bismuth, at the present time, there are insufficient structure-toxicity information and toxicity data (i.e., subchronic, chronic, and/or reproductive and developmental toxicology studies) available to derive specific questions to refine their sorting. Based on the limited available data (see Appendix 2 and the referenced articles above), we consider a class assignment of III to be protective for compounds containing covalently bound bismuth. Therefore, as a result of this evaluation, instead of being defaulted to EDT Class IV at Q5, these compounds were changed to default into EDT Class III. While Class III is likely overprotective for compounds containing covalently bound bismuth, due to the lack of toxicological data and to err on the side of caution, a Class III assignment was deemed to be reasonable.

CAS	Name	NOAEL (duration)	Reference
7440-69-9	Bismuth	1000 mg/kg bw/day (28 days)	Sano, Y., Satoh, H., Chiba, M., Okamoto, M., Serizawa, K., Nakashima, H., & Omae, K. (2005). Oral toxicity of bismuth in rat: single and 28-day repeated administration studies. Journal of occupational health, 47(4), 293–298. https://doi.org/10.1539/joh.47.293
14882-18-9	Bismuth subsalicylate	300 mg/kg bw/day (14 days)	BASF (2018) Results of a Test study in rats, oral administration with CASRN 14882-18-9 at https://chemview.epa.gov/chemview/proxy?filename=8e%2F8EHQ-18-21155_Combined.pdf
813-93-4	Bismuth citrate	1000 mg/kg bw/day (90 days)	Dupont Haskell et al. (2005) Bismuth citrate: subchronic toxicity – 90-day oral gavage study in rats. Report Dupont — 15881, July, 18 from Scientific Committee on Consumer Safety (2013) Opinion on Bismuth citrate at https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_147.pdf
1304-85-4	Bismuth subnitrate	1000 mg/kg bw/day (90 days)	Unknown, 2016. Available from ECHA at https://echa.europa.eu/da/registration-dossier/-/registered-dossier/23467/7/6/2
5892-10-4	Bismuth (III) carbonate	1000 mg/kg bw/day (90 days)	Unknown, 2016. Available from ECHA at https://echa.europa.eu/mt/registration-dossier/-/registered-dossier/23467/7/6/2

Table 5. No-effect-levels of compounds containing bismuth as a counterion

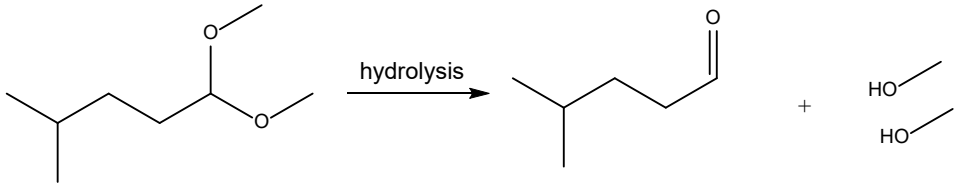
4.4.3.2 Clarifications for Q1a, Q1b, and Q1c

At Q1a), Q1b), and Q1c), the presence of certain hydrolyzable functional groups (e.g., ester) are allowed. Also, at these sub-questions, it is required that the compound described has more than one carbon atom. Based on misclassifications by the validation chemists, FDA realized that it was unclear from the original text of these sub-questions that all potential hydrolysis products (if a hydrolyzable functional group is present) must have more than one carbon atom. Therefore, for clarity, after these three sub-questions, FDA added that for compounds with hydrolyzable functional groups, all hydrolysis products must satisfy the structural requirements at Q1a), Q1b), and Q1c). If any of the potential hydrolysis products does not satisfy the structural requirements, the user responds ‘no’ at Q1a), Q1b), or Q1c). For examples:



methyl pentanoate pentanoic acid methanol

As methanol does not satisfy the structural requirements at Q1a) (it does not have > 1C), the user is asked to respond 'no' at Q1a) for methyl pentanoate.



1,1-dimethoxy-4-methylpentane 4-methylpentanal methanol

As methanol does not satisfy the structural requirements at Q1b) (it does not have > 1C), the user is asked to respond 'no' at Q1b) for 1,1-dimethoxy-4-methylpentane.

4.4.3.3 Update to Q1b

A limited number of highly specific substances are caught at Q24a) and placed into Class III. During the validation of the EDT, it came to light that some substances that should have been caught at Q24a) instead get caught at Q1b) (i.e., they never 'arrive at' Q24a)) and, as such, are placed into Class I. Therefore, to avoid capturing some compounds at Q1b) instead of Q24a), the following statement was added at the end of Q1b): "(Please also check whether your compound fits 24a). If yes at Q24a), say no at 1b), and go to Q1c))."

4.4.3.4 Updates to Q1c

Dicarbonate, peroxydicarbonate, and peroxycarbonate were added to the list of allowed structural features at this sub-question as linear aliphatic and methyl substituted alkyl dicarbonates, peroxydicarbonates, and peroxycarbonates with no other functional groups and heteroatoms (i.e., no elements other than C, H, or O) have very low systemic toxicities (see combined EDT DB in Appendix 2 for examples such as di-sec-butyl peroxydicarbonate (CAS 19910-65-7), dicetyl peroxydicarbonate CAS 26322-14-5), dimyristyl peroxydicarbonate (CAS 53220-22-7), and *t*-butylperoxy isopropyl carbonate (CAS 2372-21-6)). Moreover, for Q1c(ii)), the carbonate functional group was added as simple carbonates have low systemic toxic potentials (see Appendix 2 for examples such as potassium hydrogen carbonate (CAS 298-14-6), dimethyl carbonate (CAS 616-38-6), ethyl methyl carbonate (CAS 623-53-0), and dimethyl dicarbonate (CAS 4525-33-1)).

Q24b) aims at capturing α-hydroxy- or α-alkoxy-ethanoic acids and a variety of its hydrolytic precursors and placing them into Class III. During the validation, it came to FDA's attention that some of these precursors may get caught at Q1c(ii)) and placed into Class I instead of Class III at Q24b). Therefore, to avoid this, the following was added to Q1c(ii)): "except substances addressed at Q24b." Consequently, substances that fit the structural descriptions both at Q1c) and Q24b) will result in a no response at Q1c), and the substance is passed along to the next sub-question to ensure that it will arrive at Q24b) after some further pre-screening.

While compounds with ≥8 contiguous conjugated double bonds were exempted at Q1a) and Q1b), it was an oversight not to exempt these at Q1c). Based on their NELs (when compared to the 5th percentile Class I and Class II NELs), a Class II assignment is warranted (e.g., norbixin, see Appendix 2), while those with less than 8 contiguous double bonds warrant a Class I assignment (e.g., crocetin, see Appendix 2). Therefore, exempting compounds with ≥8 contiguous conjugated double bonds was extended to Q1c).

4.4.3.5 Clarification for Q1e

Q1e) captures monosaccharides and "hydrolyzable oligosaccharides and polysaccharides" along with some of their derivatives. To clarify that this sub-question aims at capturing oligosaccharides and polysaccharides that hydrolyze either enzymatically and/or nonenzymatically, FDA updated this question to state "monosaccharides and (enzymatically and nonenzymatically) hydrolyzable oligosaccharides and polysaccharides."

4.4.3.6 Update to Q1g

To avoid classifying formaldehyde at Q1g) as Class I and for consistency with Q1a), this sub-question was updated to specify that the compounds captured at this question “must have > 1C.” Formaldehyde is now captured at Q28 and placed into EDT Class II, as originally intended.

4.4.3.7 Clarification for Q1h

Q1h) aims at capturing lactones that undergo hydrolysis to form linear aliphatic or methyl-substituted hydroxycarboxylic acids and place them into Class I. As some of the potential hydrolysis products may undergo tautomerization to keto-acids, some of the validation chemists were unsure whether to respond yes or no at this sub-question. Therefore, FDA added a clarification to this sub-question stating that “the tautomerization will be ignored for the purposes of the EDT as these keto-acids would also be classified as Class I (at Q1c). Therefore, only the pre-tautomerization product must satisfy the structural requirements of this question.” Moreover, additional examples of structures were provided after this sub-question to ensure that the user fully understands what compounds are intended to be captured here.

4.4.3.8 Clarification for Q1i

This sub-question aims at capturing nucleotides, nucleosides, phospholipids, monophosphates of amino acids, and their hydrolysis products and placing them into Class I. During the validation, based on the available toxicological data, FDA observed that the phosphate esters of nucleotides with betaine and choline, such as citicoline (cytidine 5'-diphosphocholine), have low oral toxic potentials. For example, a NOAEL of 1,000 mg/kg bw/day was identified for citicoline in a 90-day rat study (Schauss et al., 2004; Schauss et al., 2009). Furthermore, choline chloride has a NOAEL of 500 mg/kg bw/day in a 72-week study (Shivapurkar et al., 1986), and betaine has a NOAEL of 812 mg/kg bw/day in a 90-day study (Unknown, 2001).

Consequently, FDA wanted to capture the betaine and choline derivatives of nucleotides at Q1 to enable their placement into Class I. The best sub-question to catch these compounds was identified as Q1i), as Q1i) aims at capturing related compounds. Therefore, FDA updated Q1i) to enable the capture of the phosphate esters of nucleotides with betaine and choline at this sub-question. The new sub-question now reads: “Nucleotides (and their phosphate esters with betaine and choline), nucleosides, phospholipids, monophosphates of amino acids, or their hydrolysis products.”

4.4.3.9 Clarification for Q1k

Q1k) was created to capture “Bile acids, bile salts, and alkyl esters of bile acids, but no other substances containing a steroid skeletal structure”. During the validation, FDA recognized that certain bile alcohol and bile acid conjugates with very low toxic potentials were left out of Q1k) even though they should have been included. Therefore, FDA updated “Bile acids, bile salts, and alkyl esters of bile acids” to “Bile

acids, alkyl esters of bile acids, bile acid conjugates (with the carboxyl group and the amino acids taurine or glycine), bile alcohols, and bile salts". During the validation, FDA also realized that it would be easier for future users of the paper version of the EDT if the core structures of the above compounds were provided right after the question for the users' quick reference. Therefore, core structures of bile alcohols and bile acids and their conjugates were added after Q1k).

4.4.3.10 Updates to Qs 2 and 3

Q2 was designed to capture organophosphorus compounds other than the safe ones captured at Q1 and to sort organophosphorus compounds into Classes III, V, and VI. Q3 captures a wide variety of compounds most of which are placed into Classes III-V, with a few exemptions classified as II. For the substances captured at Qs 2 and 3, the driving force of their toxicity is the organic portion of the compound and the presence of counterions exhibiting no or low toxic potentials have no effect on the toxic potential of these compounds. Therefore, in the original (pre-validation) EDT at Qs 2 and 3, the user was asked to disregard the presence of certain counterions such as sodium and calcium. The list of counterions that can be disregarded at these two questions were expanded to contain bismuth (see 4.4.3.1 for justification), ammonia, thionosulfate, sulfite, bisulfite, sulfinate, sulfamate, and phosphate, as these are not expected to be the driving force of toxicity or contribute to the toxicity of compounds captured at Qs 2 and 3.

As Qs 2 and 3 sort compounds into Classes III, V, and VI and Classes II-V, respectively, to avoid placing some compounds into a lower class (mostly Classes II, III, or IV) at either Q2 or Q3 than they should be placed, Qs 2 and 3 were updated to ask the user to cross check against the toxic elements listed in Q5 and the toxic structural features dealt with in Q6 before classifying the compound at either Q2 or Q3. If any of the structural features or elements yielding high toxic potentials addressed in Qs 5 or 6 are present in the compound of interest, the user is directed to ultimately classify the compound into the highest class it would get at any of these questions. This update was especially important when dealing with compounds with complex structural features to ensure that they get classified based on their most toxic functional group, structural moiety, or skeleton.

4.4.3.11 Clarifications for Q2c and Q2d

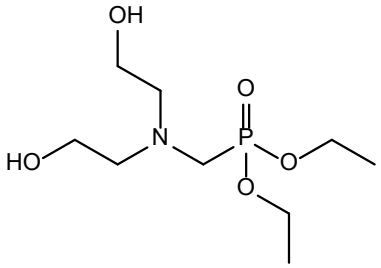
Qs 2c) and 2d) were designed to capture organophosphorus compounds with low to intermediate toxic potential and place them into Class III. It was not FDA's intention to capture compounds at these two sub-questions that have halogens present as the presence of halogens may make these compounds more toxic. Therefore, for clarity, "no covalently bound halogen may be present" was added to these sub-questions.

4.4.3.12 Creation of Q2e

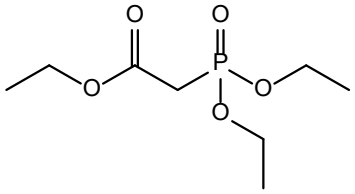
In the pre-validation EDT, compounds containing covalently bound phosphorus (yes at Q2a)) but not fitting the structural requirements at Q2b), c), or d) were defaulted into Class V. The original (pre-validation) EDT DB contained 28

substances that defaulted into Class V due to a yes response at Q2a) but a no response at Q2b), c), and d). Once the original (pre-validation) EDT DB and the external validation DB were combined, the number of organophosphorous substances defaulting at Q2 into Class V doubled. One of the validation chemists pointed out that many phosphonates default into Class V at Q2, but based on their knowledge, certain phosphonates would be overclassified as a result (i.e., a class assignment lower than V is warranted). Therefore, FDA looked at the large number of substances that defaulted into Class V at Q2. The review of the scientific literature and the data in the combined EDT DB yielded the following conclusions:

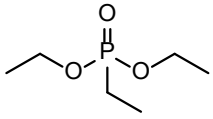
a) Alkyl phosphonates ($RCP(=O)(OR)_2$), with or without the presence of one or more -OH group(s) on the alkyl chain, with or without an ester or N in the alkyl chain (neither directly attached to the P), based on their NELs should be placed into either Class I (71%) or Class II (29%). The presence of halogens can significantly increase the toxicity of alkyl phosphonates (with some having NELs warranting a Class V assignment). Examples of alkyl phosphonates defaulting into Class V at the pre-validation EDT Q2 even though their NELs warrant a Class I or II assignment:



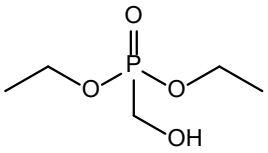
diethyl bis(2-hydroxyethyl)aminomethylphosphonate



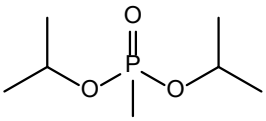
triethyl phosphonoacetate



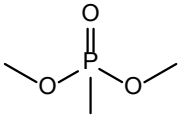
diethyl ethylphosphonate



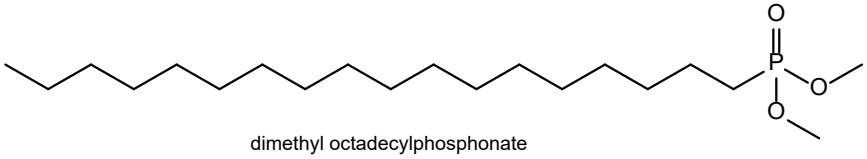
diethyl (hydroxymethyl)phosphonate



diisopropyl methylphosphonate



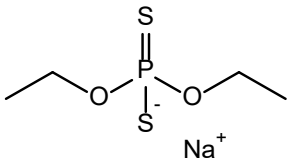
dimethyl methylphosphonate



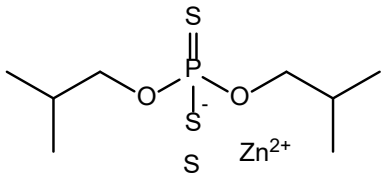
dimethyl octadecylphosphonate

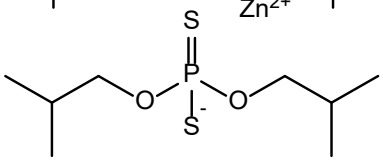
b) No aryl phosphonates were present in the combined EDT DB to impel any conclusions for their best class assignment. Phosphine oxides (3 direct C bonds to P) with 3 aryl rings had NELs warranting a Class I or III assignment and the only aryl phosphinate (two direct C bonds to P) had a NEL warranting a Class II assignment.

c) Alkyl and aryl phosphorodithioates (HSP(=S)(OR)2 or -SP(=S)(OR)2) where R= alkyl or aryl), without the presence of any good leaving groups or halogens, had NELs warranting a Class I or II assignment. Examples of alkyl and aryl phosphorodithioates defaulting into Class V at the pre-validation EDT Q2 even though their NELs warrant a Class I or II assignment are:

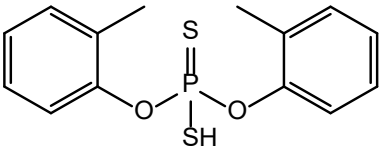


sodium O,O-diethyl dithiophosphate

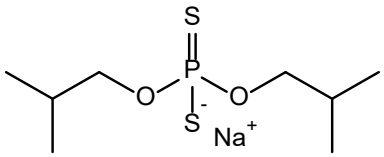




phosphorodithioic acid, mixed
O,O-bis(iso-Bu and pentyl) esters, zinc salts



O,O-ditolyl phosphorodithioate



sodium diisobutyldithiophosphate

Based on the above data and to avoid the overclassification of certain organophosphorus compounds, a new sub-question (Q2e)) was created:

Q2e) i) alkyl phosphonate ($RCP(=O)(OR)_2$) where R=alkyl, with or without one or more -OH substitution on the alkyl chain, with or without an ester or N in the alkyl chain (neither directly attached to the P), or ii) alkyl and aryl phosphorodithioate ($HSP(=S)(OR)_2$ or $^-SP(=S)(OR)_2$) where R= alkyl or aryl, without the presence of any potential leaving groups or halogens. If yes to Q2e(i)) or Q2e(ii)), the substance will be assigned to Class II.

4.4.3.13 Clarification for Q3d(ii)

Based on a few misclassifications by one of the validation chemists, FDA added a statement clarifying that the secondary amine cannot be a connector or a part of a connector between two rings as this sub-sub-question aims at capturing only linear or simply branched secondary amines. Secondary amines that are connectors between two rings were not intended to be considered at this sub-sub-question as they behave differently.

4.4.3.14 Update to Q3e

From the validation it came to FDA's attention that the EDT does not address aminothioureas, and the validation chemists were unsure whether aminothioureas should be captured at Q3e), which identifies thioamides and thioureas. As originally written, FDA's intent was to capture aminothioureas at this sub-question. For clarity, aminothiourea was added to the list of functional groups and should evoke a yes response at this question.

4.4.3.15 Updates to Qs 3f(ii) and 3f(iii)

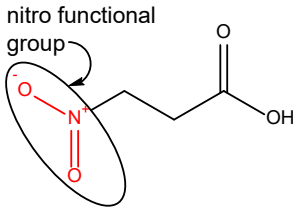
The original (pre-validation) Q3f(ii)) aimed at capturing quaternary N^+-containing compounds, with some exceptions such as nitrobenzenes, diazos, azides, and some other compounds, assigning them to Class IV. Thirty compounds using the pre-validation EDT were captured at this sub-sub-question in the original (pre-validation) EDT DB. Based on their NELs, 16 of the 30 compounds should have been

classified as either Class I or II, 11 compounds as Class III, 1 compound as Class IV, and 2 compounds had LELs that warranted a Class IV or higher classification (as no NELs are available, the exact classification is unknown). In the external validation DB, 34 substances were captured at the pre-validation Q3f(ii). Based on their NELs, 23 of the 34 substances should have been assigned to either Class I or II, 6 of the 34 should have been assigned to Class III, 2 of the 34 should have been assigned to Class IV, 2 of the 34 should have been assigned to Class V, and 1 of the 34 had only a LEL (but no NEL) that warranted a Class V or higher classification. Based on these results, a large number of compounds (39/64) were placed into Class IV even though their NELs warranted a Class I or II assignment. On the other hand, for very few compounds (2/64 for certain and 2/64 potentially (those with LEL only)), the Class IV placement was not or may not have been protective enough. Therefore, FDA reviewed whether the classification/sorting of compounds captured at the pre-validation Q3f(ii)) could be further refined.

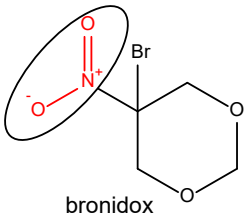
Moreover, the pre-validation EDT Q3f(iii)) captured compounds with more than one quaternary N^+ and placed them into Class V. Based on their NELs, in the combined EDT DB, 5 of the 8 compounds captured at Q3f(iii)) should have been placed into Class I or II and 3 of the 8 into Class IV indicating that reevaluating the classifications of compounds containing more than one quaternary N^+ was needed. Therefore, FDA reviewed whether the classification/sorting of compounds captured at the pre-validation Q3f(iii)) could be further refined.

Upon an examination of the structural features of and the toxicological data (see Appendix 2, all examples below along with other examples are listed in the combined EDT DB along with the best representative toxicological study for each) for the very large number of substances fitting the structural requirements at the pre-validation Q3f(ii)) and Q3f(iii)), FDA came to the following conclusions:

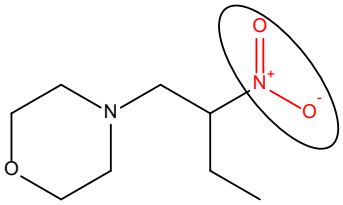
1) Compounds containing one or more nitro functional group(s) ($^-ON^+(=O)$-) (except those listed as exceptions in the original question (e.g., nitrobenzenes)) where the nitro group is neither a heteroaromatic ring substituent nor directly attached to cyclic or acyclic guanidine (-N(=N)N) or a -N- warrant a Class V assignment based on their NELs. Example compounds:



3-nitropropionic acid

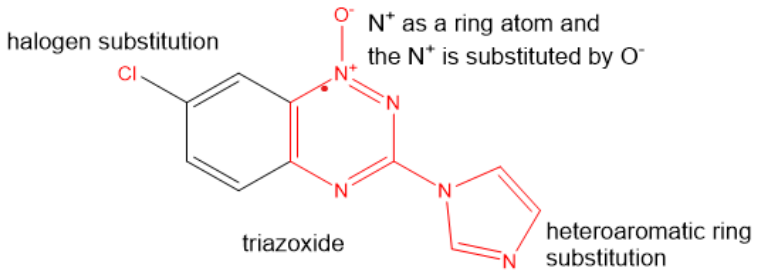


bronidox

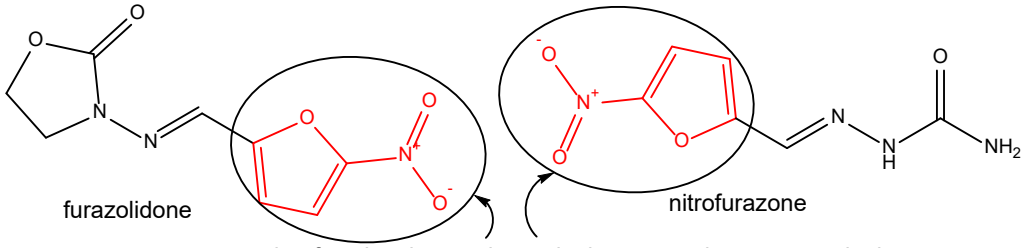


4-(2-nitrobutyl)morpholine

2) Compounds containing a heteroaromatic ring with at least one N^+ as a ring atom and the N^+ is substituted by O^-, with one or more covalently bound halogens and/or one or more additional heteroaromatic rings, warrant a Class V assignment based on their NELs. Example compound:

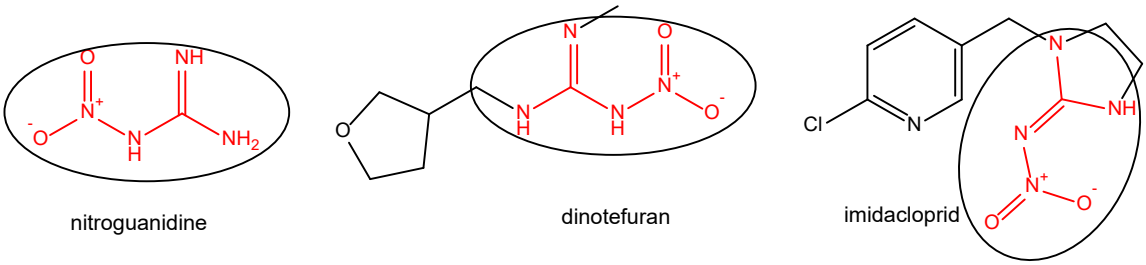


3) Compounds containing one or more nitro functional group(s) (except those listed as exceptions) in which the nitro group is a substituent on a heteroaromatic ring but the heteroaromatic ring does not contain a cyclic guanidine, warrant a Class IV assignment based on their NELs. Example compounds:



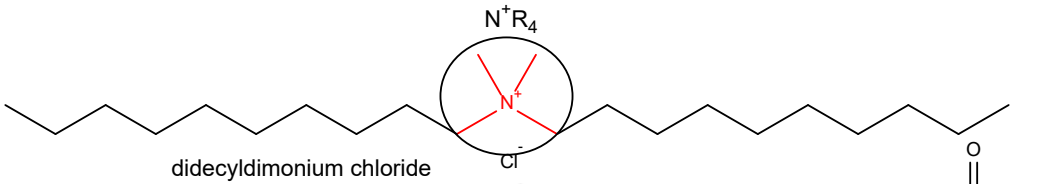
nitro functional group is a substituent on a heteroaromatic ring

4) Compounds with one or more nitro functional group(s) (except those listed as exceptions) in which the N^+ from the nitro group is directly attached to cyclic or acyclic guanidine warrant a Class III assignment based on their NELs. Example compounds:

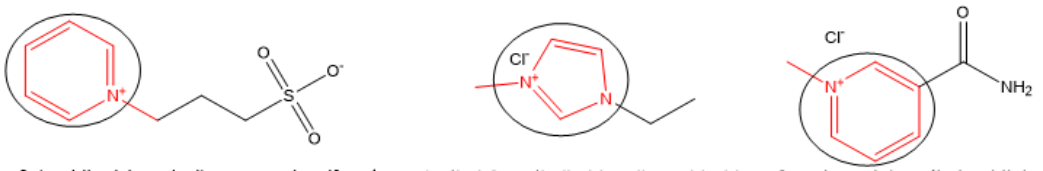


The circled regions highlight a nitro functional group in which the N^+ is directly bonded to a cyclic or acyclic guanidine moiety.

5) Compounds with one or more N^+R_4, except in any of the above forms, where the N^+ is attached to 4 Cs and the compound is either acyclic or has a maximum of 2 benzene rings or the N^+ is a ring atom in a heterocyclic (not heteroaromatic) ring. The compound may have one or more ether, alcohol, ester, carboxylic acid, secondary amide, sulfoxide, sulfone, sulfonate, sulfamate, and/or a maximum of 3 covalently bound Cl and/or Br (these covalently bound halogens may not be a substituent on a ring), without the presence of any other functional groups. The presence of a single heteroaromatic rings is also allowed, but the N^+ can neither be a ring atom in the heteroaromatic ring nor can it be a direct substituent (i.e., directly attached) to the heteroaromatic ring. Based on their NELs, compounds fitting this description are classified as Class III. Example compounds:

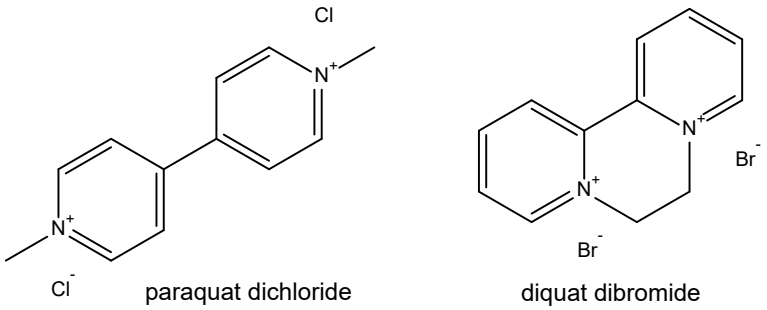


6) Compounds with a single N^+ as a ring atom in a 5- or 6-membered heteroaromatic ring where the N^+ is directly bonded to Cs only. (While more than one ring may be present, only one ring with a single ring N^+ may be present (the N^+ may not be present at the fusion point of two rings). Note that the N^+ may not be directly substituted by an O^-, and it must be directly substituted by a non-ring C. Based on their NELs, compounds fitting this description are classified as Class III. Example compounds:

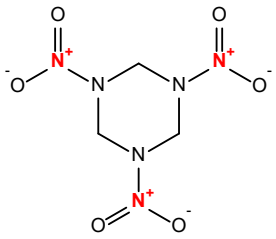


The circled regions highlight a single N^+ as a ring atom in a 5- or 6-membered heteroaromatic ring where the N^+ is directly bonded to Cs only.

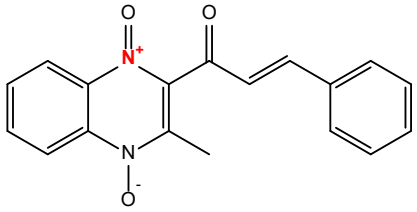
7) Compounds with at least two rings each with a single N^+ as a ring atom in a 5- or 6-membered heteroaromatic ring where all N^+s are directly bonded to Cs only. (Note that the N^+ may not be directly substituted by an O^-; it must be directly substituted by a non-ring C.) Based on their NELs, compounds fitting this description are classified as Class IV. Example compounds:



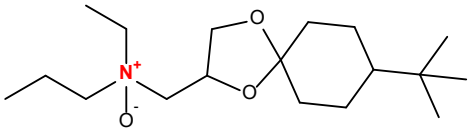
8) All other compounds containing a N^+, except in any of the above forms. Based on their NELs, compounds fitting this description are defaulted into Class IV. Example compounds:



cyclonite



quinocetone



spiroxamine-N-oxide

Based on the above observations, new sub-sub-questions were added to Q3f). Moreover, from the validation, it came to FDA's attention that betaine derivatives could be captured at Q3f). As betaine derivatives were not intended to be captured at Q3f), betaine derivatives were added to the original list of compounds (containing e.g., nitrobenzenes) that are exempted from consideration at Q3f). In addition, neonicotinoids captured at the newly created Q6e(iii)) were exempted from consideration at Q3f). (See section 4.4.3.24 for Q6e(iii)).) Finally, compounds (e.g., Guinea Green B, Fast Green FCF, Brilliant Blue FCF, Light Green SF Yellowish, and Benzyl violet 4B) containing at least two sulfonates and/or sulfamate functional groups were exempted to ensure that they reach Q47b) and Q47c). Q47b) and Q47c) deal with the effect of sulfonates and sulfamates on the toxicity of compounds. For these compounds, the presence of sulfonate and/or sulfamate decreases their toxic potential significantly (see Appendix 2 for toxicological data on all compounds captured at Q47b) and Q47c) including those listed above).

4.4.3.16 Update to Q3g(i)

Q3g(i)) was designed to capture compounds containing a single sulfonyl carbamate, sulfonyl carbohydrazide, or sulfonyl guanidine and place these compounds into Class III. During the validation, it came to FDA's attention that the pre-validation EDT did not address sulfonylhydrazines. Therefore, sulfonylhydrazine was added to Q3g(i)).

For Q3g(i)), one of the validation chemists had the following comment: "Would it make sense to add guidance for multiple of certain reactive groups? For example, electrophilic cross-linkers could be higher risk than their corresponding mono-functionalized versions." FDA notes that compounds captured at Q3g(i)) are placed into Class III. Those with more than one of the functional groups listed in Q3g(i)), for which there is no specific sub-sub-question, will default into Class IV, a class of higher toxic potential. Therefore, no change to the pre-validation EDT was needed to address this comment.

4.4.3.17 Updates to Q3g(ii) and the creation of Q3g(xv) and Q6b(iv)

Prior to validation, Q3g(ii)) captured compounds having a wide variety of N-containing functional groups including, but not limited to oximes, oxime ethers, and isocyanates. For Q3g(ii)), one of the validation chemists commented that in their experience, "oximes (especially oxime ethers) seem to be in a very different toxicological category from isocyanates." While FDA understood that the compounds captured at this sub-sub-question can have various modes of toxic action, we

estimated that their toxic potentials are similar (i.e., they are all Class IV compounds regardless of their mode of toxic action). The comment from one of the validation chemists prompted FDA to reevaluate all functional groups captured at this sub-sub-question. This reevaluation included, but was not limited to, FDA reviewing the toxicological data for oximes, oxime ethers, and isocyanates to determine whether the original (pre-validation) EDT Class IV placement was appropriate for all three congeneric groups of compounds.

Unfortunately, only limited data were available for isocyanates: only 1 compound in the original (pre-validation) EDT DB and 3 compounds in the external validation DB were classified based on the presence of isocyanate. Therefore, FDA set out to find additional isocyanates that have reproductive/developmental, subchronic, and/or chronic toxicity study data. In addition, we searched the scientific literature for structure-toxicity relationship information for isocyanates. While numerous toxicological studies were identified for isocyanates, for many, the exposure route was inhalational. The inhalational studies were not added to the external validation DB. Oral studies were identified for 6 additional isocyanates bringing the total number of isocyanates with oral toxicological data to 10.

In the combined EDT DB, 4 of the 10 compounds have 2 isocyanate functional groups and a single benzene ring. Two of these compounds have a NEL warranting a Class II assignment, and a second has a NEL warranting a Class III assignment. The 4th compound did not produce a NEL, only a LEL (the value of which is Class II, hence the NEL will likely be of a class higher than II (i.e., Class III or higher)). One of the isocyanates has a biphenyl ring where each benzene ring is substituted by an isocyanate. While no NEL is available for this substance, the value of the LEL is Class I (hence the value of NEL is expected to fall into a class of higher concern than Class I). Two of the 10 isocyanates have a single isocyanate and one benzene ring. In one of the compounds the benzene ring was substituted by 2 chlorines. Despite the factor of 10 used for adjusting the NEL for the short (14 days) study duration for this substance to estimate the chronic NEL, the NEL of this compound still warrants a Class II assignment (i.e., the NEL of this compound is higher than the 5th percentile Class II NEL). In the other compound, the benzene ring was substituted by 1 chlorine. There is no NEL for this compound, but the LEL value is Class I (hence the value of NEL is expected to fall into a class higher than Class I). One of the 10 isocyanates has two isocyanate substituents on an alicyclic bridged ring with a NEL warranting a Class II assignment. Two of the 10 isocyanates were acyclic; one acyclic compound contained a single isocyanate and an ester functionality, which had a NEL warranting a Class III assignment (but this assignment is due to the very conservative factor of 10 used for adjusting for the short (14 days) study duration). The second acyclic substance contains two isocyanate functional groups and no other function groups. This compound has no NEL and a LEL that is Class II (i.e., the LEL is higher than the low 5th percentile NEL for Class II). In summary, based on their NELs and LELs, all 10 isocyanates, despite their structural diversity, fall into Classes II or III.

According to an EPA review, “diisocyanates are extremely reactive” and “although they may affect other organ systems, the primary target of organ toxicity is the upper and lower respiratory tract” (which explains why FDA found that for a number of isocyanates the subchronic and chronic toxicity studies were conducted *via* the inhalational route only) (EPA, 2005). During its review of diisocyanates, EPA only

considered inhalational studies because i) that route is the most likely during occupational exposures to diisocyanates and because ii) “the diisocyanate moiety will hydrolyze in water” (hence exposure *via* drinking water is of very low concern). Isocyanates ultimately hydrolyze to ammonia and carbon dioxide. As the current version of the EDT was designed to sort chemicals based on/according to their relative chronic toxic potential through oral exposure only, isocyanates will be separated out from oximes (and the other N-containing functional groups caught at the original (pre-validation) Q3g(ii)), and based on the available oral toxicological data, our understanding of the target organ of isocyanates, and the rapid hydrolysis of isocyanates to ammonia and carbon dioxide, all isocyanates will be placed into Class III in the post-validation EDT at the newly created Q3g(xv). Aromatic and acyclic isocyanates are not going to be separated (i.e., captured at different sub-sub-questions) as they seem to have similar toxic potentials based on the limited number of oral studies that are available and based on EPA’s conclusion that “In general, there appears to be little or no difference between aromatic and aliphatic diisocyanates for the above listed” (respiratory tract toxicity, oncogenicity, respiratory and dermal sensitization, and dermal irritation) “end-points” (EPA, 2005).

In addition to reviewing the toxicological and structure-toxicity data for isocyanates, FDA reviewed the data for oximes and oxime ethers (see Appendix 2). For oximes, the duration adjusted NELs (see section 2.2 “Criteria for Data Collection and Derivation of Duration Adjusted NELs”) ranged from 3.3 to 10 mg/kg bw/day, but the duration adjusted LELs ranged from 0.06 to 7.17 mg/kg bw/day. While all NELs for oximes were higher than the pre-validation Class III 5th percentile NEL, the LEL for a single oxime (with no NEL) indicated the need for a likely Class V assignment (i.e., the LEL was so low, that the NEL is estimated to fall into the NELs covered by Class V). For oxime ethers, the duration adjusted NELs ranged from 0.005 to 36 mg/kg bw/day, a very broad range. The lowest NEL for oxime ethers indicated a need for a Class V assignment, but all other NELs indicated a Class IV or lower (III or II) assignment. As 15 oxime ethers were available, we tried to identify what features (other than the oxime ether) may affect their toxic potential.

The number of oxime ethers (1, 2, or 3) and the presence or absence of halogens in these molecules did not seem to have a significant effect on their NEL. What stood out for the substance siponimod, the most toxic oxime ether in the combined EDT DB, was the presence of an azetidine ring. Therefore, FDA set out to better understand the contribution of azetidine ring to the toxic potential of chemicals.

As there was only one substance containing an azetidine available in the combined EDT DB, FDA set out to locate toxicological data for additional azetidines. We only managed to locate *in vivo* repeat-dose toxicological data for eight compounds in addition to Siponimod. These compounds were added to the external validation DB. The same inclusion/exclusion criteria (see section 2.2) were followed as for all other substances in the EDT DB.

Based on their NELs, two compounds had NELs warranting a Class III and two compounds had NELs warranting a Class IV assignment. Two compounds had no NELs, but based on their LELs, they are expected to fall into Class III or into a higher class (as the NELs are unknown, the appropriate class assignment is also unknown. While the appropriate class is unknown, these substances are expected to have toxic potentials in the intermediate (Class III) to very high toxic potential (Class V) range).

Three of the nine azetidines had NELs warranting a Class V assignment. These nine azetidines had highly varying structural features. The number of rings present varied between 1 and 5. Some had one or more halogens, others did not have halogens. Based on the limited toxicological data that exists for these azetidines and the lack of structure-toxicity information available in the scientific literature, considering that a third of azetidines should be classified as Class V, to err on the side of caution, all azetidines will be classified as Class V at the newly created Q6b(iv)) in the post-validation EDT. If in the future additional *in vivo* repeat-dose toxicity studies for additional azetidines become available, this sub-sub-question (Q6b(iv))) will be further refined when sufficient data are available.

Regarding oximes and oxime ethers, based on the NELs for these substances (see Appendix 2), their class assignment will remain Class IV, except for those with a monobactam or azetidine skeleton. Monobactams are classified at Q6b(iii)) as Class II (see section 4.4.3.30). As at the end of Q3, all compounds are crosschecked against Q6 and are assigned to the highest class they would get at either Q3 or Q6, azetidines will be classified as Class V at Q6b(iv)) instead of a Class IV assignment at Q3g(ii)) if they also contain oxime or oxime ether.

4.4.3.18 Updates to Q3g(ii)

One of the validation chemists noted that while isocyanates and isothiocyanates were addressed in Q3g), cyanates were not included. Before adding cyanates to any sub-questions, FDA set out to review the toxicological data available for organic cyanates. While a large amount of data is available for organic isocyanates and isothiocyanates, no subchronic or chronic toxicological data were located for organic cyanates. According to the Human Metabolome Database “The cyanate ion is relatively non-toxic in comparison with cyanides.” (HMDB, n.d.). Due to the lack of toxicological data for organic cyanates and to err on the side of caution, organic cyanates will be placed into Class IV, the major default class for data poor substances with potential for toxicity, in the post-validation EDT at Q3g(ii)) where a variety of N-containing functional groups are captured and placed into Class IV. If toxicological data become available for organic cyanates in the future, the classification of cyanates will be updated.

For Q3g(ii)), for clarity, language was added to indicate that for the purposes of the EDT, C(=O)N(R)N(C=O)R when connecting two benzene rings is also considered to be a hydrazide (normally $C(=O)N(R)NR_2$).

4.4.3.19 Creation of Q3g(v, vi, vii, viii, ix, x, and xiv)

In the pre-validation EDT, Q3a(iii)), Q3g(iii)), and Q3g(iv)) were designed to address the toxicity of nitriles. The major sorting was done based on the number of nitriles present. Nitriles were placed into either Class IV or V at these sub-sub-questions. One of the validation chemists recommended nitriles, based on their knowledge, be placed into a lower class. Therefore, FDA carefully reevaluated how the pre-validation EDT handled nitriles.

There are 37 nitriles in the original (pre-validation) EDT DB and an additional 45 in the external validation DB bringing the total to 82 nitriles. As the number of nitriles available for evaluation greatly expanded, FDA reexamined whether nitriles

were appropriately sorted by the original (pre-validation) questions. In the first step, due to their large numbers and to allow the observation of structure-toxicity relationships, nitriles were grouped based on their structural features. Grouping was done based on the number of nitriles, presence or absence of oxygenated functional groups, halogens, and N- and/or S-containing functional groups. The groups were further subdivided based on whether the substance was acyclic or not, and the type of rings (if any) present. The following was observed when reviewing all toxicological data in the combined DB (see Appendix 2 for the underlying toxicological data):

a) The number of nitriles present is not a major determinant of the toxic potential of these substances. A major determinant of their toxic potential is whether one or more halogens are present. The presence of halogen(s) increased the toxic potential of nitriles substantially. The presence of rings, especially aromatic or heteroaromatic ring(s), increased the toxic potential of a nitrile compared to an acyclic nitrile.
b) For acyclic substances, when one or more nitrile functional group(s) was(were) present either with no other functional group or with only oxygenated functional group(s), all substances had a NEL that warranted a Class III or lower assignment (i.e., the most toxic substances fell into Class III) except for a few nitriles that warranted a Class IV assignment. The most striking feature of these more toxic nitriles was that the nitrile triple bond was conjugated with a double bond.
c) For acyclic substances, without the presence of halogens, but with either nitrogen- (other than nitrile) and/or sulfur-containing functional groups, all substances had a NEL that warranted a Class IV or lower assignment (i.e., the most toxic substances had a NEL that warranted a Class IV assignment).
d) For acyclic substances, when one or more nitrile functional group(s) was(were) present along with one or more halogens, all substances had a NEL that warranted a Class V or lower assignment (i.e., the most toxic substances had a NEL that warranted a Class V assignment)
e) For compounds with at least one ring and one or more nitrile functional group(s) but without any halogens, all substances had a NEL that warranted a Class IV or lower assignment.
f) For compounds with at least one ring and one or more nitrile functional group with at least one halogen, all substances had a NEL that warranted a Class V or lower assignment.

Based on the above data and pattern, Q3g(iii)) and Q3g(iv)) looking for the presence of one or more than one nitrile, respectively, classifying nitriles as IV and V, respectively, were deleted. The following 7 sub-sub-questions were added to Q3g) to better address the toxic potentials of nitriles (note that the class assignment is based on the NELs of the large number of substances that are captured at each of the newly created sub-sub-questions below (for NELs, see the combined EDT DB in Appendix 2)):

- 3g(v)): acyclic substance with one or more nitrile functional group(s) and at least one covalently bound halogen (Class V)

- 3g(vi)): acyclic substance with one or more nitrile functional group(s) either with no other functional group or with only oxygenated functional group(s) and the nitrile triple bond is not conjugated with another triple bond or double bond (Class III)
- 3g(vii)): acyclic substance with one or more nitrile functional group(s) and the nitrile triple bond is not conjugated with another triple bond or double bond and have either one or more N- and/or S-containing functional group(s) with or without additional oxygenated functional groups (but no other functional groups) (Class IV)
- 3g(viii)): one or more nitriles with at least one ring and at least one covalently bound halogen present (except substances (i.e., neonicotinoids) that fit the structural descriptions at Q6e(iii)). For these substances, say no at Q3g(viii)) as they will be classified at Q6e(iii)).) (See section 4.4.3.24 for neonicotinoids) (Class V)
- 3g(ix)): one or more nitriles and at least one ring (other than azetidine) without the presence of any covalently bound halogens (Class IV)
- 3g(x)): acyclic nitrile with no covalently bound halogens where the nitrile triple bond is conjugated with another triple bond or double bond (Class IV)
- 3g(xiv)): all other nitrile-containing compounds (Class III)

4.4.3.20 Creation of Q3g(xi)

The original (pre-validation) EDT questions did not directly address the biguanidine functional group. Based on data from the external validation DB and the scientific literature, the biguanidine functional group was added as Q3g(xi)) to the post-validation EDT placing these compounds of relatively low toxic potential into Class II (see the combined EDT DB in Appendix 2 for examples such as phenformin hydrochloride (CAS 834-28-6), metformin hydrochloride (CAS 1115-70-4), polyhexamethylene biguanidine hydrochloride (CAS 32289-58-0), and 1-(*o*-tolyl)biguanide (CAS 93-69-6)).

4.4.3.21 Creation of Q3g(xii)

During the validation, it came to FDA's attention that oxamides were not addressed by any questions in the pre-validation EDT. Upon the review of the compounds in the original (pre-validation) EDT DB, two oxamides were identified. Only one oxamide was identified in the external validation DB. Therefore, FDA performed an extensive search of a large number of toxicological DBs to see whether additional oxamides could be identified to support the class assignment of oxamides. Chronic toxicological data were identified for only two additional oxamides, bringing the total number of oxamides to five (see Appendix 2).

Unfortunately, no additional publicly available reproductive/developmental, subchronic, or chronic toxicological data were found for oxamides. Comparing the duration adjusted NELs (mg/kg bw/day) to the pre-validation 5th percentile class NELs, three of these substances have NELs that warrant a Class II placement, and one has a NEL warranting a Class I placement. On the mmol/kg bw/day basis, due to its relatively high MW and lower NEL, one substance (CAS 480449-71-6) warrants a Class IV assignment as its NEL is just lower than the Class III 5th percentile NEL when using the unit of mmol/kg bw/day. When using the Class median MWs to transfer the

NEL from mmol/kg bw/day to mg/kg bw/day, this is a substance warranting a Class III placement.

While acute toxicity data indicated that seven additional oxamides had acute toxic potentials lower than that of aspirin, a compound of low acute toxic potential (see PubChem for LD_{50} values of aspirin), (El-Koussi et al., 1978), the currently available data are just not enough to confidently place these compounds into a specific EDT class. To err on the side of caution and based on the presently available data, these compounds will be defaulted into Class III, the class of intermediate toxicity, despite their relatively high NELs and LD_{50}s.

4.4.3.22 Creation of Q3g(xiii)

One of the validation chemists recommended that carbodiimide should be added to the reactive moieties listed at Q3g). Only one carbodiimide was present in the original (pre-validation) EDT DB. As *in vivo* repeated-dose toxicological data for only two carbodiimides were available in the external validation DB, FDA did an extensive search to find data for additional carbodiimides. Toxicological data for only one additional carbodiimide were located. Moreover, we tried to locate *in vivo* toxicological data for sulfur diimides.

While the data for carbodiimides are very limited (see Appendix 2), based on the duration adjusted NELs, one of the carbodiimides has a duration adjusted NEL (0.1 mg/kg bw/day) that is lower than the pre-validation Class IV 5th percentile NEL of 0.29 mg/kg bw/day. No toxicological data was available for sulfur diimides. Therefore, to err on the side of caution, both carbo- and sulfur-diimides will be placed into Class V at the newly created Q3g(xiii)).

4.4.3.23 Creation of Q3g(xvi)

The pre-validation EDT placed sulfonyl isocyanates into Class III at Q3g(i). One of the validation chemists noted that according to their knowledge, sulfonyl isocyanates are highly reactive electrophiles, and the chemist was surprised that they are placed into Class III. The chemist recommended FDA take a second look at the classification of sulfonyl isocyanates.

This input prompted FDA to reexamine whether the classification of sulfonyl isocyanates as Class III appropriately reflects their toxic potentials. Unfortunately, a single sulfonyl isocyanate was present in the original (pre-validation) EDT DB and none in the external validation DB. The only substance for which we had toxicological data is 2-chlorobenzenesulfonyl isocyanate (CAS 64900-65-8) with a 28-day gavage study that produced a LOAEL of 50 mg/kg bw/day and no NOAEL (DuPont, 2010). Therefore, FDA set out to find additional reproductive/developmental, subchronic, and chronic oral toxicological data for sulfonyl isocyanates.

During our review of the scientific literature for sulfonyl isocyanates, we found that, “Owing to the polar sulfonyl group attached to the cumulative double bond system, the reactivity of the isocyanato group toward nucleophilic attack on the center carbon atom is vastly enhanced in sulfonyl isocyanates. Also, the polarization of the C=N double bond is sufficiently influenced by the sulfonyl group to change the regular reaction pattern observed in isocyanates.” (Ulrich, 1965). Because of the very limited oral toxicological data available for sulfonyl isocyanates and as isocyanates are

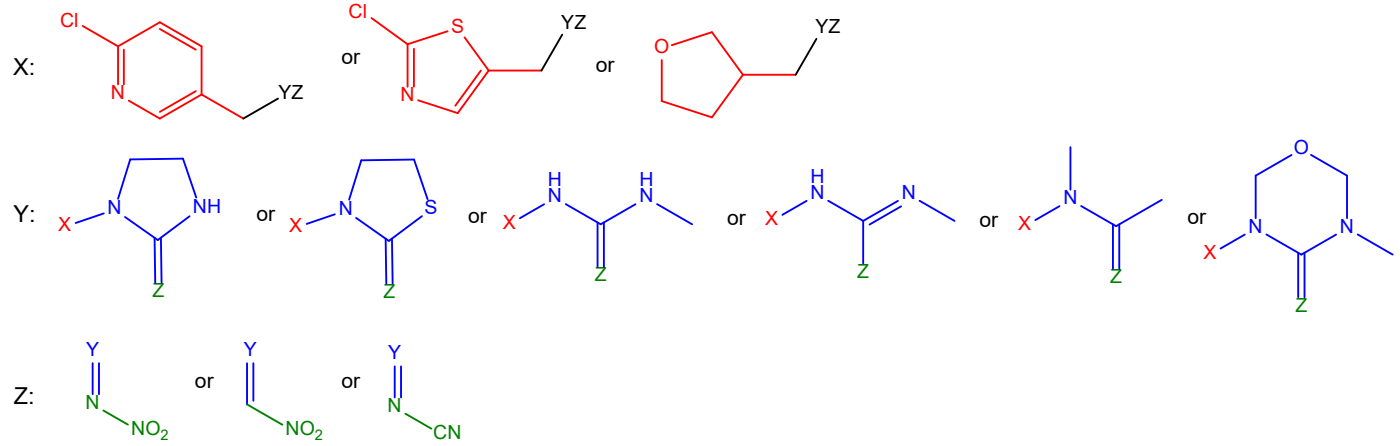
expected to be more toxic than cyanates (Class III compounds), to err on the side of caution, sulfonyl isocyanates will be classified as Class IV instead of Class III going forward at the newly created Q3g(xvi)). If additional toxicological data become available in the future, FDA will reexamine the classification of sulfonyl isocyanates.

4.4.3.24 Creation of Q3g(xvii) and 6e(iii)

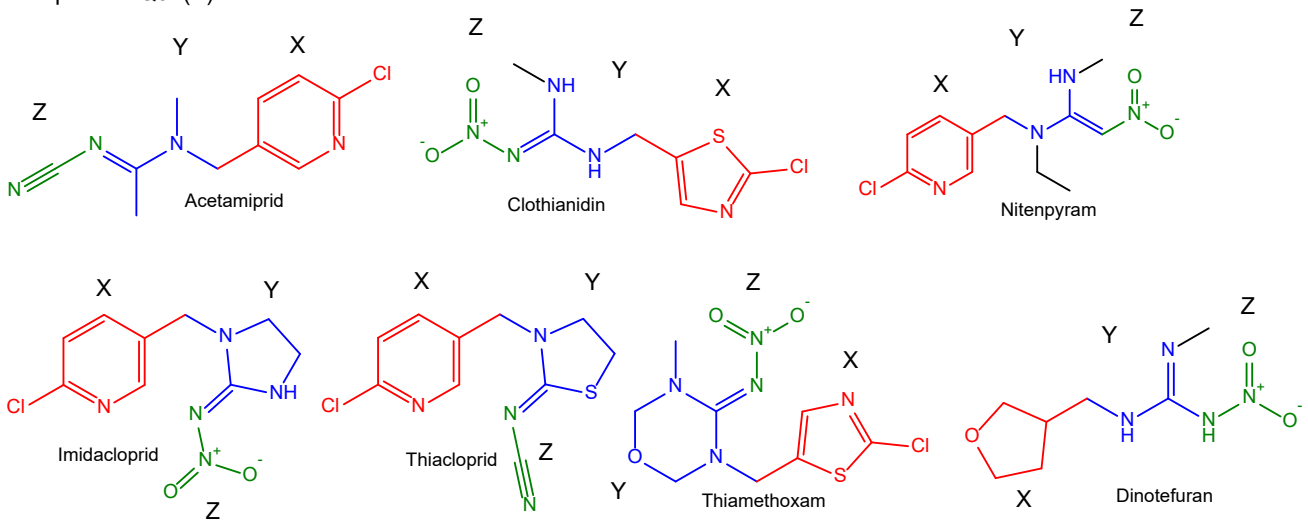
Amidines were handled by Q3g(ii)) in the pre-validation EDT. After a review of the classifications of amidines, FDA noted that some amidines warrant a Class V classification based on their NOAELs (hence the original (pre-validation) classification of IV was not protective enough for some of the amidines) (see Appendix 2 for toxicological data). Based on the currently available limited toxicological data and knowledge of structure-toxicity relationships, FDA is unable to refine the classification of amidines to capture less toxic amidines at a different sub-sub-question than more toxic amidines allowing the placement of less toxic amidines into a lower EDT class. Therefore, to ensure that the TTCs are protective for all amidines, in the post-validation EDT, amidines are placed into Class V instead of the original (pre-validation) Class IV at the newly created Q3g(xvii)). FDA notes that some amidines that are neonicotinoids, a class of insecticides, will still be placed into Class IV at the newly created Q6e(iii)) as enough toxicological data are available for neonicotinoids to show that Class IV is protective for all neonicotinoids captured at Q6e(iii)).

Q6e(iii)) is a newly created question to address the number of additional neonicotinoids found in the external validation DB. The 7 neonicotinoids in the DB have similar mode of toxic action but varied toxic potential (1 of the 7 neonicotinoids has a NEL warranting a Class I placement, 4 of the 7 neonicotinoids have a NEL warranting a Class II placement, and 2 of the 7 neonicotinoids have a NEL warranting a Class IV placement). Based on the publicly available data and information, at this point, FDA found no information on why some of these structurally closely related neonicotinoids are more toxic than others. Due to their structural similarities, mode of toxic action (Seifert, 2014), and somewhat still limited data available (see Appendix 2), neonicotinoids with the structural features described below will be classified at the newly added Q6e(iii)) as Class IV; a class that is protective to all neonicotinoids described in this newly created sub-sub-question.

Neonicotinoid skeletal structures:



Examples for Q3e(iii):



4.4.3.25 Updates to Q3h: creation of multiple sub-sub-questions

The pre-validation Q3h) aimed at capturing isothiocyanates, ureas, and ureides. One of the validation chemists noted that isothiocyanates are more reactive than the other two groups and, therefore, should be separated from ureas and ureides. FDA had four isothiocyanates in the original (pre-validation) EDT DB (and none in the external validation DB) that fit the description of the pre-validation sub-question. A yes response at Q3h) for an isothiocyanate, urea, or ureide resulted in a Class III assignment. For two of the isothiocyanates, the best representative studies only yielded a LEL but not a NEL. For the other 2 substances, when their NELs were compared to the pre-validation 5th percentile NELs of each EDT class, one had a NEL that would have warranted a Class II assignment and the 2nd one had a NEL that would have warranted a Class IV assignment (though the NEL of this compound was only very slightly below the 5th percentile Class III NEL). Not enough data were available to deduce what would make an isothiocyanate more or less toxic based on

its structural features in addition to the isothiocyanate functional group. Therefore, to err on the side of caution, isocyanates will default into Class IV in the post-validation EDT at the newly created Q3h(i)).

The pre-validation EDT Q3h) looked for the presence of ureide but had the structural features of ureas described within brackets. Therefore, FDA was asked to clarify whether we are looking for ureas, ureides, or both at this question. In addition, this question did not allow the presence of additional oxygenated functional groups, which one of the validation chemists found confusing. To address these notes from the validation chemists, FDA first reviewed all data for ureas and ureides to see whether they should be grouped together. In addition, FDA noticed that sulfonylureas, guanylureas, semicarbazones, and certain other related functional groups are not addressed directly at any questions. Therefore, FDA evaluated whether the addition of one or more sub-sub-questions at Q3h) to address these functional groups were warranted and the appropriate class assignments for these compounds.

The pre-validation Q3h) divided ureides (-C(=O)NC(=O)NC-), when the ureide is not part of a ring, into two groups: i) substances containing one or more aromatic ring(s) with at least one halogen substituent or substances containing at least one heteroaromatic ring and ii) substances containing neither a halogen substituted aromatic ring nor a heteroaromatic ring. A yes response at Q3h(i)) resulted in a Class IV assignment, and a yes response at Q3h(ii)) resulted in a Class III assignment. Upon the review of the toxicological data in the combined EDT DB (see Appendix 2), these are appropriate classifications (i.e., comparing the NELs of these substances to the pre-validation Class low 5th percentile NELs, they were sorted into their appropriate classes). In the post-validation EDT, ureides that contain at least one aromatic ring and at least one ring halogen and ureides that contain at least one heteroaromatic ring are addressed at Q3h(iii)) (still Class IV). All other ureides are now addressed at Q3h(vii)) (still Class III) (i.e., the original (pre-validation) sub-sub-questions stayed the same along with the class assignment, just the positions of the sub-sub-questions were shifted within the sub-question.

Pre-validation Q3h) divides ureas (-CNC(=O)NC-) the same way as ureides at the same sub-sub-questions (Q3h(i)) and Q3h(ii))). A total of 9 ureas in the combined EDT DB satisfied the structural requirements at Q3h(i)). Based on their NELs, 3 of the 9 substances classified at this sub-sub-question have a NEL warranting a Class II assignment, 4 of the 9 substances have a NEL warranting a Class III assignment, and 2 of the 9 substances (Diuron and Fluometuron) have a NEL warranting a Class IV assignment. Therefore, Class IV is the most protective class assignment for the compounds that contain urea and either an aromatic ring substituted with halogen or a heteroaromatic ring. Hence, the class assignment for these compounds did not change and are still captured together with ureides containing at least one aromatic ring with at least one ring halogen substituent or containing at least one heteroaromatic ring.

Based on the review of toxicological data for almost three dozen ureas (other than sulfonylureas) and a review of the literature, it was clear that acyclic aliphatic ureas with no other functional groups (e.g., isobutylidene diurea and 1,1'-propane-1,3-diylbis(3-octadecylurea)) have low toxic potentials (see Appendix 2). While these used to be placed into Class III at the pre-validation Q3h(ii)), based on the data in the combined validation DB, it was clear that this is an overclassification. Therefore, a new

sub-sub-question, Q3h(vi)) was created to sort these acyclic aliphatic ureas with no other functional groups and no halogens into Class II instead of Class III.

A review of the non-acyclic compounds that got captured at the pre-validation Q3h(ii)) and placed into Class III showed that this class assignment was appropriate. A new sub-sub-question, Q3h(vii)), was created to capture these compounds (to separate them from the less toxic acyclic ureas) and place them into Class III.

In the pre-validation EDT, ureas with an oxygenated functional group on the alpha-carbon of the urea or an -O- attached to the urea N were excluded at Q3h) and mostly defaulted in Class IV elsewhere. A review of the NELs of these compounds (e.g., Dichloralurea, Metobromuron, and Linuron; see Appendix 2) yielded that they should be classified as Class IV. To better enable the EDT to support read-across, instead of allowing these compounds to default into Class IV, a new sub-sub-question, Q3h(ii)), was added to the post-validation EDT to capture these compounds together.

Sulfonylureas were treated the same as ureas in the pre-validation EDT and were placed into either Class III or IV. Even though there are 27 sulfonylureas in the combined EDT DB, upon the review of the data for these compounds, FDA found it difficult to make any conclusions on structure-toxic potential relationships. What we found is that sulfonylureas with at least one heteroaromatic ring tend to be more toxic and the presence of halogen tends to increase the toxic potential (but noting that there are a few compounds that despite of not having halogen are amongst the most toxic sulfonylureas). On the other hand, the presence of $-S(=O)_2-$ (in addition to the one that is part of the sulfonylurea functional group), $-NS(=O)_2-$, and/or amide decreases the toxic potential of sulfonylureas. Therefore, sulfonylureas with at least one heteroaromatic ring without $-S(=O)_2-$, $-NS(=O)_2-$, and/or amide (e.g., Express, Triasulfuron, and Oxasulfuron) will be placed into EDT Class IV, sulfonylureas with a heteroaromatic ring with $-S(=O)_2-$, $-NS(=O)_2-$, and/or amide but without any covalently bound halogens (e.g., rimsulfuron, amidosulfuron, and orthosulfamuron) will be placed in Class III, and all other sulfonylureas (e.g., Pergafast 201, Chlorpropamide, and Tolazamide) into EDT Class II. Sulfonylureas in Classes IV, III, and II will be addressed by the newly created Qs 3h(iv)), 3h(v)), and 3h(viii)), respectively.

Semicarbazones (C=NNC(=O)N), carbohydrazides (NNC(=O)NN, NNC(=O), and C(=O)NNC(=O), guanylureas (NC(=O)N=C(NR)N were not specifically addressed in the pre-validation EDT, and it caused some confusion among the validation chemists how they should be treated when running them through the EDT during the validation. Based on their NELs, the two semicarbazones (diflufenzopyr and metaflumizone) were Class II and III, two of the three carbohydrazides were Class II and one of the three carbohydrazides was Class III (Carbohydrazide, Chromafenozide, and Irganox 1024), and the single guanylurea (guanylurea phosphate) was Class I. Based on the limited data available and the limited scientific literature, these substances seem to exhibit relatively low toxic potential. As the data for these substances are very limited, to err on the side of caution, they will be placed into Class III, a class assignment protective for all of these compounds, at the newly created Q3h(vii)). If additional data becomes available for these substances in the future, their sorting and class assignment will be refined.

None of the pre-validation EDT questions addressed thiocyanates. This was overlooked originally as the original (pre-validation) EDT DB, unlike the external validation DB, did not contain any compound with a thiocyanate functional group.

During the validation this shortcoming came to light as the external validation DB contains compounds with thiocyanate functional group. Therefore, Q3h(ix)) was added in the post validation EDT to enable the classification of thiocyanates such as methylene bis(thiocyanate).

4.4.3.26 Update to Q4

At Q4, sulfinate and thionosulfate were added to the post-validation EDT to ensure that these substances reach Q26 where their presence was allowed in the pre-validation EDT.

4.4.3.27 Creation of Q5e

The pre-validation EDT defaulted compounds containing either a tin counterion or covalently bound tin into Class IV at Q5. The original (pre-validation) EDT DB contained only 5 compounds containing tin. Three of these five compounds contained Sn^{2+} as a counterion to oxalate, tartrate, or oleate and have relatively high non-duration adjusted NOAELs (50 mg/kg bw/day (28 days), 50 mg/kg bw/day (28 days), and 500 mg/kg bw/day (28 days), respectively) (see Appendix 2). FDA notes that oxalate, tartrate, and oleate have very low toxic potentials and are considered to be safe (all Class I compounds). The other two compounds in the original (pre-validation) EDT DB are dioctyltin di(isooctyl thioglycolate) and tributyltin oxide with NOAELs of 1.5 mg/kg bw/day (>98 days) and 0.19 mg/kg bw/day (742 days), respectively (see Appendix 2); NOAELs that are lower than those compounds containing tin as a counterion and not in the covalently-bound form. In the external validation DB, toxicological data for an additional 15 tin-containing substances became available. As organotin compounds are widely used for agricultural, industrial, and biomedical applications, FDA decided to review, and if possible, refine the classification of tin-containing compounds. To achieve this, FDA actively searched for toxicological data on additional tin-containing compounds and found data for another 6 tin-containing compounds bringing the total to 26 compounds. Based on the review of the above toxicological data in the combined EDT DB (see Appendix 2) and articles on structure-toxicity relationships for organotins, FDA concluded the following:

a) The toxicity of organotin compounds increases with the number of alkyl groups attached (Nath, 2008). Trialkyltin compounds are the most toxic, followed by the dialkyl- and monoalkyl-tin compounds, with the ethyl derivative in each group being reported as the most toxic. In general, as the length of the alkyl group increases, its toxicity decreases (Nath, 2008; Fait et al., 1994).
b) Triphenyltin compounds are moderately toxic, but less toxic than trialkyltin compounds with shorter alkyl chains (Nath, 2008; Ostrakhovitch, 2022). The aryltin compounds are less soluble and less toxic than the alkyl tin compounds (Kimbrough, 1976).
c) Chlorinated organotins are highly toxic. At and above pH 6.0, the Sn–Cl bond in most organotin halides is hydrolyzed and tin can attack electron donor atoms (Pagliarani et al., 2013). Based on the data available in the EDT DBs, chlorinated tins are more toxic than their corresponding (based on alkyl chain length) non-chlorinated counterparts.

Based on the above data and information, organotin compounds will be sorted/classified based on the following rules at Q5:

- Q5e(i)): tin (Sn) as Sn^{2+} counterion(s) to an organic molecule. (Class II) (Note: the classification will also depend on the organic counterion: the compound containing Sn^{2+} will be assigned to the highest class that either Sn^{2+} or the organic counterion has.)
- Q5e(ii)): organotin compounds containing one or more covalently bound halogen(s) where the halogen is directly bonded to the tin. (Class VI)
- Q5e(iii)): mono-, di-, or tri-alkyltin (these 1-3 alkyl chains must be directly attached to the Sn) with each alkyl chain having a chain length of maximum 4 carbons. In addition to the direct alkyl substitution(s), the tin may have any number of direct hydroxyl, alkyl ester, alkyl ether, -S-, and/or sulfate substitution along with a single benzoic acid ester (i.e., benzoate) substitution, but no other substitutions. If two or more direct Sn-S substitutions are present and the chain length of these -S- linked substitutions exceeds 4 atoms, say no at this sub-sub-question. (Class V)
- Q5e(iv)): mono-, di-, tri-, or tetra-alkyltin (these 1-4 alkyl chains must be directly attached to the Sn) with each alkyl chain having a chain length of 5 or more carbons. The tin may have any number of direct hydroxyl, alkyl ester, alkyl ether, -S-, and/or sulfate substitution along with a single benzoic acid ester substitution but no other substitutions. (Class IV)
- Q5e(v)): phenyltin or cyclohexyltin with the phenyl or cyclohexyl ring(s) directly attached to the tin, with or without the tin having direct hydroxyl, ether, -S-, and/or sulfate substitution but no other functional groups or halogens. (Class V)
- Q5e(vi)): all other organotin compounds. (Class IV)

4.4.3.28 Expansion of elements considered at Q6

At Q6, the list of elements that were allowed to be present were expanded to also contain F and I, as there was no reason why these two elements should be excluded here as F and I are not expected to greatly influence the toxic potential of the compounds that are addressed at Q6.

4.4.3.29 Clarification for Q6a

Q6a) aims to capture highly bioactive compounds with a steroidal nucleus such as testosterone and estrogen. When additional rings that are fused, spiro fused, or bridged to the steroidal nucleus are present, it may decrease the bioactivity of the compound. Therefore, as these compounds were not intended to be captured here, the following clarification was added to Q6a): “The additional rings, if present, cannot be fused (except for cyclopropyl ring), spiro fused, or bridged to the steroidal nucleus.”

4.4.3.30 Creation Q6b(iii)

Certain monobactam antibiotics of low toxic potential used to get classified at Q3g(ii)) as Class IV due to the co-presence of oxime ether (C=NOR) functionality when using the pre-validation EDT while others were caught elsewhere. To capture all

monobactams together (to better facilitate the read-across function of the EDT) and sort them according to their relatively low toxic potentials (see Appendix 2), a new sub-sub-question, Q6b(iii)), was created. Moreover, to avoid certain monobactams from being captured at Q3g(ii)) as Class IV due to the co-presence of oxime ether, the following was added at the end of Q6: “Note that at Q3 the user is asked to cross-check against Q6 and assign the substance to the highest class it would get either at Q3 or Q6. For Q6b(iii)), ignore this instruction and assign the substance according to the instructions at Q6b(iii)) (i.e., Class II).”

4.4.3.31 Creation of Q6d(i), Q6d(ii), Q6d(vi), and Q6d(v)

Aflatoxins, brevetoxins, and ciguatoxins were captured at pre-validation Q6d(i)) along with other compounds with expected very high toxic potentials and classified as Class V. A review of the data in the combined EDT DB (see Appendix 2) yielded that for some aflatoxins, brevetoxins, and ciguatoxins, Class V may not be protective enough. Therefore, two new sub-sub-questions were created to capture aflatoxins (post-validation Q6d(i))) and brevetoxins and ciguatoxins (post-validation Q6d(ii))) to place these toxins into Class VI. All other toxins that were intended to be captured at the pre-validation Q6d(i)) are now captured at post-validation Q6d(iii)) and still placed into Class V.

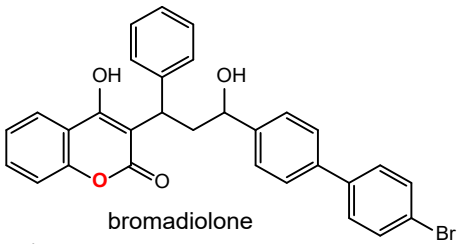
Ochratoxins were not captured at a specific pre-validation EDT question but, unintentionally, defaulted into Class IV at Q47. Upon the review of the toxicological data, FDA realized that Class IV is not protective enough for all ochratoxins. For example, based on its chronic NOAEL (NTP, 1989), Ochratoxin A (OTA), should have been classified as Class V as its NOAEL is much lower than the Class IV pre-validation 5th percentile NEL. In the case of ochratoxins, the kidney is the primary target for toxicity (Dickman and Grollman, 2010). In addition to nephrotoxicity, in general, ochratoxins, depending on the species, can act as hepatotoxins, immunotoxins, neurotoxins, teratogens, or carcinogens (O’Brien and Dietrich 2005; Gupta et al., 2018).

A review of the very large number of compounds that were captured at the pre-validation Q6d(i)) also revealed that compounds containing a phthalocyanine skeletons (e.g., C.I. Pigment Blue 15 (CAS 147-14-8) and C.I. Pigment Green 7 (CAS 1328-53-6)), despite very low toxic potentials (see Appendix 2), were captured at the pre-validation Q6d(i)) and placed into Class V. To avoid this gross overclassification, a new sub-sub-question, Q6d(v)), was created to capture compounds containing a phthalocyanine skeleton and place them into Class I.

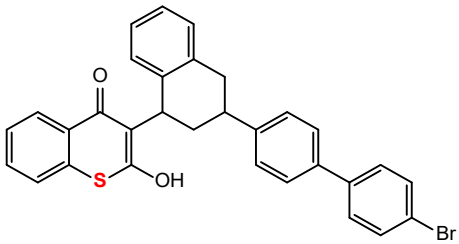
4.4.3.32 Expansion of Q6f(i)

Pre-validation Q6f(i) was designed to capture anticoagulants with the following structural features: 4-hydroxycoumarin system substituted at the 3-position either by an alkyl chain (the chain can be a part of an alicyclic ring) containing 1-phenyl or 1-phenyl-3-keto (or hydroxy) substituent. During the validation it came to FDA’s attention that compounds with the above structural features where the ring -O- in the coumarin ring system is replaced with an -S- still retain their anticoagulant properties (e.g., difethialone, an anticoagulant rodenticide). Therefore, the sub-sub-question was

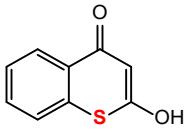
updated to capture compounds not just with a 4-hydroxycouarin ring system, but also with a 4-hydroxylthiocoumarin and 2-hydroxythiochromen-4-one ring system.



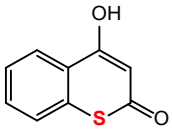
bromadiolone
(captured with pre- and post-validation EDT)



difethialone
(captured with the post-validation EDT)



2-hydroxythiochromen-4-one



4-hydroxylthiocoumarin

4.4.3.33 Clarification for Q6g(i)

At Q6g(i)), after one of the validation chemists was unclear on whether additional rings may be present (i.e., in addition to the two benzene rings requested by the question), the question was updated from “two benzene rings connected by a 2- or 3-carbon chain (connector, with or without unsaturation)” to “two benzene rings connected by a 2- or 3-carbon chain (connector, with or without unsaturation) (no additional rings may be present)” for clarity.

4.4.3.34 Creation of Q6h(ii)

Barbiturates were not directly addressed by the pre-validation EDT. Dependent upon structural features, they defaulted into various classes. To avoid this and to enhance the read-across capabilities of the EDT, a new sub-sub-question, Q6h(ii)), was created to capture all barbiturates and place them into Class IV.

4.4.3.35 Creation of Q6h(iii)

One of the substances in the external validation DB is Misoprostol with a LOAEL of 0.0067 mg/kg bw/day, no NOAEL, in a 3-day study (Kim et al., 2015) indicating a very high potential for toxicity. Misoprostol is a synthetic prostaglandin (i.e., prostaglandin analogue) medication and its uses include prevent and treat stomach and duodenal ulcers, induce labor, the induction of medical abortions, and treat postpartum bleeding. Prostaglandins are physiologically active lipid compounds that have diverse hormone-like effects in animals.

Misoprostol defaulted into Class IV using the pre-validation EDT. Depending on their structural features, prostaglandins would have defaulted into Classes II or IV, which, based on their toxic potential was an under-classification. To not default prostaglandins and prostacyclins at various questions into various classes, to ensure their proper classification, and to enhance the EDT’s read-across capability, Q6h(iii)) was created to capture these compounds at the same sub-sub-question. To determine the most appropriate classification, FDA set out to find additional publicly available oral toxicological data for these substances.

The publicly available oral toxicology data for prostaglandins were very limited. Most publicly available studies FDA found were via the intravenous

administration route. For epoprostenol, a prostaglandin that can inhibit platelet aggregation, no NOAEL was available. The LOAEL was 0.0018 mg/kg bw/day (Unknown (a), n.d.) when administered intravenously. Dinoprostone, an oxytocic (capable of inducing contraction or greater tonicity of the uterus) prostaglandin, is used to induce labor, terminate pregnancy, and for other medical indications. In a 3-month oral study in rats, at the lowest dose of 10 mg/kg bw/day loosening of the feces and increases in stomach weight were observed (Unknown (b), n.d.). In a 3-month intravenous study in dogs, the NOAEL for Latanoprost was established as 0.001 mg/kg bw/day based on clinical signs (Unknown (c), n.d.). In rabbits, latanoprostene bunod, when administered intravenously from gestational day 7 to 19, was found to cause reproductive and developmental toxicity even at the lowest tested dose of 0.00024 mg/kg bw/day (Unknown, 2016). In a 13-week intravenous study, a LOAEL of 0.1 mg/kg bw/day, no NOAEL, was established for travoprost with the bone as the primary target organ (Unknown, 1998).

Based on the literature review and the above toxicological data, it is clear that prostaglandins have very high biological activities. Unfortunately, most toxicological data originates from intravenous studies, and only very limited oral toxicological data are available publicly to aid us in classifying them. Extensive metabolism occurring during absorption is often the reason why they are not used orally when used as drugs (Magee et al., 1973). Regardless, many prostaglandins are active orally. Based on the limited, publicly available, oral preclinical toxicological data, prostaglandins will be captured at the newly created Q6h(iii) and placed into Class VI, to err on the side of caution until further oral safety data become available to refine their sorting and classification.

4.4.3.36 Updates to Q7

Based on comments from one of the validation chemists, Q7b(i)) and Q7b(ii)) were expanded to allow the presence of I in addition to F, Cl, and Br as not including I was not scientifically justifiable considering that these sub-sub-questions sorted compounds into Classes IV and V, respectively, and saturated acyclic or alicyclic iodine containing compounds would have defaulted into Class IV at the end of Q8 anyway. The presence of I remained not allowed at Q7b(iii)) and Q7(iv)), as these questions place compounds into Classes II and III, respectively, and the I-containing versions of the compounds addressed at these two sub-sub-questions are expected to have higher toxic potentials than their corresponding Cl- and/or Br-containing counterparts. Moreover, FDA added that the presence of I in addition to other halogens are also allowed at Q7c(ii)), Q7e), and Q7f), as excluding I at these sub-sub- or sub-questions was not justifiable scientifically; I-containing counterparts of the substances described at these questions are expected to be at least as toxic as their other halogen-containing counterparts, and these questions place substances into EDT Class V. If I-containing compounds were not caught here, they would mostly default into Class IV.

4.4.3.37 Clarifications for Q7c and Q7d

At Q7c) and Q7d), after one of the validation chemists misunderstood that “a benzene ring substituted by…” does not allow for the presence of any other rings,

these two sub-questions were updated to “benzene (i.e., a single benzene molecule, no other rings may be present) substituted by…” for clarity.

4.4.3.38 Changing class assignment for Q7g(iv)

Compounds captured at Q7g(iv) were placed into Class IV by the pre-validation EDT. We reassigned compounds captured at this sub-sub-question to Class V. This reassignment was based on a thorough review of the toxicological data for these compounds. The compounds in this sub-sub-question are primarily toxic due to the reactivity of the halogen atoms (F, Cl, Br, I) attached to the carbon atoms bearing ether oxygens, indicating a high potential for toxicity.

For example, chloro(methoxy)methane (CAS 107-30-2) produced a Lowest Effect Level (LEL) of 450 µg/kg bw/day, with no No Effect Level (NEL) identified (Laskin et al., 1975). While the NEL is expected to be below this value, how far below is unknown. Considering that the post-validation 5th percentile NEL for Class IV is approximately 288 µg/kg bw/day, the potential NEL for this compound is likely significantly lower than the Class IV 5th percentile NEL. The post validation 5th percentile NEL for Class V is around 5.2 µg/kg bw/day. Therefore, Class V is the most appropriate/protective for this substance.

Other highly toxic and suspected carcinogenic substances captured at the same sub-sub-question and included in this classification are bromo(methoxy)methane (CAS 13057-17-5), iodo(methoxy)methane (CAS 13057-19-7), (chloromethoxy)ethane (CAS 3188-13-4), and (bromomethoxy)ethane (CAS 53588-92-4). Some halogenated ethers are recognized for their neurotoxic effects; for instance, (chloromethoxy)ethane is known to be both a neurotoxin and hepatotoxin (see https://pubchem.ncbi.nlm.nih.gov/compound/18523#section=Toxicity).

4.4.3.39 Clarification and addition to Q11

In the pre-validation Q11, no allowance was made on what to do if the compound is a cyclic methylenedioxy fused to an aromatic ring that has an ester, thioester, hemiacetal, acetal (other than cyclic methylenedioxy), hemiketal, ketal, sulfate, mono- or poly-glycoside, carbonate, anhydride and/or polysulfide. To correct for this oversight, the user is now instructed to do the following: “If yes to Q11 and the compound is a cyclic methylenedioxy fused to an aromatic ring, hydrolyze or reduce the functional groups listed in the question (but not the methylenedioxy fused to the aromatic ring). Send the cyclic methylenedioxy fused to the aromatic ring to Q33 and all other hydrolysis products to Q1. Assign the compound to the highest class any of its hydrolysis and/or reduction products may have.”

Moreover, the following new instruction was added at the end of Q11: “If yes to Q11 and the compound is an epoxidized triglyceride, send the compound to Q14 without performing any hydrolysis (i.e., do not hydrolyze the triglyceride).” This was done to enable the recognition of epoxidized triglycerides (that is, to allow for distinguishing epoxidized triglycerides from epoxy fatty acids) at the post-validation Q14 where epoxidized triglycerides and epoxy fatty acids get classified at different sub-sub-questions (see 4.4.3.38).

4.4.3.40 Updates to Q14

Epoxidized soybean oil (ESBO) was originally assigned, unintentionally, as Class V at the pre-validation Q14 due to the presence of multiple epoxides. FDA observed that epoxidized triglycerides get hydrolyzed at Q11 before being sent to Q12, Q13, and then Q14, making it harder to recognize them. Therefore, to enable the recognition of epoxidized triglycerides, the user is asked not to hydrolyze these at the end of Q11 (see 4.4.3.37).

Moreover, based on the review of the toxicological data for ESBO, the substance should have been assigned to Class I (Bendele et al., 2018). The non-publicly available toxicity data for epoxidized linseed oil (ELSO), a related substance to ESBO, was reviewed by FDA (but not included in the combined ET DB due to its confidential nature). Based on the NOAEL for ELSO, it should also be an EDT Class I compound. A review of the scientific literature on epoxidized fatty acids and their esters indicated that some epoxidized fatty acids and esters may exert both desired and undesired biological activity (i.e., toxicity) (Kuksis and Pruzanski, 2017). Except for ESBO and ELSO, FDA was unable to locate in vivo reproductive/developmental, subchronic, and/or chronic/carcinogenicity studies for other epoxidized fatty acids and their esters. The in vivo studies on ESBO and ELSO and the in vitro toxicity studies of some epoxidized fatty acids indicated low toxic potentials (Kitaguchi et al., 2020). Other in vitro studies indicated the potential for toxicity for some epoxidized fatty acids (Green et al., 2000). Leukotoxin (C18) is stated to target white blood cells (Kachlany, 2010) and isoleukotoxin (C18) may contribute to organ failure (Moran et al., 1997). Based on the available data, toxicity of the epoxides of fatty acids and fatty acid esters may be due to their diol metabolite. Unfortunately, no oral studies are available for these to determine what oral dose levels may result in adverse effects. Therefore, to err on the side of caution, epoxidized triglycerides will be placed into Class II at post-validation Q14a(i)) and all other epoxidized fatty acids and their esters will be placed into Class III at post-validation Q14a(ii)). Once more data becomes available for these substances, FDA will reevaluate their classifications.

4.4.3.41 Clarification for Q17

The original (pre-validation) Q17 asked “Does the substance contain one or more heteroaromatic rings?” To clarify that compounds that have tautomers that are heteroaromatic should prompt a yes response here, the question was updated to: “Does the substance contain one or more heteroaromatic rings or may have one or more tautomers that is heteroaromatic?” Additionally, the definition section on tautomerization was expanded and additional examples were included. These changes were made based on input from the validation chemists.

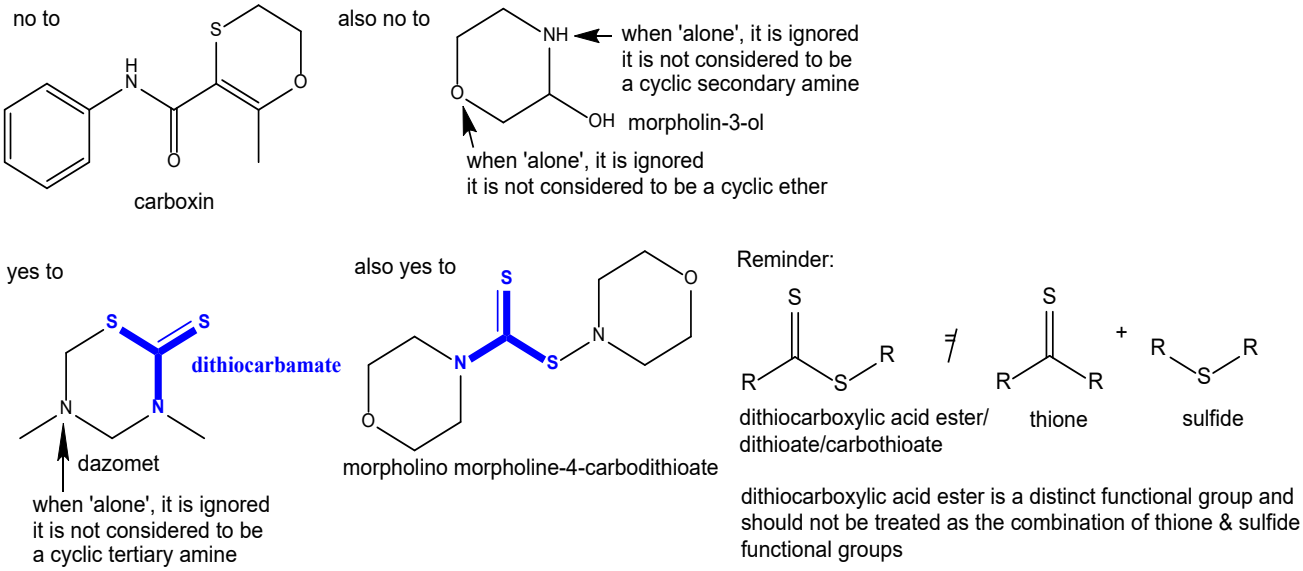
4.4.3.42 Clarifications for Q18 and expansion of Q18b

Q18a(i)) aims at capturing compounds with dibenzo-*p*-dioxin skeletal structure and place them into Class V. Q18a(ii)) is intended to capture compounds with at least three rings that are fused, bridged, spiro-fused, and/or singly bonded and send them onto other questions for further sorting before final classification. As compounds intended to be captured at Q18a(i)) can also fit the structural requirements at Q18a(ii)),

one of the validation chemists recommended FDA clarify that compounds captured at Q18a(i)) should not be considered at Q18a(ii)) to avoid confusion on what directions to follow if a substance fits the structural requirements at both Q18a(i)) and Q18a(ii)). Therefore, Q18a(ii)) was updated from “at least three rings that are fused, bridged, spiro-fused, and/or singly bonded” to “at least three rings that are fused, bridged, spiro-fused, and/or singly bonded (other than dibenzo-*p*-dioxins as they are captured at 18a(i))”.

Pre-validation EDT Q18a(ii)), as mentioned above, was looking for heterocyclic compounds with at least three rings that are fused, bridged, spiro-fused, and/or singly bonded. Based on input/request from one of the validation chemists, asking how the additional rings may be connected to the three rings described in the sub-sub-question, the following clarifying statement was added in the post validation Q18a(ii)) in parenthesis: “(additional rings connected in any way may be present, but at least 3 rings need to be fused, bridged, spiro-fused, and/or singly bonded)”.

At Q18b), one of the validation chemists found the statement “the heteroatoms contained within the ring (i.e., ring atoms) are not considered substituents” confusing. To clarify, the following statement was added to Q18b): “(while ring heteroatoms ‘alone/by themselves’ are not considered substituents, if they are a part of a functional group, the functional group must be considered in its entirety (see dazomet below))”. To aid the user, additional example structures were added after Q18b):



At Q18b), the presence of “linear or simply branched aliphatic chains of ≤6 Cs” was allowed in the pre-validation EDT. The validation chemists found it confusing as it was unclear whether the “≤6 Cs” restriction applied to each chain or whether it was a limit on the total number of carbons in all chains combined. To clarify that the restriction applied to per chain, the structural requirement was updated to “linear or simply branched aliphatic chains of ≤6 Cs (each)”.

At Q18b), based on input from another validation chemist, the list of functional groups was expanded to include peroxide, hydroperoxide, peroxyester, peracid, diacylperoxide, cyclic urea, and cyclic ureide. There was no scientific justification for excluding these originally. These functional groups do not yield very

high toxic potentials, but, if their presence were not allowed, heterocycles with low or intermediate toxicities (Classes II and III, respectively) would have been caught as Class IV (high toxicity) due to omitting these functional groups at Q18.

A yes response at Q18a(ii)) and Q18b) required the user to send the compound under evaluation to Q47 where compounds were placed into Classes I-IV depending on their structural features. Based on the results of the validation, it became clear to FDA that a very few compounds that should have been caught as Class V at Q28, do not reach Q28 due to directing compounds from Q18a(ii)) and Q18b) directly to Q47. Moreover, some compounds that should have been placed into Classes II or III at Q28 based on their structural features were placed into Class IV instead at Q47. Therefore, in the post-validation EDT, the user is instructed to proceed to Q28 if yes at Q18a(ii)) or Q18b) and assign the substance to a class it would get at Q28a) through Q28s). If no at Q28, the user is directed to go to Q47. FDA notes that crosschecking a compound against multiple questions before assigning to a class is very important as compounds can be complex and have multiple functional groups, moieties, and skeletal features contributing to their toxicities. Compounds should be assigned to a class based on their most toxic functional group, moiety, and/or skeleton. Crosschecking ensures that compounds are assigned based on their most toxic structural features.

4.4.3.43 Clarification for Q19b

Q19b) looks for heteroaromatic compounds containing “a 5- or 4- methyl- or ethyl- imidazole ring”. Based on a few incorrect classifications made by one of the validation chemists, at Q19b), after “a 5- or 4- methyl- or ethyl- imidazole ring”, “(with no other substitutions in the 4 or 5 position)” was added.

4.4.3.44 Clarifications for Q20

Sub-questions at Q20 sort heteroaromatic compounds mostly based on how many rings are present, whether the rings are substituted, and if they are substituted by additional rings, how these rings are connected, and the types of additional rings.

Q20b(ii)), Q20c(ii)), and Q20d(ii)) allowed for the substitution of the rings specified in the question. The validation chemists questioned whether the ‘substitution’ allowed substitution by additional rings that were not specified in the sub-sub-questions, and if so, how the additional rings were allowed to be connected to the rings specified at these sub-sub-questions. Therefore, FDA added the following clarifying statement after these three sub-sub-questions: “(note: substitution by additional rings is allowed, but these additional rings should not be fused, spiro fused, bridged, singly bonded, or connected by an -C(=O), -O-, -N-, or -S- to the two rings specified in this sub-sub-question)”.

Q20e) looks for a “substituted or unsubstituted ring system composed of any combination of at least three aromatic and heteroaromatic rings or at least 3 heteroaromatic rings if no aromatic rings are present”. The validation chemists recommended to add a clarification statement after this sub-question on how these three rings may be connected. Therefore, FDA added the following statement at the end of Q20e): “note that these three rings may only be fused, spirofused, bridged,

singly bonded, or connected by an -C=C-, -O-, -C(=O)-, -N- (but not an amide connector), or -S-)".

After a yes response at Q20e) or Q20f), the pre-validation EDT instructed the user to go to Q47 for final classification if a sulfonate or sulfamate was present. If no sulfonate or sulfamate was present, the compound was assigned to Class IV at Q20. This way of treating compounds prevented the placement of a few compounds into Class V that also fit the structural requirement at Q43c). Therefore, in the post-validation EDT, if one or more aniline (also diaminobenzene), nitroaniline, and/or (di)nitrobenzene moiety is present, the user is instructed to crosscheck against Qs 43c) and assign the substance to the highest class it would get at either Q43c) or Q47.

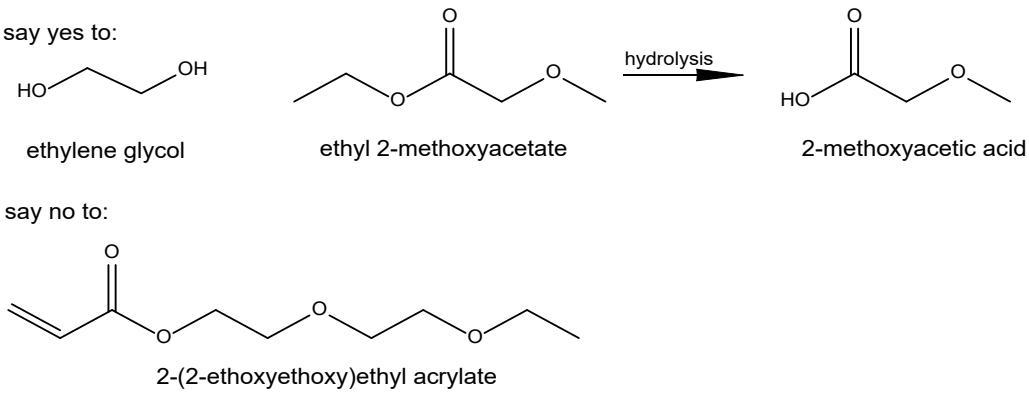
4.4.3.45 Update to Q22

The allowable functional groups were expanded by carboxylic acid and ether at Q22 as their exclusion could not be justified. At the end of Q22, the user is asked to crosscheck against Q43c) before assigning the compound to a class at Q47 if the substance contains at least one aniline (also diaminobenzene), nitroaniline, and/or (di)nitrobenzene moiety for the same reasons as at Q21.

4.4.3.46 Expansion of Q24a and clarification for Q24b

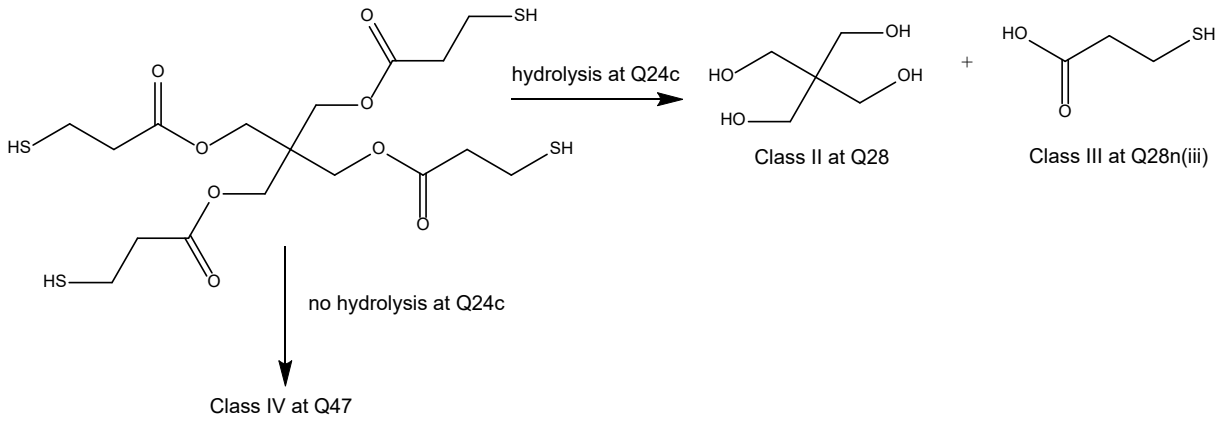
Q24a(i)) looks for a primary alcohol, the primary alcohol's corresponding aldehyde or carboxylic acid, with no other functional groups, and a main chain (containing the alcohol, aldehyde, or carboxylic acid) length of 5-8 Cs containing only one 2-alkyl substituent (2-4 Cs). Q24a(ii)) looks for hydrolytic precursors of compounds captured at Q24a(i)). In the pre-validation EDT, the hydrolytic precursors included esters, acetals, and hemi-acetals that hydrolyzed to a compound that Q24a(i)) aimed at capturing. As the possible hydrolytic precursors include carbonate, peroxide, peroxyester, peroxyacid, diacylperoxide, hydroperoxide, peroxycarbonate, peroxydicarbonate, or any other peroxides, these were added to the list of hydrolytic and reductive precursors in Q24a(ii)).

Q24b) looks for a compound that is "an α-hydroxy- or α-alkoxy-ethanoic acid, its corresponding alcohol or aldehyde, or an ester, acetal, or hemi-acetal that hydrolyses to an α-hydroxy- or α-alkoxy-ethanoic acid, its corresponding alcohol or aldehyde where the alkoxy substituent adjacent to the above oxygenated functional groups has ≤4 Cs. To ensure that compounds containing polyoxyethylene are not captured here, the following statement was added at the end of the sub-question: "(note that no repeating units of polyoxyethylene [-CH_2CH_2O-] should be present)." In addition, an example was added at the end of the sub-question for clarity.



4.4.3.47 Hydrolysis at Q24 and creation of Q24c

Q24 to Q27 (and some sub-questions within Q28) sort and classify acyclic compounds. During the classification of compounds in the validation DB, FDA realized that without having a sub-question in the 'acyclic block' of questions that asks for the hydrolyzation of some esters, hemiacetals, acetals, and carbonates, certain compounds (such as esters hydrolyzing to acrylic acid) that should have reached and been captured at Q28 never reach Q28 and instead are sent onto Q47 and default into Class IV instead of being appropriately classified at Q28 according to their true toxic potentials. To avoid this, Q24c) was added to the post-validation EDT. Q24c) looks for "an ester*, hemiacetal*, acetal*, or carbonate (including dicarbonate) (*other than those listed in" Q24a) and Q24b)). At the end of Q24, if yes to Q24c), the user is asked to hydrolyze the functional group listed in the sub-question. Any fragments that may result in a yes at Q1 can be disregarded. The user is asked to evaluate all other fragments starting at Q25. If all fragments would be classified as Class I at Q1, the substance is assigned to Class I. An example of a compound that would get misclassified if Q24c) were not added to the post validation EDT is pentaerythritol tetrakis(3-mercaptopropionate). If hydrolysis is allowed at Q24c), this substance gets classified as Class III based on its most reactive hydrolysis product (3-mercaptopropanoic acid). If no hydrolysis were allowed at Q24c), this compound would end up as Class IV at Q47. Based on the duration adjusted subchronic NEL available for pentaerythritol tetrakis(3-mercaptopropionate), it is a Class II substance. While being placed in Class III is a slight overclassification (the finalized Class III TTC is about 1/4th of the Class II TTC), Class IV would be a clear overclassification (the finalized Class IV TTC is about 1/16th of the Class II TTC). FDA notes that while quite some conservatism is built into the EDT classifications and the TTCs to err on the side of caution, we also aimed at avoiding placing compounds into a class that is too restrictive or too overprotective when predicting the relative toxic potential of a compound.



In summary, adding hydrolysis to Q24 ensures that all compounds get classified based on their true toxic potential and some do not default into Class IV at Q47 due to the lack of a question asking for the hydrolysis of hydrolyzable functional groups. This concept is applied throughout the EDT.

4.4.3.48 Clarification for Q25

The pre-validation EDT Q25 asked: “Is the substance a a) primary and/or tertiary aliphatic amine (if tertiary, only one tertiary amine may be present) or b) primary, secondary, and tertiary amide and both a) and b) of a chain length ≥12 Cs or a combination of carbons, oxygens, and nitrogens (for tertiary amines N is counted as part of the chain) with or without oxygenated functional groups but no other functional groups”. It was unclear for one of the validation chemists whether the ≥12 Cs applied to the whole molecule (i.e., to the total number of carbons present in the main (longest) chain and any of its branches). Therefore, for clarification, FDA added the following statement: “the chain length requirement applies to the longest continuous chain that contains the amine or amide, the side-chain atoms (branching) should not be counted toward the 12 Cs/atoms).”

4.4.3.49 Expansion of Q26

The pre-validation EDT Q26 looked for a wide variety of functional groups that either yield low toxic potential (Class II) or are addressed at Q28 (and placed into Classes III, IV, or V). Based on the validation results, it became clear to FDA that some functional groups should have been listed in Q26 to ensure that compounds that need to reach Q28, because they are either of low toxic potential or contain a functional group or moiety that is addressed at Q28, would reach Q28 for proper classification. Not adding these functional groups to Q26 would have resulted in the compounds being sent to Q47 and defaulted into Class IV instead of being classified as either Class II, III, IV, or V based on their structural features that are captured at Q28. Therefore, the list of functional groups and moieties that ultimately sends a compound to Q28 were expanded to contain peroxide, hydroperoxide, peroxycarbonate, peroxydicarbonate, peroxyester, peracid, diacylperoxide, N^+ in betaine and choline derivatives only, sulfate, and sulfinate.

A no response at Q26 means that either the compound does not have low toxic potential (not a Class II substance) or it does not have any of the structural

features that would result in a Class III, IV, or V placement at Q28. To err on the side of caution, even for compounds with a no response at Q26, the user is asked to crosscheck against Q28 before assigning the compound to a class at Q47 and assign the compound to the highest class it would receive at either Q28 or Q47. This is done to ensure that a compound that should receive a Class V assignment at Q28 is not missed and placed into Class IV at Q47 instead. Once again, this is done to ensure that all compounds are classified based on their most toxic structural feature.

4.4.3.50 Update to Q27

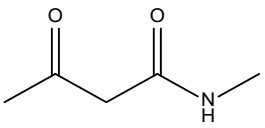
Based on the results of the external validation, FDA determined that some compounds that should be classified as Class V at Q28c(i) never reach this sub-sub-question, because they are classified as Class IV when a user responds yes at Q27. This was not the intention of FDA. Instead of placing all compounds into Class IV, if there is a yes response at Q27, in the post-validation EDT the user is instructed: “If yes, and the substance meets the structural requirements in 28c(i)), assign to Class V. In all other cases, if yes, assign to Class IV.” Once again, this is done to ensure that all compounds are classified based on their most toxic structural feature.

4.4.3.51 Clarification for Q28d

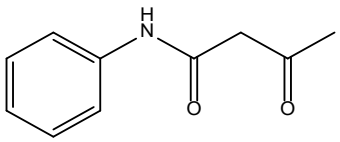
Sentences clarifying in which positions the methyl and/or methoxy substitutions may be present were added. From the pre-validation EDT, it was unclear where these substitutions may occur and still allow the substance to display γ-diketone type neurotoxicity.

4.4.3.52 Clarification for Q28j

The pre-validation EDT Q28j) looked for “aliphatic β-diketone or β-ketoamide moiety (may be a substituent on a ring).” FDA did not intend to classify compounds here where the aliphatic β-diketone or β-ketoamide moiety is a connector between two rings. FDA also did not intend to capture compounds here where the two ketones in the β-diketone are both substituents on the same ring (i.e., cyclic β-diketones). Therefore, after seeing misclassifications by the validation chemists, for clarity, the question was updated with the following language: “aliphatic β-diketone or β-ketoamide moiety (may be a substituent on a ring but not a connector between two rings or a cyclic β-diketone).” This clarification ensures that compounds with the same mode of toxic action are captured at Q28j) and compounds that behave differently are not captured here. The example section after the question was also expanded to further clarify what kind of compounds are intended to be captured at Q28j).

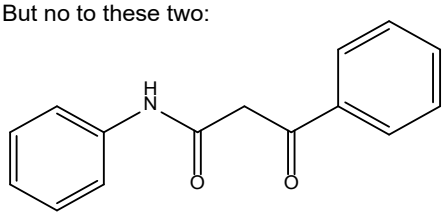


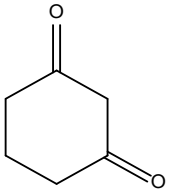
N-methylacetoacetamide



acetoacetanilide

But no to these two:





4.4.3.53 Clarification for Q28s

The pre-validation EDT Q28s) looked for a “linear or simply branched-chain aliphatic acyclic hydrocarbon with or without one or more $=CH_2$ branches that has a terminal diene and i) ≤ 6 Cs or ii) > 6 Cs.” Some of the validation chemists found the restriction on carbon number confusing. Moreover, they were unsure whether the whole compound must be an aliphatic acyclic hydrocarbon or the compounds that FDA aims at capturing here may have rings and the above description may apply for a ring substitution considering that this moiety may also be reactive when it is a substituent/sidechain on a ring. Therefore, for clarity, FDA added the following statement at the end of this sub-question: “((i) and (ii) may also be a substituent on a ring). Please note that the restriction on carbon number applies to the chain containing the terminal diene and not the whole molecule.”

4.4.3.54 Creation of Q28t

One of the validation chemists noted that according to their knowledge, sultones (cyclic sulfonic esters) are mutagenic, carcinogenic, and/or show reproductive and developmental toxicities. The validation chemist thought that sultones are ‘underclassified’; that is, they should be placed into a class of higher concern. Therefore, FDA reviewed the data and information available for sultones.

According to the scientific literature, some sultones are strong alkylating agents (e.g., 1,2-propanesultone was shown to sulphoalkylate DNA in vitro) (Bolt and Golka, 2004; Roberts and Williams, 1987). Both 1,3-propane sultone and 1,4-butanesultone, two common sultones, were found to be mutagenic and carcinogenic (Zimmermann et al., 1984; Weisburger et al., 1981; Van Duuren et al., 1974). Based on our current understanding of the reactivity of aliphatic and aryl sultones (Soile, 2014), FDA created a new sub-question, Q28t, to capture aliphatic sultones of very high toxic potential and place them into Class V.

4.4.3.55 Update of classification of heterocycles at Q28

In the pre-validation EDT, at the end of Q28, cyclic peroxides (i.e., the peroxide is a part of the ring and not a ring substituent) and cyclic ozonides defaulted into Class II. One of the validation chemists noted that based on their knowledge, these compounds are reactive and should be placed into a higher class of concern.

While FDA had no subchronic or chronic oral toxicity data for these types of compounds, FDA acknowledges that these compounds are expected to be more reactive than some other heterocycles of low concern that FDA intended to default into Class II here. Therefore, in the post-validation EDT, at the end of Q28, unsubstituted and some substituted cyclic peroxides and cyclic ozonides are defaulted into Class III if they do not contain any reactive functional groups and/or moieties that are captured at Q28 and, therefore, placed into Classes IV or V.

To avoid defaulting certain heterocycles containing at least two metabolic handles, which decrease their toxicity, into Class III instead of Class II upon a no response at the end of Q28, the following statement was added at the end of Q28: "Finally, assign the heterocycle to Class II if it contains at least two hydroxy, methoxy, aldehyde, and/or carboxylic acid functional groups."

4.4.3.56 Increasing the granularity of Q30a

There are two groups of structurally closely related substances that are found in the original (pre-validation) EDT DB and the validation DB. All these passed through Q30a). Based on their structural features, these compounds can be described as:

A) Substances with two alicyclic rings containing one ring double bond per ring, substituted with exactly 3 methyl groups per ring with or without one ketone and/or one hydroxy substitution per ring, and connected by a simply branched hydrocarbon chain with alternating double bond (extended conjugation). No additional rings or substituents are present. Example compounds include canthaxanthin, astaxanthin, meso-zeaxanthin, β-carotene, and lutein.

B) Substances with one alicyclic ring containing one ring double bond, substituted with exactly 3 methyl groups **and** i) a chain of <6 Cs with double bond and one ketone substituent on the carbon chain (e.g., β-ionone, α-ionone, α-isomethylionone), or ii) a chain of ≥6 Cs but ≤12 Cs (in total or if an ester is present, then between the ester -O- and the ring) with alternating double bonds and either a terminal carboxylic acid substituent or its corresponding alkyl ester (e.g., all-trans-retinoic acid, 13-cis retinoic acid, retinol acetate) or iii) a chain of > 12 C with alternating double bonds and either a terminal carboxylic acid substituent or its corresponding alkyl ester, and terminal aldehyde, or a ketone (e.g., apocarotenal, β-apo-8'-carotenoic acid methyl ester, and citranaxanthin). No additional rings or substituents are allowed to be present in i), ii), and iii).

Compounds fitting the structural requirements in A), B(i), and B(ii) shared a common path through the pre-validation EDT questions: 1N, 2N, 3N, 4N, 6N, 7N, 9N, 10N, 23N, 29N, 30aY, 31N, 32N, 28N and were defaulted into Class II at Q28. Upon the review the NELs of all compounds in the combined EDT DB originally classified as Class II employing the pre-validation EDT, FDA found that the NELs of some of these compounds were lower than the low 5th percentile NEL of all compounds found in Class II in the combined EDT DB.

Based on their NELs (in mmol/kg bw/day), some substances that meet the structural requirements of A) should be placed in Classes I or II and others into Class

III. The difference in structures of the Class III compounds (e.g., canthaxanthin and astaxanthin) from the Class I and II compounds (e.g., β-carotene, lutein, and meso-zeaxanthin) is that in the Class III substances, the ring double bond is conjugated with both a ring substituent ketone and the double bonds in the long chain linking the two rings. For the compounds that warrant a Class I or II assignment, either the ring double bond is not conjugated with the double bonds in the linker chain and/or the ring double bond is not conjugated with a ring substituent oxygenated functional group.

Based on their NELs, substances meeting the structural requirements in B(ii) should be placed into Class IV.

Compounds fitting the structural requirements in B(iii), as their substituent chain length exceeds 12 Cs, followed the following path: 1N, 2N, 3N, 4N, 6N, 7N, 9N, 10N, 23N, 29N, 30N, 47N and were defaulted into Class IV at Q47. Based on the review of the expanded toxicological data in the combined EDT DB, substances satisfying the structural requirements in B(iii) are appropriately defaulted into Class IV at Q47 after a no response at Q30a).

Based on the above data and information, the sorting of classifications of compounds passing through Q30a) needed to be refined. To properly sort and classify all compounds that were captured at the pre-validation Q30a), FDA created three sub-sub-questions within Q30a) (having no sub-sub-questions pre-validation):

Q30. Does the alicyclic substance contain a) i) one cycloalkane ring containing a single ring double bond, substituted with exactly 3 methyl groups and a linear or simply branched chain of ≥6 Cs but ≤12 Cs (in total or if an ester is present, then between the ester -O- and the ring) with alternating double bonds (extended conjugation) and either a terminal carboxylic acid substituent or its corresponding alkyl ester; ii) two cycloalkane rings containing a single ring double bond per each ring, substituted with exactly 3 methyl groups per ring with or without one ketone and/or one hydroxy substitution per ring, and connected by a linear or simply branched hydrocarbon chain with alternating double bonds. These alternating double bonds must be further conjugated with the ring double bond, which must be further conjugated with the ring ketone substitution; or iii) one, two, or three alicyclic rings containing ≤30 ring Cs (with or without a single ring double bond per each ring), unsubstituted or substituted with or without linear or simply branched aliphatic chains each of ≤12 Cs per ring* with or without one or more of only the following functional groups: alcohol, aldehyde (except for vicinal dialdehydes), acetal, carboxylic acid, ester, ether, ketone (including ring ketone), ketal, peroxide, hydroperoxide, peroxyester, peracid, peroxycarbonate, peroxydicarbonate, diacylperoxide, thiol, sulfide (mono-di- or poly-sulfide), sulfoxide, primary or tertiary amine, or primary or secondary amide (*If one long chain connects two rings, the chain can contain up to 24Cs.) (note that no additional rings, substituents, or functional groups other than those listed in i), ii), and iii) are allowed to be present).

Compounds captured at Q30a(i)) are placed into Class IV, and compounds captured at Q30a(ii)) are placed into Class III. A yes at Q30a(iii)), just like before, would send these types of compounds onto Q31, Q32, and then finally to Q28 to be placed into Class II. Compounds not fitting any sub-sub-question within Q30a) or

Q30b) are sent onto Q47 and mostly default into Class IV. Moreover, post-validation, a no response to all sub-questions of Q30, before proceeding to Q47, the user is asked to crosscheck against Q28 and is directed to assign the substance to the highest class it would receive either at Q28 or Q47. This is done to ensure that if a substance contains any of the structural features yielding very high toxic potential that would result in a Class V assignment at Q28 is not mistakenly placed into Class IV at Q47 instead of Class V at Q28. This, once again, is in alignment with our policy of ensuring that a compound gets classified based on its structural feature yielding the highest toxic potential. In summary, all updates to Q30 allow the proper classifications of not only the compounds described by the structural requirements at the start of this section under A) and B), but also the proper classification of all substances passing through Q30a). These changes improved the granularity and accuracy of the EDT.

4.4.3.57 Addition of cross-checking at Q32

The block of questions between Q29 and Q32 deal with the sorting and classification of alicyclic compounds. This ‘alicyclic’ block did not have a hydrolysis component in the pre-validation EDT. At the end of Q32, a no response here prompted the user to pass the compound to Q28 for final classification. At Q28, unless a functional group or moiety listed at Q28 yielding increased toxic potential was present, all alicyclic compounds were classified as Class II at the end of Q28. During the classification of compounds in the external validation DB, it came to FDA’s attention that some side chains (substitutions on the alicyclic ring) had the potential to hydrolyze with one of the hydrolysis products being an acyclic compound with higher toxic potential (Class III) that would have been caught in the ‘acyclic block’ at either Q24a) or Q24b) if no alicyclic ring were present. Due to the setup of the pre-validation EDT, these acyclic compounds that are captured at Q24a) and Q24b) never got to these two sub-questions. To correct this and avoid placing these compounds into Class II at the end of Q28 instead of placing them into Class III at the end of Q24, the instruction at the end of Q32 was updated from “If no to a), b), c), and d), proceed to Q28.” to “If no to a), b), c), and d), and the substance does not contain a hydrolyzable functional group, proceed to Q28. If no to a), b), c), and d) and the substance contains a hydrolyzable functional group, before assigning the substance to a Class at Q28 crosscheck whether any of the hydrolysis products satisfy the structural requirements at Q24a) or Q24b). Assign the substance to the highest class it would get at either Q24a), Q24b), or Q28.” This update ensures that all alicyclic compounds are classified based on their most toxic structural features.

4.4.3.58 Clarification for Q33c

Q33c) looks for certain polyaromatic compounds of high toxic potential and places them into Class V. The allowable functional groups are listed in the sub-question. One of the validation chemists was unsure whether this was a list of functional groups that must be present while the presence of other, unlisted, functional groups was also allowed. Therefore, for clarity, FDA added the following statement to the sub-question after the list of allowable functional groups: “(no other substituents should be present)” to ensure that it is clear that only the presence of the listed functional groups is allowed, and no other functional groups should be present.

Another validation chemist noted that the presence of sulfonate functional group is allowed at Q33c) (Class V). The chemist felt that this goes against the 'spirit' of Q47b), where the presence of sulfonate ("≥2 sulfonate ($-S(=O)_2OH$) or sulfamate ($-OS(=O)_2NH_2$) substituents where there is at least one sulfonate or sulfamate for every ≤10 Cs (note: but no azo functionality") results in a Class I assignment. FDA notes that the compounds that we aim at capturing at Q33c) either have no sulfonate functional group (e.g., CAS 2564-65-0, CAS 40495-42-9, CAS 78776-41-7, CAS 568-75-2, and CAS 57-97-6, see Appendix 2) or have one sulfonate functional group at most and they were not intended to fit the structural requirement: ≥2 sulfonate ($-S(=O)_2OH$) substituents where there is at least one sulfonate for every ≤10 Cs; hence their high toxic potential. To ensure that some relatively safe compounds are not accidentally/unintentionally captured at Q33c) and placed into Class V, the following statement was added at the end of Q33c): "If ≥2 sulfonate ($-S(=O)_2OH$) substituents where there is at least one sulfonate for every ≤10 Cs are present, say no at Q33c)." This statement ensures that these compounds reach Q47, and the effect of sulfonate is taken into consideration when classifying them. If this statement were not added at the end of Q33c), a relatively safe compound such as C.I. Solvent green 7 (CAS 6358-69-6) with a NOAEL of 81.40 mg/kg bw/day in a 91-day rodent study (Unknown, 1978) would have been unintentionally caught as Class V at Q33c) instead of Class I at Q47b).

4.4.3.59 Update to Q34a(i) and creation of Q34a(iii)

The pre-validation Q34a(i)) aimed at capturing *o*-phthalates that have much lower or no reproductive and/or developmental toxic potentials compared to other *o*-phthalates. During the validation process, FDA realized that the sub-sub-question may not capture certain *o*-phthalates of much lower or no reproductive and/or developmental toxic potential. Therefore, in addition to reviewing the toxicological data for *o*-phthalates in the external validation DB and the original (pre-validation) EDT DB, FDA undertook a thorough, updated, literature search on the structure-activity relationships of *o*-phthalates.

In the case of *o*-phthalates, reproductive and/or developmental concerns mostly arise when the linear portion of the alkyl side chain has between 3 and 6 carbons (Saillenfait et al., 2006). According to this paper "Chain length appears to be a critical factor in the toxicity of phthalates. Transitional phthalates, such as DEHP, DnBP and BBP, have 4 to 6 carbons in the linear portion of the alkyl side chains. Compared to other phthalates, they show the greatest potency as developmental and reproductive toxicants. The short methyl and ethyl phthalates, and phthalates with much longer side chains (e.g., di-n-octyl-, diisononyl-, and diisodecyl-phthalates) demonstrate lower or no developmental toxicity in rodents." According to Martino-Andrade and Chahoud (2010), "Another important aspect of phthalate toxicity is the marked structure-activity relationship for the induction of reproductive effects. The testicular toxicity of these compounds depends in part on the length of the alcohol moiety (side chain) of the ester molecule. In general, phthalates with medium- (e.g., dibutyl phthalate [DBP]) or branched long-side chains (e.g., DEHP) induce degenerative testicular lesions while those with short- (diethyl phthalate (DEP)) or linear long-side chains (di-n-octyl phthalate) are inactive" (for clarity, inactive here

means that they do not cause degenerative testicular lesions). A review of the toxicological data of *o*-phthalates found in the combined EDT DB and in an internal FDA memo and draft publication also indicated that the *o*-phthalates with no or minimal reproductive and/or developmental toxicity concern are dimethyl phthalate, diethyl phthalate, and the longer-chain phthalates such as diisodecyl phthalate.

Therefore, based on the above data and information, we updated Q34a(i)) to look for an *o*-phthalate diester that either contains at least one alcohol moiety with more than 7 carbons in the linear portion of the alkyl side chain or contain two alcohol moieties each containing a maximum of 2 carbons in the linear portion of the alkyl side chain in the aims of capturing *o*-phthalates of lower toxic potential in comparison to other *o*-phthalates. Substances captured at the post-validation Q34a(i) will be placed into EDT Class III.

Other *o*-phthalates simply defaulted at other questions in the pre-validation EDT. As most *o*-phthalates that are not captured at Q34a(i) have reproductive and/or developmental toxicity concerns associated with them, and to enhance the read-across capability of the EDT, FDA created a new sub-sub-question, Q34a(iii)), to capture *o*-phthalates that may have reproductive and/or developmental concerns at the same sub-sub-question. While compounds captured at the newly created Q34a(iii)) have different potentials to cause reproductive and/or developmental toxicity, based on the available NELs and LELs (when no NEL was available), a Class IV assignment was protective for all substances captured at this sub-sub-question.

4.4.3.60 Expansion of Q34b

Q34b) looks for aromatic ring substituents with hydrolyzable functional groups. At the pre-validation Q34b), the user was asked to assume the hydrolysis of any ester, orthoester, thioester, acetal, ketal, hemiacetal, hemiketal, sulfate ester, and anhydride and evaluate the toxicity of each hydrolysis product individually. The compound of interest would be assigned to the highest class of any of its hydrolysis products. One of the validation chemists suggested that carbonate should be added to the list of hydrolyzable functional groups at Q34b). As this sub-question looks for the presence of hydrolyzable functional groups and carbonate satisfies this requirement, carbonate (including dicarbonate) was added to the list of hydrolyzable functional groups. In addition, FDA added sulfite ester, peroxide, hydroperoxide, peroxyester, peracid, diacylperoxide, peroxycarbonate, and peroxydicarbonate to the list of hydrolyzable and reducible functional groups at this sub-question as these would also be expected to hydrolyze or go through reduction when on a side chain.

4.4.3.61 Clarifications for Q36

At Q36c), examples of allowed connections between rings were listed. To help the user and comply with the recommendation of the validation chemists, all allowed connections are listed in the post-validation EDT and not just examples of allowed connections.

One of the validation chemists noted that the original text at Q36d) is confusing due to the presence of the word “unfused” when in effect the rings specified in the question will be fused rings based on the structural requirements detailed at this question. FDA agreed and removed the word “unfused.”

4.4.3.62 Clarifications for Q40b

Q40b) looks for certain ring substituted benzoic acids, benzaldehydes, or benzyl alcohols. It was unclear for one of the validation chemists whether the substitutions, especially the hydroxy substitution, listed in this question were in addition to the hydroxy substitution that is already present in benzyl alcohol. To clarify this, the following statement was added to this sub-question: “Cs (note this hydroxy substitution on benzyl alcohol is in addition to the alcohol that makes/defines benzyl alcohol and unlike the hydroxyl group in benzyl alcohol, this hydroxy group is a direct aromatic ring substituent).”

4.4.3.63 Clarifications for Q41

Q41 originally (i.e., prior to validation) read as “Does the substance have only one or a maximum of two aromatic ring(s) and is substituted by not more than one phenolic -OH per aromatic ring and….” One of the validation chemists noted that grammatically this could mean that substitution by no -OH is also allowed even though the chemist was sure that that’s not what was meant here. Therefore, to remove ambiguity, the question was updated to “Does the substance have only one or a maximum of two aromatic ring(s) and is substituted by at least one phenolic -OH, but not more than one phenolic -OH per aromatic ring and….”

Moreover, at Q41, one of the validation chemists thought that it was somewhat unclear whether functional groups and moieties other than those listed were allowed to be present. Therefore, for clarification, FDA added “(no N and/or S containing functional groups and no halogens may be present)” to both Q41a) and Q41b).

Q41a) lists what substitutions are allowed. These were “one or more *o*- and/or *p*- (to the phenolic -OH) alkyl substituents of ≥ 4 Cs.” Based on a misclassification by one of the validation chemists, FDA thought that future users could benefit from clarifying that ≥ 4 Cs applied to at least one of the alkyl substituents not to the total number of carbons of all alkyl substituents. Therefore, for clarity, the question was updated to “one or more *o*- and/or *p*- (to the phenolic -OH) alkyl substituents, one of which must have ≥ 4 Cs.”

4.4.3.64 Clarifications for Q42

Q42 lists the allowable functional groups that can be present in the compounds this question aims at capturing. One of the validation chemists asked to clarify whether other functional groups may be present, and if the presence of additional functional groups is not allowed. Therefore, FDA added “with no N- and/or S-containing functional groups or halogens” to Q42a) and “no other functional groups or halogens” to Q42b).

4.4.3.65 Clarifications and updates to Q43a(i) and 43c, clarification for Q43a(ii), and the creation of Q43a(iii)

Some of the validation chemists were confused whether counting a -N- connector between two rings when determining whether a substance is a diaminobenzene or nitroaniline. To avoid confusion, FDA added “The presence of only a single benzene ring bearing the amino- and/or nitro- substitution is allowed.” to both Q43a(i)) and Q43a(ii)) along with “(note: an -N- connector between two aromatic rings (Ar-N-Ar) should be disregarded when determining whether the compound is a (di)aminobenzene).”

A relatively large number of compounds (e.g., trifluralin and *m*-dinitrobenzene) were captured at the pre-validation Q43a(i)) and Q43a(ii)) and assigned to Class V. After a careful review* of all substances classified at these two sub-sub-questions in the combined EDT DB (see Appendix 2 for the underlying data), FDA found that for compounds captured here a Class IV assignment was protective, and it was not warranted to place them into Class V. Therefore, the class assignment at Q43a(i)) and Q43a(ii)) was changed from V to IV. (*Upon the completion of the validation, substances captured at every question, sub-question, and sub-sub-question were examined to determine whether the question, sub-question, and sub-sub-question put these substances into an appropriate class.)

During the review of dinitrobenzenes, FDA found that some compounds that contained two unfused benzene rings where one of the benzene rings is a dinitro benzene and both benzene rings are substituted by one or more halogens and/or -CF_3 defaulted into Class IV at Q47 even though they needed a Class V assignment based on the comparison of their NELs with the pre-validation Class IV 5th percentile NEL. (Note that they did not match Q43a(i)) because they had more than one benzene ring present.) To address this shortcoming, new sub-sub-question, Q43a(iii)), was created to capture these compounds. Compounds captured at Q43a(iii)), such as bromethalin and fluazinam, are placed into Class V.

Q43c) looks for compounds with two benzene rings that can be connected various ways (e.g., alkyl chains, -O-, -S-, -C(=O)-, and -N-) substituted by one or two amino and/or nitro groups with or without additional alkyl, methoxy, and/or halogen substituents but not substituted by any other functional group. One of the validation chemists was confused as to whether certain connectors between the two benzene rings count as functional groups the presence of which are not allowed. For clarity, FDA added “(note that the -C(=O)-, -N-, -O-, -S-, and -$S(=O)_2$- in the connector between the rings do not count as functional groups for the purpose of this question)” at the end of Q43c). Moreover, compounds where one of the benzene rings is replaced with a heteroaromatic ring, but otherwise fitting the structural requirements at Q43c) are expected to behave similarly, the allowable types of rings were extended to include heteroaromatic rings, not only benzene rings.

4.4.3.66 Clarifications for Q44

Q44 is intended to identify substituted anilines and nitrobenzenes. As per the request of one of the validation chemists, for clarity, it was added to this question that the presence of only a single benzene ring is allowed (i.e., the aniline or the nitrobenzene should not be substituted by another ring).

Q44b) is intended to capture anilines and nitrobenzenes with alkyl substituents and no additional functional groups should be present. Based on a

misclassification, FDA found it necessary to clarify at the end of this sub-question that the phrase “no additional functional groups” also meant that no halogens may be present anywhere in the molecule.

Q44c) looks for anilines and nitrobenzenes with certain functional groups containing oxygen with or without the presence of alkyl substituents. Based on misclassifications at this question by one of the validation chemists, FDA found it necessary to clarify at the end of this sub-question that no other functional groups, including no halogens, may be present to ensure that only compounds intended to be captured at this sub-question are captured here.

4.4.3.67 Clarification for and expansion of Q45

Q45 aims at separating aromatic compounds that should be classified at Q28 from those that should be classified at Q47 based on their structural features. Q45 lists a large number of structural features with the purpose of properly directing compounds to either Q28 or ultimately to Q47. During the validation, based on some unintentional paths down the EDT for some compounds due to inappropriate directing at Q45, it became clear that the list of functional groups and moieties at Q45 had to be expanded to properly direct compounds to their final destinations. The expansion of functional groups and moieties at this question include aldehyde, sulfone, sulfonamide (but only when the sulfonamide is either a connector between two rings or non-terminal substituent (terminal: $-S(=O)_2NH_2$), sulfonate (but only when it is a connector between two rings), cyclopropylamine moiety, and the presence of -O- in an aliphatic chain.

Moreover, at Q45, to improve clarity, it was recommended by one of the validation chemists to edit “linear, simply branched aliphatic chain(s), and/or alicyclic ring(s).” Therefore, this was revised to “linear aliphatic chain, simply branched aliphatic chain(s), and/or alicyclic ring(s).”

4.4.3.68 Reevaluation of 47a(ii)

The pre-validation Q47a(ii)) looked for substances with one or more azo groups and one or more sulfonate, sulfamate, or carboxylate, but not on each fragment (fragments were separated by the azo groups). For the compounds fitting this structural requirement, the user was asked to assume the reductive cleavage of the azo function(s). The user was then asked to run the various fragments through the pre-validation EDT. The original (pre-validation) EDT DB had 19 substances fitting the structural requirement at Q47a(ii)). Based on their NELs (when compared to each of the six pre-validation 5^{th} percentile Class NELs), 4 of the 19 warranted a Class I assignment, 6 of the 19 warranted a Class II assignment, and 5 of the 19 warranted a Class III assignment. Four substances had no NEL, only LEL. Two of the four substances had a LEL that was higher than the Class I 5^{th} percentile NEL, one of the four substances had a LEL that was above the Class II 5^{th} percentile NEL (but below the Class I 5^{th} percentile NEL), and of the four substances had a LEL that was above the Class III but below the Class II 5^{th} percentile NELs (hence, the classes for NELs were expected to be higher than those for the LELs). Despite their relatively low to intermediate toxic potential, all were placed into Classes III, IV, or V based on the classification of their fragments. The validation DB had an additional 11 substances

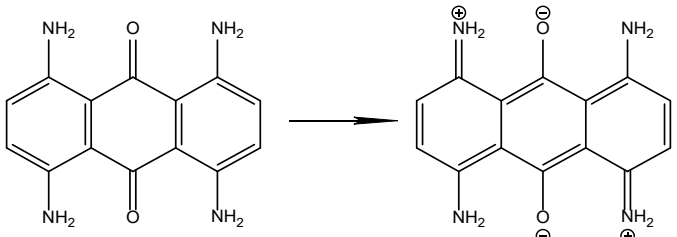
that got caught at Q47a(ii)). Based on their NELs (when compared to the Class 5th percentile NELs), 4 of the 11 substances warranted a Class I assignment, 5 of the 11 substances warranted a Class II assignment, and 2 of the 11 substances warranted a Class III assignment. The most toxic of the 30 compounds with a LEL higher than the Class III 5th percentile NEL, unknown NEL (possible Class IV or V) was C.I. Acid Red 114 (CAS 6459-94-5) that produced a LEL of 4 mg/kg bw/day in a 2-year rodent carcinogenicity study (NTP, 1991). During the azo reduction of this compound, the production of 3,3'-dimethylbenzidine was expected. This substance gets classified at Q43c(ii)) as Class V. Substances captured at Q43c(ii)) (a biphenyl, methylenebis(phenyl) and homologues with linkages of ≤4 Cs (a maximum of 4 Cs linking the two phenyl rings, the presence of -C(=O)- and/or -N- in the link is allowed), diphenyl ether, diphenylthioether, or diphenylsulfuryl containing diamine (or N-acetyl derivative), nitroamine (or N-acetyl derivative), or dinitro groups at the 4,4'- positions with or without additional alkyl, methoxy, and/or halogen substituents but not substituted by any other functional group are highly toxic. On the other hand, another substance, Direct Blue 6 (CAS 2602-46-2), produced a NEL of 18.75 mg/kg bw/day (a NEL warranting a Class II assignment) in a 91-day study (NCI, 1978) even though a potential reduction fragment of this compound also fits Q43c(ii)).

Except for the fact that these 30 compounds have one or more azo functionalities, all fit the structural criteria at Q47b) (Class I assignment) or Q47c) (Class II assignment). Q47b) and Q47c) capture compounds containing sulfonate and sulfamate, and depending on the ratio of sulfonate or sulfamate to the number of carbons present in the compound, they place compounds into Classes I and II, respectively. Clearly, while the presence of sulfonate and sulfamate reduces the toxicity of the 30 compounds captured at Q47a(ii)), some azo reduction products make them more toxic compared to what their toxicity would be if no azo functional groups were present (hence no azo reduction products formed). On the other hand, even the compounds with the highest toxic potential within this group of related substances are not as toxic as some of their individual reduction products are on their own. While compounds captured at Q47a(ii)) may fall into classes that are overprotective due to the requirement to reduce the azo group, FDA, to err on the side of caution, will continue to require the reduction of the azo functionality here to ensure that the toxic potentials of these compounds are not underestimated. In the future, as more data and information become available, Q47a(ii)) will be revisited.

4.4.3.69 Change of class assignment for Q47d

The pre-validation EDT Q47d) placed compounds with three or more fused aromatic and/or heteroaromatic rings that can extend conjugation through ring substituents (N or C=O) with the formation of a zwitterion (e.g., N^+ and O^-) (see example of C.I. Disperse Blue below) into Class III. A review of all substances in the combined EDT DB (see Appendix 2) showed that 7 of the 10 substances that get classified at this sub-question have NELs warranting a Class I assignment (i.e., their NELs are higher than the pre-validation 5th percentile Class I NEL). Three of the ten substances have relatively high (Class I) non-duration adjusted NELs (when compared to the 5th percentile Class I NEL) that only changed to Class II (i.e., their NELs were lower than the Class I 5th percentile NEL but higher than the Class II 5th percentile

NEL) once adjusted for study duration. Therefore, the pre-validation Class III assignment was changed to Class II for the substances captured at this sub-question to better reflect their true toxic potential.



C.I. Disperse Blue zwitterion

4.4.3.70 Creation of Q47f(ii)

During the validation of the EDT, it came to FDA's attention that steviol and its glycosides of very low toxic potential (see Appendix 2) would, unintendedly, default into Class IV at Q47. To avoid this gross overclassification, a new sub-sub-question, Q47f(ii)), was created to capture and place steviol and its glycosides into Class I.

4.4.3.71 Change of class assignment for Q47g

The pre-validation Q47g) aimed at capturing certain structurally closely related compounds (mostly pigments) of low toxic potential. As in the original (pre-validation) EDT DB, toxicological data were available for only two substances (diarylanilide yellow (CAS 6358-85-6) and Pigment yellow 83 (CAS 5567-15-7)) that fit the structural requirements at this question. Despite their NELs [mg/kg bw/day] warranting a Class I assignment, to err on the side of caution due to the limited toxicological data available, they were placed into Class II. During the validation, toxicological data became available for an additional 12 substances fitting the structural requirements at this question. All 14 substances have very high NELs, even when the study was adjusted for duration, warranting a Class I assignment. Therefore, in the post-validation EDT, all compounds captured at this sub-question are now placed into Class I as now an adequate amount of toxicological data are available to confidently assign these compounds to Class I.

4.5 The Finalized Post-validation EDT

4.5.1 Preparation for Using the Post-validation EDT

In Appendix 2, we provide the combined EDT DB (the combination of the original (pre-validation) EDT DB and the external validation EDT DB). The combined EDT DB contains 3,138 compounds. For each substance in the combined EDT DB, we show its structure, the path it takes through the EDT, and its final class assignment. This DB can help the user become accustomed to and proficient in using the EDT.

Common understanding of the scientific chemical terms is needed to reliably evaluate substances through the EDT. We attempted to provide clear definitions of terms to aid users. Some definitions are composed specifically for questions in the EDT and do not have the same meaning in the general literature. For example, the term "aliphatic" encompasses all non-aromatic compounds in the general literature. For the purposes of the EDT, aliphatic

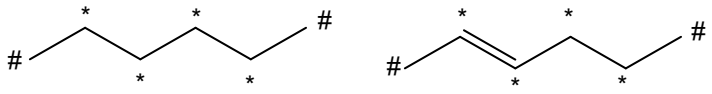
includes alkane, alkene, polyalkene, but not allenes, alkynes, polyalkynes, or alicyclic compounds. Novel definitions, such as that for aliphatic compounds, are utilized to enable simplification of many of the EDT questions. Therefore, we ask the user to review the guidelines and definitions in section 4.5.2 prior to and during the application of the EDT.

4.5.2 Applicability Domain and Definitions for Using the Post-validation EDT

Although most common chemistry terms in the EDT are used as they are in scientific literature, some terms have been modified to simplify the language of the EDT questions. The following definitions were employed during the development of the EDT questions to facilitate its application and assist in resolving issues related to class assignment:

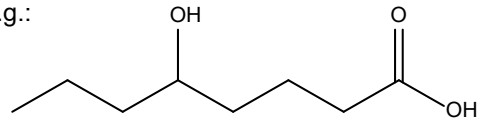
A. Applicability domain of the EDT: all compounds except unhydrolyzable polymers, proteins, elements, inorganic substances, and substances with undefined structures. Please note that ingested particles may have varying bioavailability and toxicity depending on their size. The EDT is not designed to estimate safe intake levels (i.e., TTCs) based on particle size and should only be applied to substances within its applicability domain. While there is no cutoff for molecular weight (MW) when applying the EDT, the MW range of substances in the combined EDT DB is 30.03-2285.61 Da. Some of the hydrolyzable polymers within the structural applicability domain of the EDT may have MWs that exceed this range. In case of hydrolyzable polymers, the EDT assumes complete hydrolysis to monomeric units. Additionally, please note that the EDT is designed specifically to sort compounds based on/according to their relative chronic toxic potential through oral exposure only.
B. **Skeleton/skeletal structure**: The skeletal structure of an organic compound is the series of atoms bonded together that form the essential structure of the compound. The skeleton can consist of chains, branches, and/or rings of bonded atoms. Skeleton and skeletal structure are used interchangeably throughout the EDT.
C. **Linear** means that the chain has no carbon branching (i.e., each carbon in the chain is connected to one or two other carbon(s)). **Simply branched-chain** substances may have any number of methyl substituent(s) and/or up to two n-alkyl branches of two or more carbons at not more than two points along the main chain (these n-alkyl branches cannot be on the same carbon) with no additional branching (e.g., 3,4-diethyloctane). **Branched-chain** means that the substance contains more than two branches along the main chain that has two or more carbons. Examples:

linear:



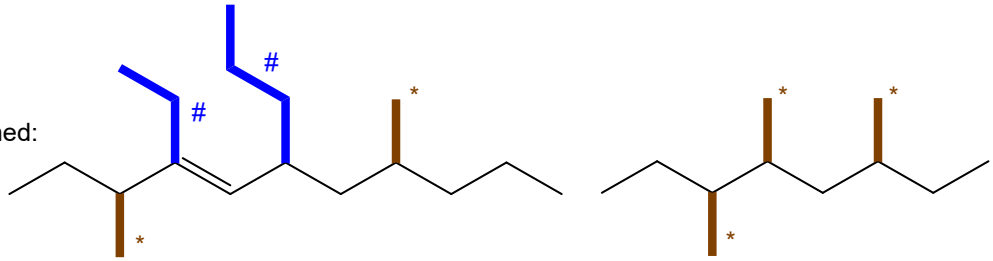
#: connected to one other carbon *: connected to two other carbons

e.g.:



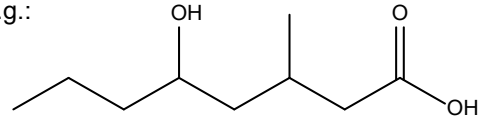
for the purposes of the EDT, the above hydroxycarboxylic acid is 'linear'

simply branched:



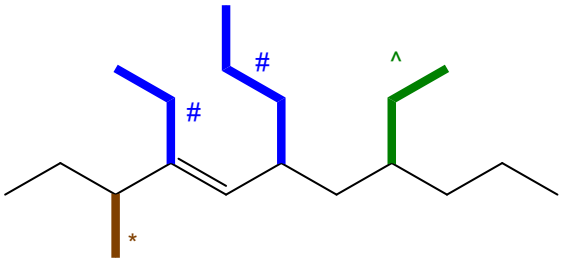
brown (also marked with *): any number of methyl substituents;
blue (also marked with #): up to 2 n-alkyl branches of 2 or more carbons at not more than 2 points along the chain

e.g.:



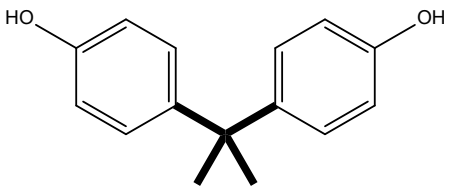
for the purposes of the EDT, the above hydroxycarboxylic acid is 'simly branched'

branched:

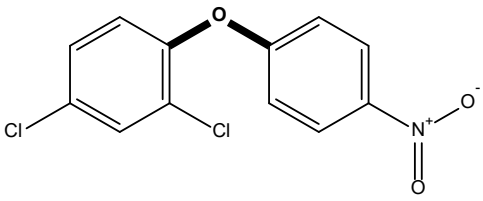


green (also marked with ^): due to 3rd branching that is other than methyl, the compound is no longer simply-branched

D. A **connector** is a structural element that links two distinct rings or fragments in a molecule through chains and/or functional groups, without fusing the rings together. Examples:

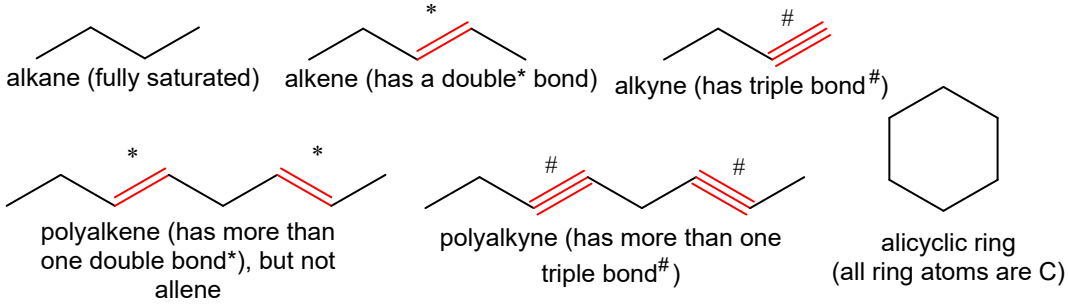


bisphenol A
(**connector is bolded**)

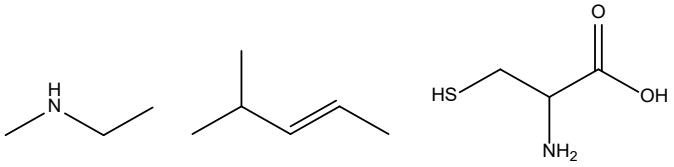


nitrofen
(**connector is bolded**)

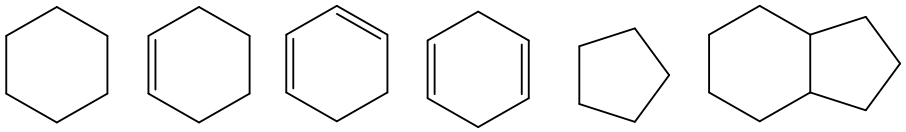
E. **Aliphatic** includes alkane, alkene, polyalkene, but not allene (C=C=C), alkyne, polyalkyne, or alicyclic compounds.



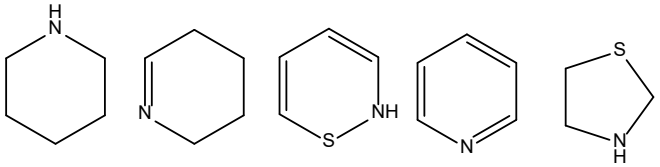
F. **Acyclic** means the absence of a ring (i.e., the molecule is open-chained).



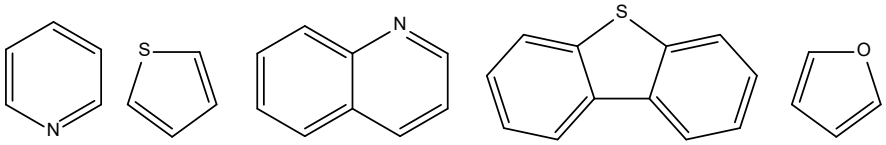
G. **Alicyclic** refers to a molecule where all rings are composed solely of carbon atoms. These rings may contain ring alkenes but do not form an aromatic ring.



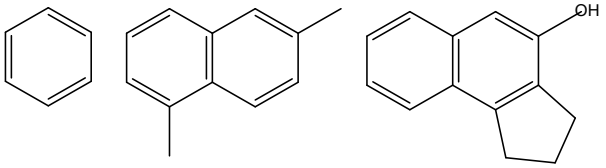
H. **Heterocyclic** refers to a molecule that contains at least one ring structure where at least one of the ring atoms is not carbon, commonly nitrogen (N), oxygen (O), or sulfur (S).



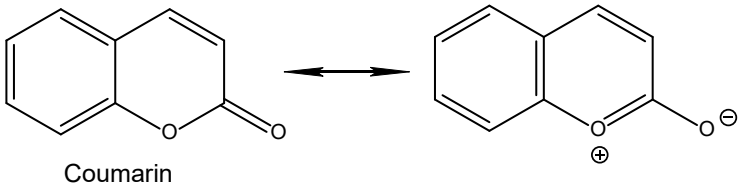
I. **Heteroaromatic** refers to a substance that contains at least one ring with at least one ring heteroatom (commonly N, O, and/or S) and a fully conjugated cyclic array of [4n+2]π electrons (e.g., furan, pyrrole, 1,3-imidazole, thiazole, and pyridine). Heteroaromatic compounds are a specific subgroup of heterocyclic compounds.



J. **Aromatic** or **aryl** (Ar) means that the substance contains at least one aromatic ring (ring with a fully conjugated cyclic array of [4n+2]π electrons) regardless of whether the aromatic ring is fused or bonded to another ring and regardless of any substitution. The aromatic ring cannot contain any ring heteroatom(s) (e.g., O, N, and S).

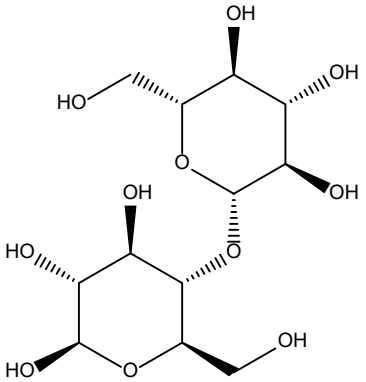


K. For the purposes of the EDT, a **pseudo-aromatic** ring means a ring that can only achieve a completed cyclic array of [4n+2]π electrons by incorporating the electron pair of a functional group into an enolic double bond, such as a lactone or lactam. Example:

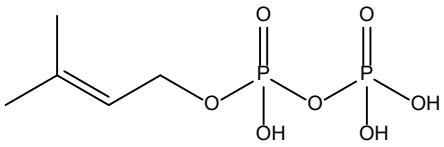


Coumarin

L. A **dimer** refers to a molecule that is formed by the combination of two identical or similar smaller molecules (monomers) through a chemical reaction. Examples:

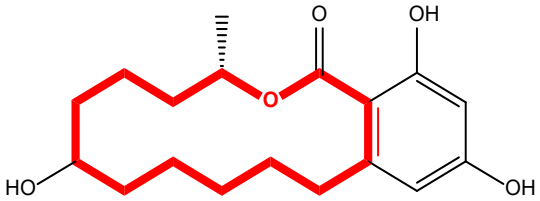


cellobiose (dimer of glucose)

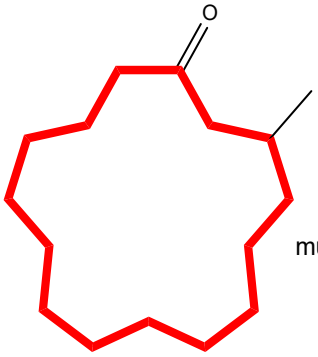


dimethylallyl diphosphate (a phosphate dimer)

M. For purposes of the EDT, a **macrocyclic** ring is a completed cyclic array of any combination of ≥11 C, O, or N atoms, with or without ring alkenes.



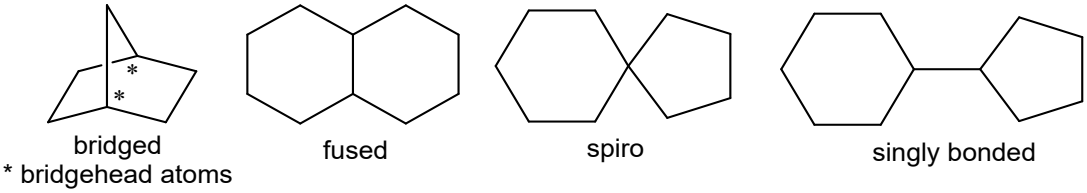
zearalanol



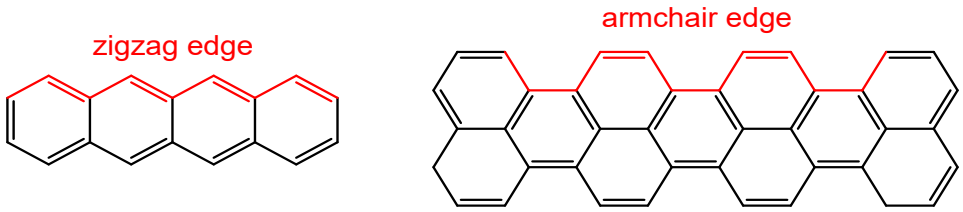
muscone

N. **Bridged** compounds have two or more rings (a ring system) that contain a bridge (i.e., a single atom or an unbranched or **branched chain** of atoms that connect two bridgehead atoms). **Bridgehead** atoms are defined as any atom that is not a hydrogen and that is part of the skeletal framework of the molecule bonded to three or more other skeletal atoms. The presence of the bridge connecting the bridgehead atoms distinguishes bridged compounds from **fused** ring compounds, which have two rings linked by two adjacent atoms, and from **spiro** compounds, which have two rings linked by a single

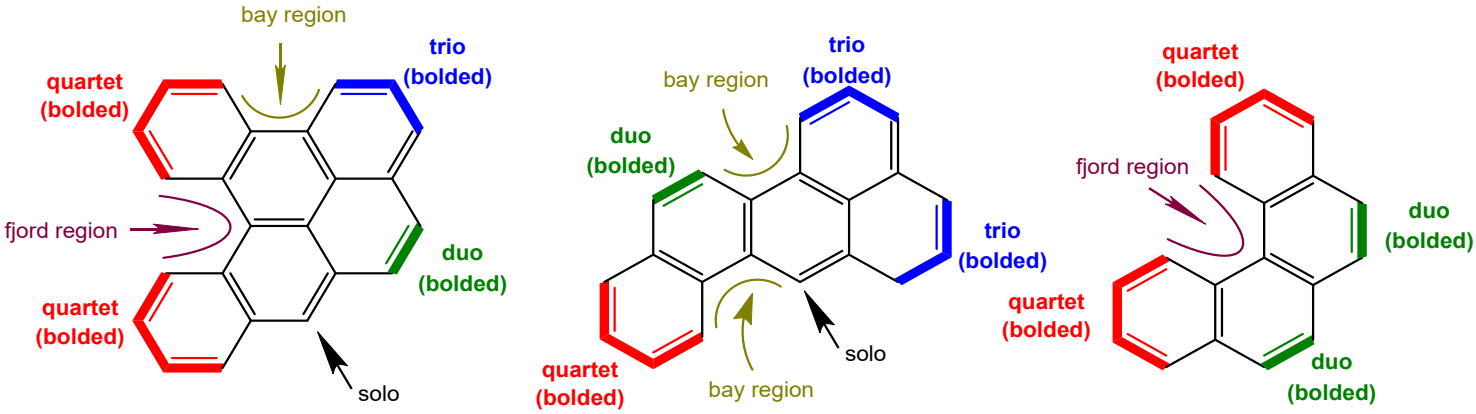
atom. **Singly bonded** rings share a bond between one atom on each ring. Examples:



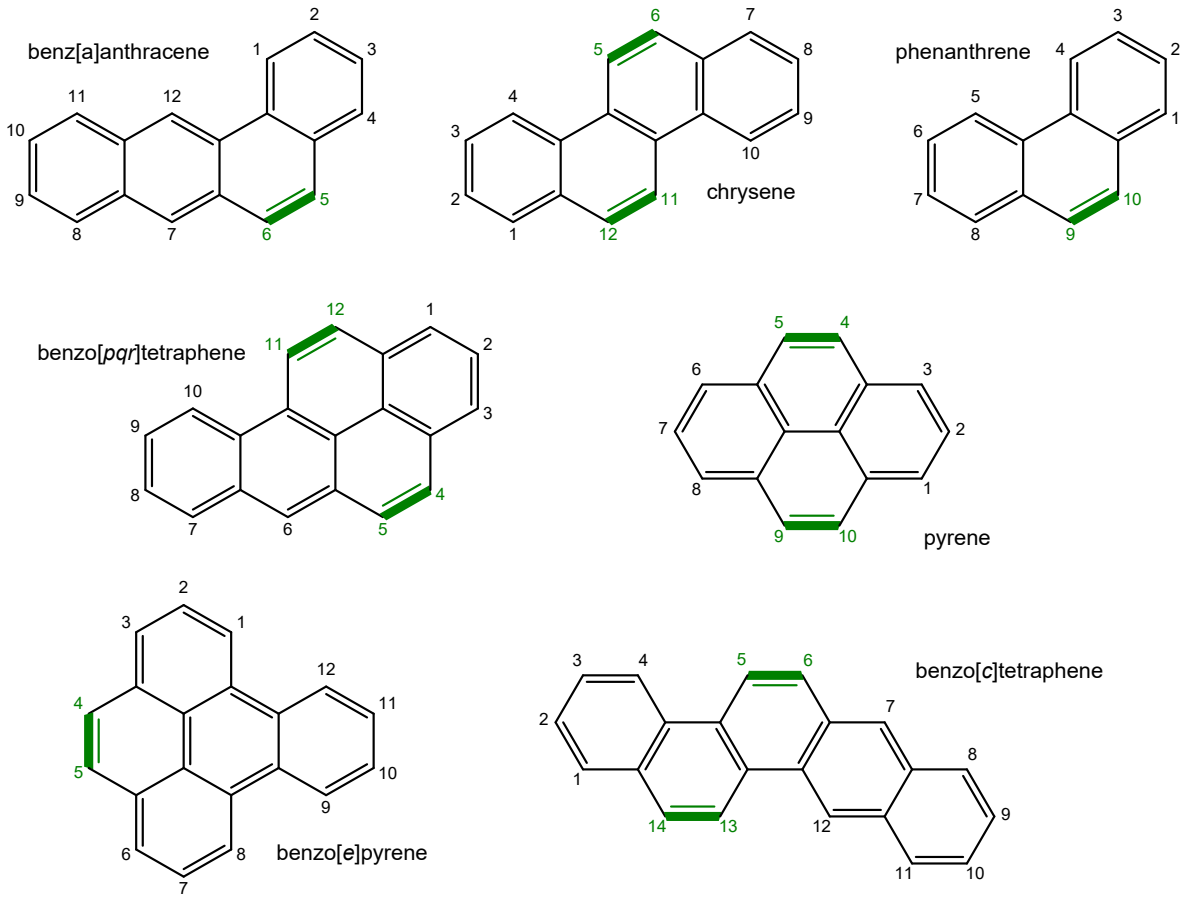
O. **Zigzag** and **armchair edge**: Zigzag edge is present when the aromatic rings fuse in a linear configuration. Armchair edges form as a result of angular fusion (i.e., angled fusion/angular configuration) of aromatic rings.



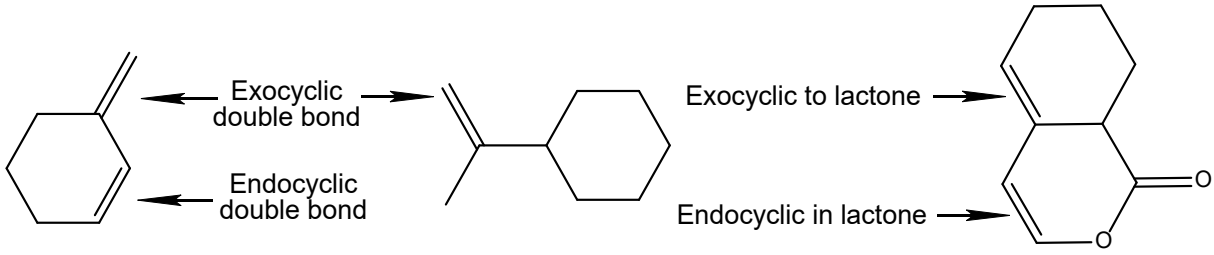
P. **Solo, duo, trio, quartet**: In polycyclic aromatic hydrocarbons (PAHs), the terms solo, duo, trio, and quartet refer to configurations where one, two, three, or four adjacent carbon atoms, respectively, are bonded to atoms other than those within the aromatic ring. For example, in the provided structures, each of the three 'trio' carbons are bonded to a hydrogen atom. That is, these trio carbons are bonded to hydrogen atoms outside the aromatic ring structure. **Bay** and **Fjord regions**: The bay region is characterized by the presence of a "bay" or "indentation" in the aromatic system. The fjord region refers to a structural feature in a molecule where there is a pronounced "fjord" or "trough" between two aromatic rings.



Q. **K-region**(s) is/(are) the convex **armchair** edge(s) of polyaromatic hydrocarbons that are joined together by angular fusion. The K-region is made of a duo (displayed in green below). For examples:

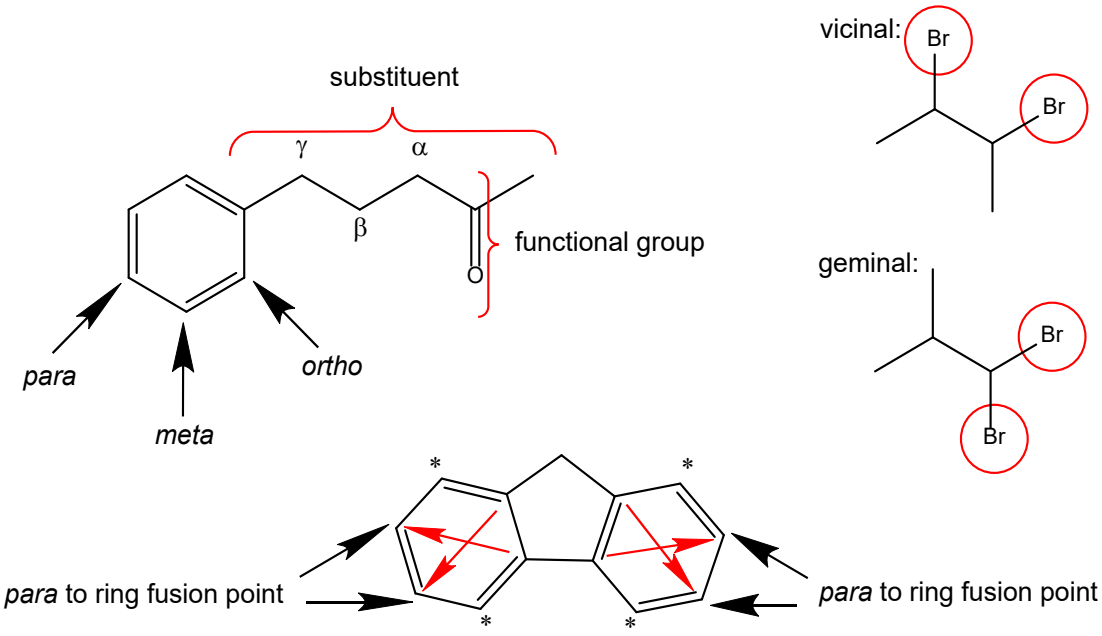


R. **Endocyclic** and **exocyclic** double bonds: If both carbon atoms connected by a double bond are members of the same ring, the double bond is said to be **endocyclic**. If at least one of the carbon atoms connected by a double bond is not a member of the same ring, the double bond is said to be **exocyclic**. Examples:



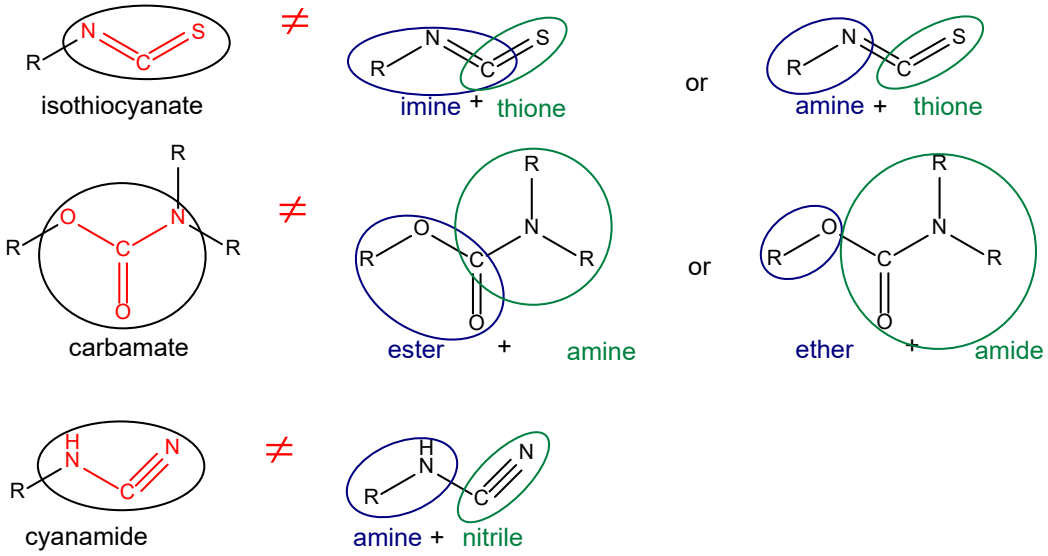
S. Positions:

a. α, β, and γ carbon; *ortho-, meta-*, and *para*-substitution; and definitions of vicinal and geminal
b. **α** carbon is the first carbon that attaches to a functional group, **β** is the second, **γ** is the third, and **δ** is the fourth.
c. For six-membered aromatic or heteroaromatic rings, ***ortho***-substitution means that the two non-hydrogen substituents occupy adjacent ring atoms; ***meta***-substitution means that the substituents are separated by one ring atom; and ***para***-substitution means that the substituents are separated by two ring atoms.
d. **Vicinal**: two functional groups or atoms attached to two adjacent atoms, and **geminal**: two functional groups or atoms attached to the same atom.



* *ortho* to ring fusion point

T. **Functional group** means a group of covalently-bound atoms of two or more elements, one of which is not hydrogen or carbon. Each functional group undergoes a characteristic set of well-known reactions independent of its individual fragments. It is important to treat the functional group as an entire molecular entity and not as a fragment (e.g., #1: R-N=C=S is an isothiocyanate and not an imine (R-N=C) and a thione (-C=S) or #2: ROC(=O)N(R_1)R_2 is a carbamate and not an ester (ROC(=O)-) and an amine (-N(R_1)R_2)). Examples:



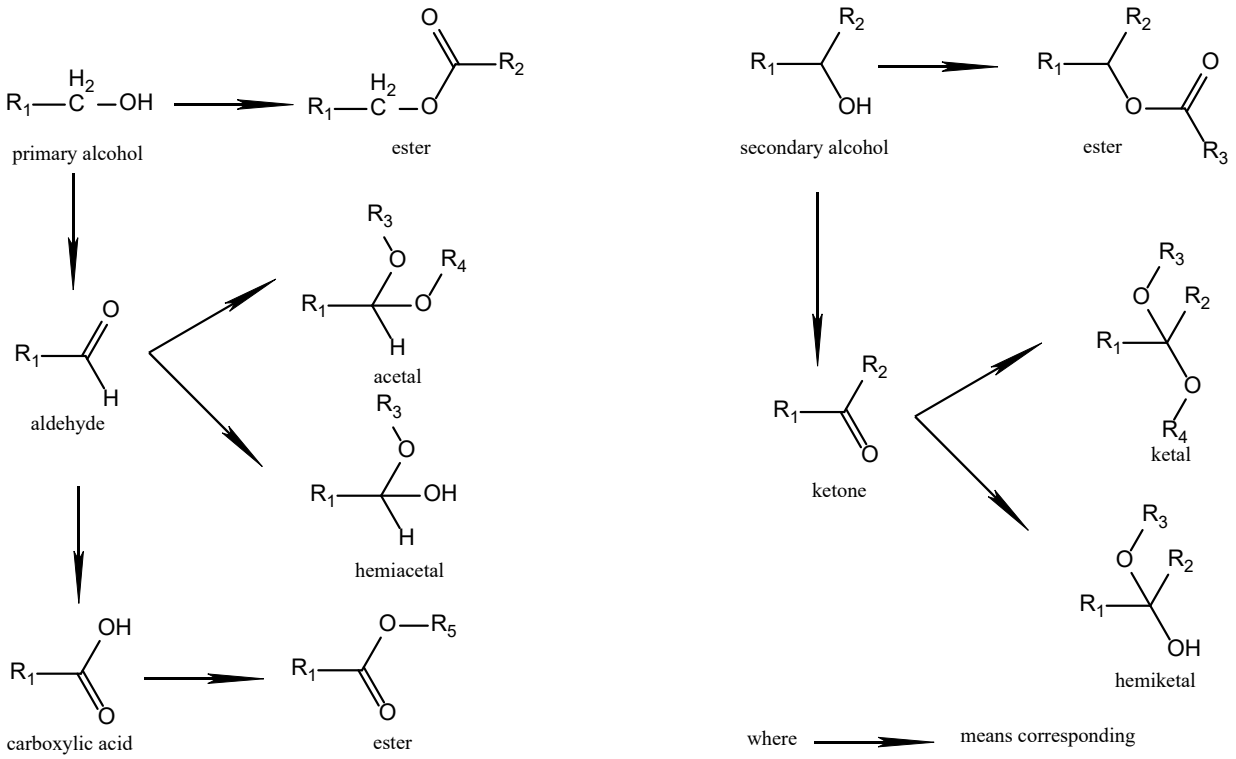
U. **Oxygenated functional group** means any of the following: alcohol (primary, secondary, or tertiary), ketone, aldehyde, carboxylic acid, ether, ester, acetal, ketal, hemiacetal, or hemiketal.

V. At Q2b only, the user is asked to identify potential **leaving groups** that are bound to phosphorus**.** Leaving groups are those atoms or groups of atoms that develop a stable negative charge following a nucleophilic substitution reaction due to inductive or resonance effects. Resonance effects operate through delocalization of π electrons present in adjacent double bonds, and inductive effects operate by polarization of electrons in sigma bonds. Both effects increase the ability of the bond to cleave and the leaving group to leave. In general, resonance effects are stronger than inductive effects and lead to more rapid bond cleavage. Resonance effects play a vital role in increasing the reactivity (and toxicity) of organophosphates in their substitution reactions with acetylcholinesterase. For the purpose of the EDT, leaving groups include, but are not limited to, those with inductive effects, such as -F, -Cl, -Br, and -SR; and those that form resonance-stabilized anions as products of a substitution reaction (e.g., -CN, -SCN, and -OCN, -O-C=C, -O-C=O, $O=P(OR)_2O$-, O=P(OR)O-, $(O=)_2S(OR)O$-, or O=S(OR)O-). Because the number of possible leaving groups that can be synthesized by today's modern organic chemist is limitless, the user is encouraged to review these topics (i.e., leaving group, resonance and inductive effects) in greater depth in a standard organic chemistry text.

W. **Electron pair donors** are atoms or groups of atoms that can donate electron density. For the purpose of the EDT, these are: $-O^-$, -OR (ether), -OH (alcohol), -OC(=O)R (ester), -C(=O)OH (carboxylic acid), $-C(=O)O^-$ (carboxylate), $-NH_2$ (primary amine), -NHR (secondary amine), $-NR_2$ (tertiary amine), -NHC(=O)R (amide), -SR (thiolate), and -SH (thiol). Question regarding electron pair donors is found only in Q6d.

X. **Organyl** refers to a general class of organic fragments that contain a carbon-based structure. Specifically, it often denotes an organic group or substituent derived from an organic molecule. While organyl can apply to various types of organic groups or substituents, such as alkyl groups (e.g., methyl, ethyl), aryl groups (e.g., phenyl, tolyl), or more complex structures, the organyl group or substituent is always based on organic carbon structures.

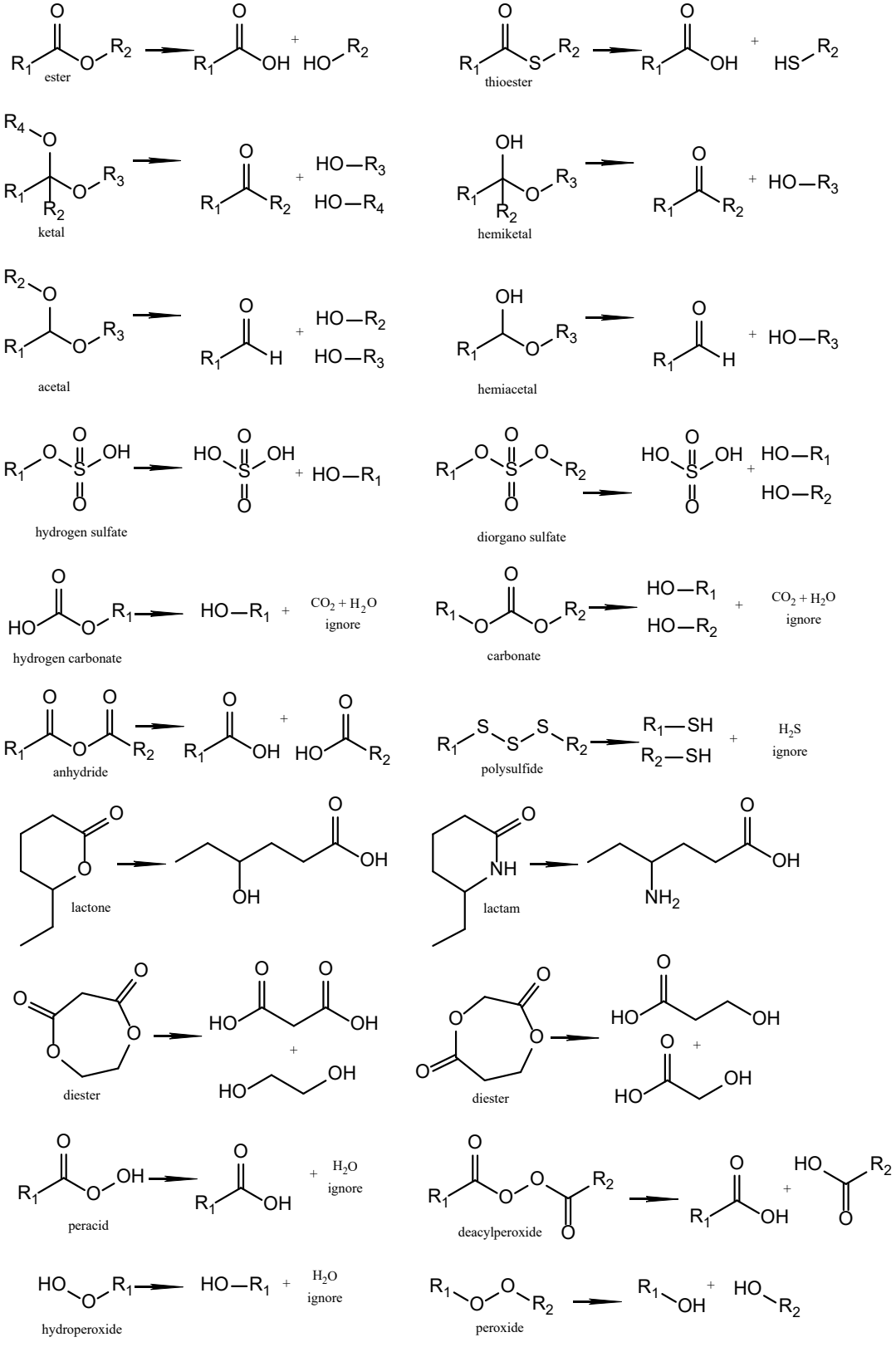
Y. The term **corresponding** refers to:
   a. A primary alcohol (e.g., 1-propanol) and its related compounds, that is, its corresponding aldehyde (i.e., propanal), carboxylic acid (i.e., propanoic acid), or an acetal, hemiacetal, or ester that hydrolyzes to yield the parent primary alcohol, or the corresponding aldehyde or carboxylic acid.
   b. A secondary alcohol (e.g., 2-butanol) and its related compounds, that is, its corresponding ketone (i.e., 2-butanone), or any ketal, hemiketal, or ester that hydrolyzes to yield the parent secondary alcohol or corresponding ketone. Examples:



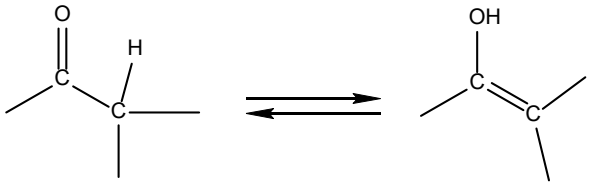
Z. The term **related** means:
   a. A member of an acyclic homologous series (e.g., 2-heptanone) different by not more than two carbons from another substance (i.e., 2-nonanone or 2-pentanone for this example) in the series.
   b. Substances with the same functional groups (e.g., ethyl 3-ketobutanoate and propyl-3-ketopentanoate) that are expected to participate in common metabolic pathways (i.e., hydrolysis to ketoacid, β-cleavage to yield acetyl CoA and the CoA ester of the acid fragment, and complete oxidation to carbon dioxide and water).
   c. Acetal, hemiacetal, ketal, hemiketal, or ester that hydrolyzes to members of a homologous series (e.g., 2-phenylethyl acetate hydrolyzes to phenylethanol and acetic acid and 4-phenyl-1-butyl acetate hydrolyzes to 4-phenyl-1-butanol and acetic acid).

AA. Multiple questions in the EDT relate to expected **hydrolysis** or **reduction** of functional groups. Hydrolysis adds the element(s) of water to a molecule leading to either a different molecule (e.g., lactones with one cyclic ester hydrolyze to hydroxycarboxylic acids) or more than one molecule (e.g., aliphatic monoesters hydrolyzed to a carboxylic acid and an alcohol, and cyclic diesters hydrolyze to either two hydroxycarboxylic acids or to a diol and a dicarboxylic acid) (see drawing of hydrolysis reactions after this paragraph). Reduction is a chemical reaction where a species undergoes a gain of electrons or a decrease in its oxidation state. This process can involve the addition of hydrogen atoms or the removal of oxygen atoms from a molecule. Reduction is typically associated with the transfer of electrons from another substance that is being oxidized. All hydrolysis and reduction products should be evaluated using the EDT as instructed at specific questions and the structural class for the parent structure assigned based on the highest EDT class of its component molecules (e.g., if one of the hydrolysis or reduction products gets assigned to Class II and the second product to Class IV, assign the parent

compound to Class IV). See figure below for the hydrolysis and reduction reactions of common functional groups.

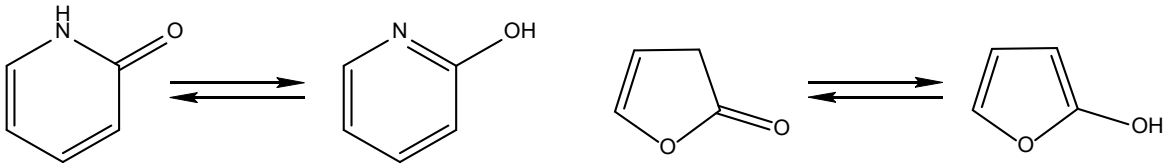


AB. **Enolization** means the interconversion (**tautomeri**sm) between a keto form and an enol form. Example:

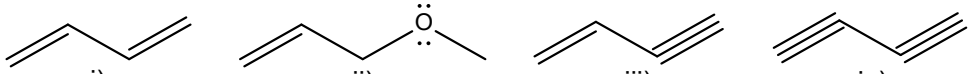


Ketone Enol

Tautomerization of heterocycles:



AC. In chemistry, **conjugated** refers to a specific arrangement of alternating single and double bonds within a molecule. This arrangement involves the overlap of p-orbitals across adjacent bonds, allowing for the delocalization of electrons across the entire system. When a double bond is adjacent to a single bond and the single bond is connected to a nitrogen or oxygen atom with lone pair electrons, these lone pairs can participate in conjugation. The lone pair on the nitrogen or oxygen can overlap with the π-system of the adjacent double bond. This interaction is often referred to as lone pair conjugation or n → π interaction and can affect the molecule's electronic structure, influencing properties such as reactivity and stability. In an alternating double bond-single bond-triple bond configuration, true conjugation does not occur because the triple bond does not participate in p-orbital overlap with the double bond in the same manner. However, there can be some electronic interaction between the double and triple bonds, though it is generally not as extensive or stabilizing as true conjugation and may affect the molecule's properties. Similarly, in a triple bond-single bond-triple bond configuration, the triple bonds do not conjugate with each other through the single bond. While there is no true conjugation here either, there may be some electronic effects or inductive interactions that can influence the molecule's stabilization and chemical properties. For the purposes of the EDT, to simplify its language, the following configurations are referred to as conjugation: i) double bond-single bond-double bond, ii) double bond-single bond-nitrogen or oxygen atom with lone pair of electrons, iii) double bond-single bond-triple bond, and iv) triple bond-single bond-triple bond.



i) ii) iii) iv)

AD. For the purposes of the EDT, we consider the moiety -CF_3 to be equivalent to one halogen. For example, if the compound has three -CF_3 moieties, we consider the compound to have a total of three halogens.

Terms in **bold letters** in the EDT questions below (section 4.5.4) indicate that they have been defined explicitly in section 4.5.2.

4.5.3 How to Use the Post-validation EDT

Based on the chemical structures, definitions, and guidelines provided, the questions are answered in sequence with “yes” or “no” responses until reaching an assignment to one of the six EDT classes: Class I – VI. To help with classification, we provide one or more example structure(s) following each question. Moreover, in Appendix 2, we provide over 3,100 compounds and show how they traverse through the EDT to help the user get accustomed using the EDT.

4.5.4 The post-validation EDT schema

To help visualize the flow of the EDT questions and how they are interconnected, please see Figure 2 below (for users of assistive technology or readers requiring a text-based version, see Appendix 3 for a description of Figure 2.):

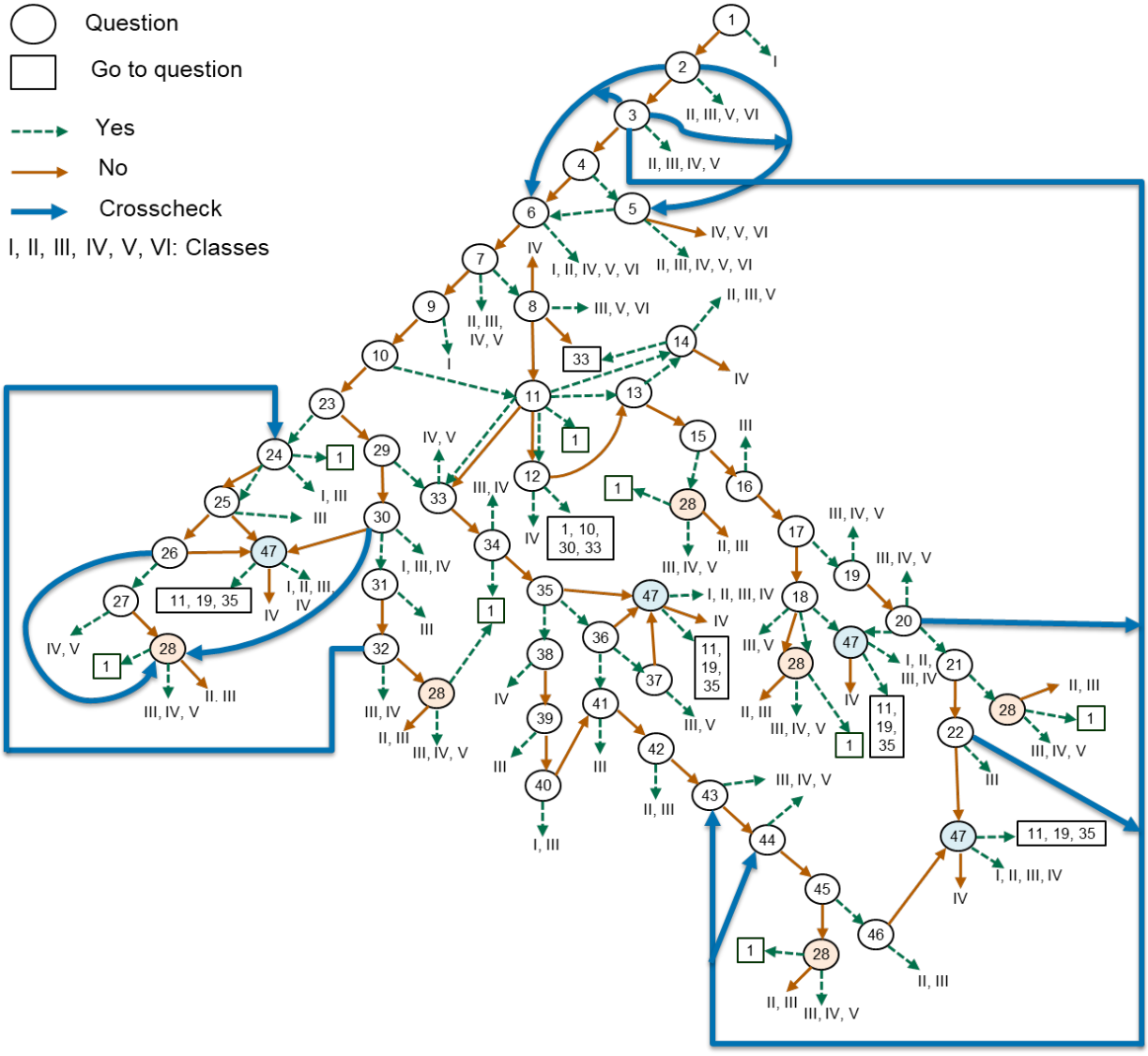
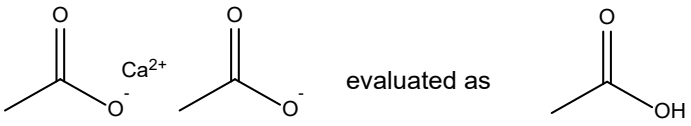


Figure 2. Organization of the post-validation EDT

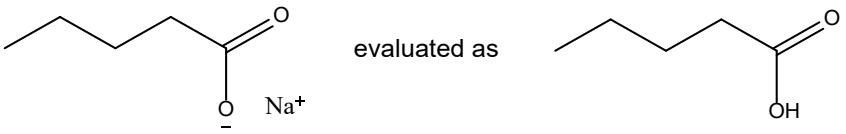
4.5.5 The Post-validation, Finalized EDT Questions

All text that was updated as a result of the external validation are in red font[3] to help the reader identify all changes compared to the pre-validation EDT.

1. Note: In Q1 only, disregard the following commonly encountered and relatively nontoxic or of low toxicity i) metal counterions: sodium, potassium, calcium, magnesium, barium, aluminum, titanium, zinc, manganese, copper, iron, and bismuth; and ii) nonmetal counterions: fluoride, chloride, bromide, phosphate, and sulfate, and evaluate the compound of interest in its neutral form. Examples:



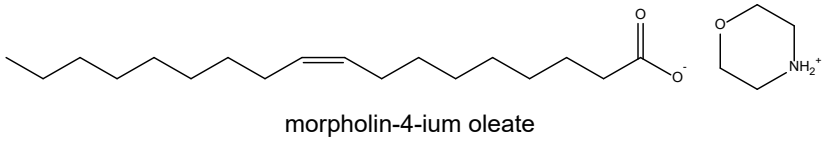
calcium acetate acetic acid



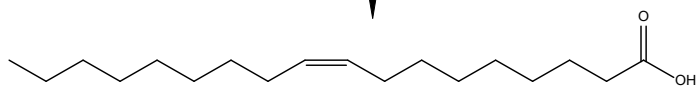
sodium pentanoate pentanoic acid

For compounds having other nonmetal counterions, evaluate each counterion in its neutral form in Q1 (e.g., morpholin-4-ium oleate is evaluated as morpholine and oleic acid). Disregard any counterions in subsequent questions that get classified as Class I by Q1 (in our example, oleic acid) and pass along all other counterions (morpholine (that does not get classified at Q1)) to Q2. If none of the counterions in a substance is classified in Q1 (e.g., morpholine and cinnamic acid in the case of morpholin-4-ium cinnamate), pass the substance in its original form (e.g., morpholin-4-ium cinnamate) to Q2.

[3] Please note that while the red color used in the EDT may not be visible to all readers, all changes are clearly described in Section 4.4.3. Hence, being able to see the red is not necessary to understand or identify the updates made.

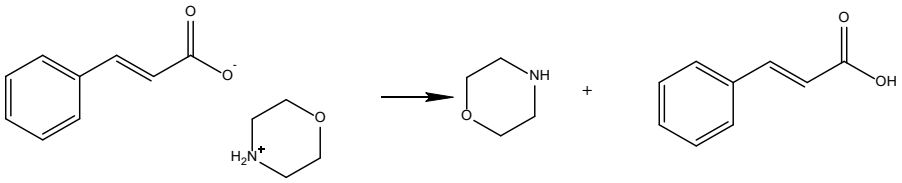


morpholin-4-ium oleate



oleic acid (will be classified in Q1)

morpholine
(at the end of Q1, it will go to Q2)



morpholin-4-ium cinnamate
(at the end of Q1, it will go to Q2)

morpholine

cinnamic acid

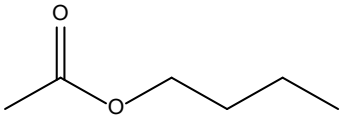
Does the substance belong to one of the structurally defined chemical categories in 1a) through 1k)?

a) A **linear aliphatic** (>1 C) primary alcohol, aldehyde, carboxylic acid, or corresponding hemiacetal, acetal, ester, CoA ester, carbonate (including dicarbonate), or orthoester formed from any of the above alcohols, aldehydes, and carboxylic acids except

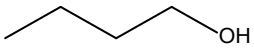
i) **linear** unsubstituted **α,β**-unsaturated aldehydes with <10 Cs or their corresponding acetals and hemiacetals (they will be addressed at Q28p, see example structures there, if needed),

ii) methallyl alcohol, allyl alcohol, or crotonyl alcohol and their corresponding acids (methacrylic acid, acrylic acid, or crotonic acid), esters, carbonates, orthoesters, acetals, hemiacetals, ketals, or hemiketals (they will be addressed at Q28, see example structures there, if needed), and

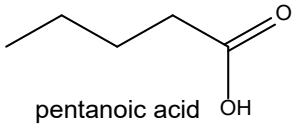
iii) compounds that fit Q1a but have ≥8 contiguous **conjugated** double bonds, or



butyl acetate

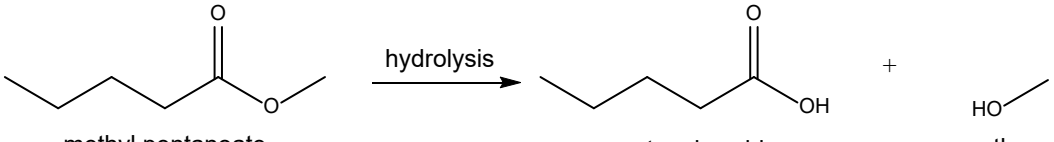


butan-1-ol



pentanoic acid

Please note that if one of the potential hydrolysis products of a hydrolyzable functional group listed in Q1a) does not fit the description above, say no at Q1a). Example:



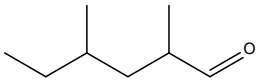
methyl pentanoate

pentanoic acid

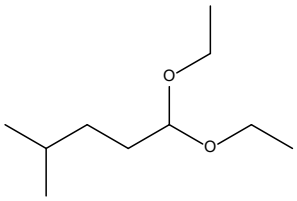
methanol

Please note that methanol does not satisfy the following requirement: >1 C

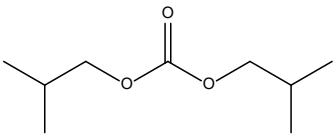
b) **Aliphatic** (>1 C) primary alcohol, aldehyde or carboxylic acid or the corresponding hemiacetal, acetal, ester, or CoA ester, carbonate (including dicarbonate), or orthoester, formed from any of the above alcohols, aldehydes, or carboxylic acids with one or more methyl substituents, except compounds that fit exceptions listed in Q1a ii) and iii). (Please also check whether your compound fits 24a). If yes at Q24a), say no at 1b, and go to Q1c)), or



2,4-dimethyl-1-hexanal

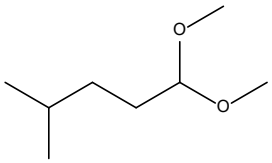


4-methylpentanal diethyl acetal

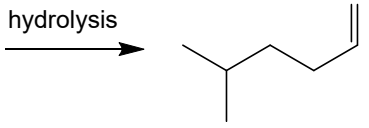


diisobutyl carbonate

Please note that if one of the potential hydrolysis products of a hydrolyzable functional group listed in Q1b) does not fit the description above, say no at Q1b). Example:



1,1-dimethoxy-4-methylpentane

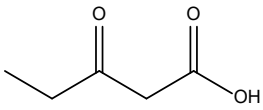


4-methylpentanal

methanol: Please note that methanol does not satisfy the following requirement: >1 C

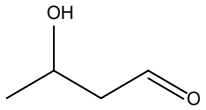
c) **i) Linear aliphatic** (>1 C) or methyl substituted (except substances addressed in Q24b) and compounds that fit the exception listed in Q1iii), dicarbonate, peroxydicarbonate, peroxycarbonate, hydroxycarboxylic acid, hydroxyester, ketoacid, ketoester, corresponding ketal, mono- and di-carboxylic acid, mono- and di-ester, and/or CoA ester, **ii) linear aliphatic** (>1C) or methyl substituted (except substances addressed in Q24b)), substance that contains a single alcohol, ketone or corresponding ketal, one or more ester(s), or CoA ester in addition to the primary alcohol, aldehyde, carboxylic acid(s), carbonate(s) (including dicarbonate(s)), or ester(s), or **iii)** a tricarboxylic acid or a triester where one of the carboxylic acids or esters is either a substituent on a linear carbon chain (a secondary carboxylic acid) or at the end of a side chain of a simply-branched compound (primary carboxylic acid). Note: just as in Q1a) and Q1b), any potential hydrolysis products must have >1 C), or

i:



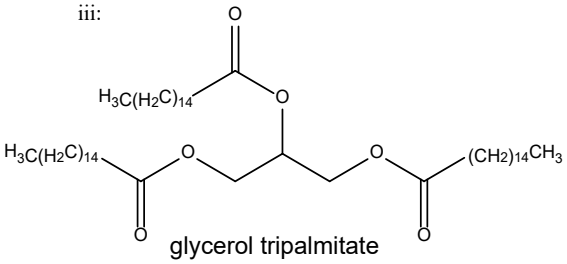
3-ketopentanoic acid

ii:



3-hydroxybutanal

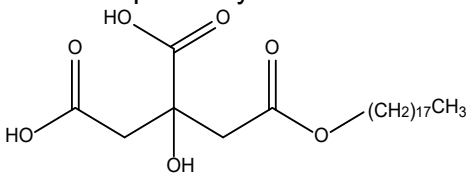
iii:



glycerol tripalmitate

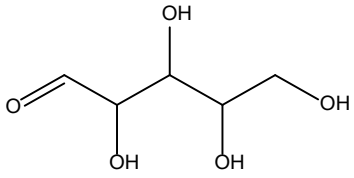
d) Substances in the fatty acid pathway, glycolysis pathway, pentose phosphate pathway, and citric acid cycle (e.g., short-chain fatty acids (C_1 to C_{10}): acetoacetate, 3-hydroxybutyrate, 2-butenoate, carnitine, glyceraldehyde, glycerol, dihydroxyacetone, lactate, malate, malonate, succinate, citrate, isocitrate, pyruvate, oxaloacetate, α-

ketoglutarate, glutarate, or gluconate), and their corresponding esters formed from alcohols and carboxylic acids specified in 1a), 1b), or 1c), or CoA esters. (Note: To further help identify these intermediates, the reader is referred to Salway (2016).), or

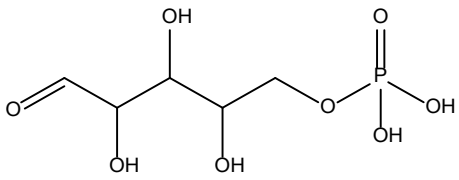


stearyl citrate

e) A monosaccharide (triose, tetrose, pentose, or hexose), an (enzymatically or nonenzymatically) hydrolyzable oligosaccharide, or a hydrolyzable polysaccharide in addition to simple monosaccharide derivatives. Simple monosaccharide derivatives are **i)** phosphate esters (e.g., triose phosphate, ribose 5-phosphate, and glucose 6-phosphate), **ii)** deoxy sugars (one of the hydroxyl groups in the parent monosaccharide is replaced by an H, e.g., L-fucose (6-deoxy-L-galactose) and L-rhamonose (6-deoxy-L-mannose)), **iii)** amino sugars (one of the hydroxyl groups in the parent monosaccharide is replaced by an amino group with or without acetylation, e.g., D-glucosamine, D-galactosamine, and D- mannosamine), or **iv)** mono- and poly-methylated, sulfated, and sulfonic acid derivatives of monosaccharides and monosaccharide derivatives (e.g., 3,4-di-O-methyl-alpha-L-rhamnose, 6-O-methyl-D-glucose, glucosamine sulfate, and 6-deoxy-6-sulfo-D-glucopyranose). These substances may also exist as the hemiacetal, acetal, hemiketal, ketal, or ester form, or as acid, or

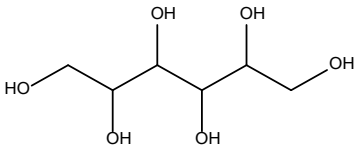


xylose

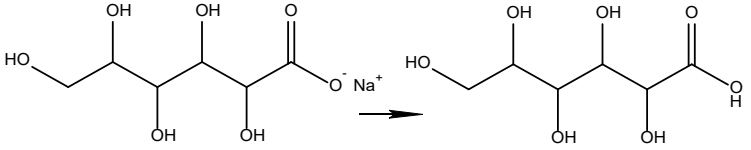


ribose-5-phosphate

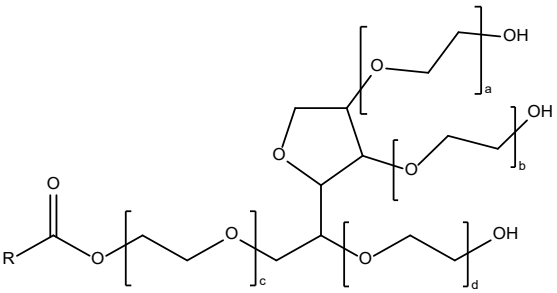
f) Sugar alcohols (e.g., glycerol, erythritol, sorbitol, xylitol, galactitol, inositol, or mannitol) or sugar acids or their corresponding esters (i.e., monosaccharides with a carboxyl group, such as aldonic acids (e.g., gluconic acid), ulosonic acids (e.g., neuraminic acid), uronic acids (e.g., glucuronic acid), and aldaric acids (e.g., tartaric acid)), in addition to derivatives of sugar alcohols that are both alkoxylated and esterified (e.g., polysorbates), or



galactitol (sugar alcohol)

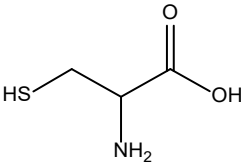


Note that the user is asked to disregard some salts at the begining of Q1.Therefore, for example, sodium gluconate is evaluated as gluconic acid (sugar acid).

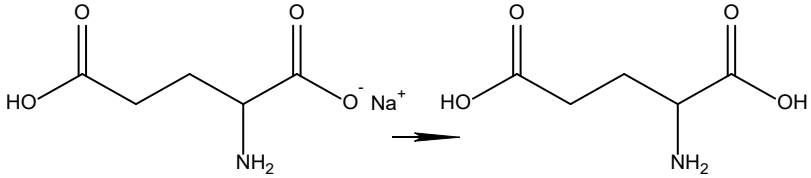


general structure of polysorbates

g) One of the twenty α-amino acids, all other naturally occurring amino acids, **related** CoA esters, and esters formed from **aliphatic** alcohols; N-acetyl derivatives; di- or tri-peptides and/or simple **aliphatic** esters thereof. (Simple aliphatic esters are those that hydrolyze to an amino acid (or di- or tri-peptide) and a compound that gets captured at Q1.) Intermediates and products in the synthesis of essential amino acids and in the transamination and transsufuration pathways and degradation of essential and non-essential amino acids (must have >1 C). For instance, intermediates in the ornithine cycle (e.g., citrulline, ornithine, and 4-hydroxyphenylpyruvate) and intermediates in the biosynthesis and degradation of non-essential amino acids (e.g., α-ketobutyrate, β-mercaptopyruvate, homocysteine, 3-thiopyruvate, α-ketoadipate, α-methylacetoacetate, 2-anthranilic acid, and α-ketoglutarate). (Note: To further help identify these intermediates, the reader is referred to Salway (2016).), or

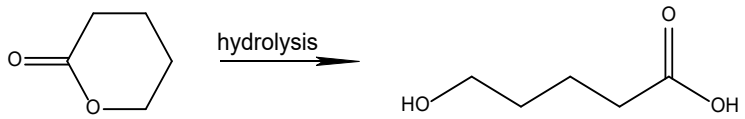


cysteine



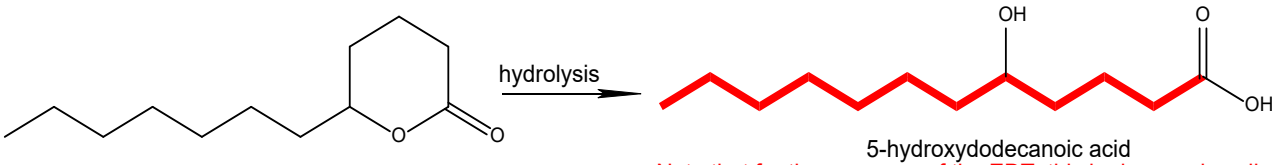
sodium glutamate that is evaluated as glutamic acid after disregarding Na^+

h) Lactones (i.e., monocyclic esters but not an α- or β-lactone) that undergo **hydrolysis** to form **linear aliphatic** or methyl-substituted hydroxycarboxylic acids. Note: Hydroxycarboxylic acids are carboxylic acids containing one or more hydroxy (alcohol) functional groups. Also note that the compounds captured here will be classified as Class I. While some hydrolysis products (hydroxy-acids) of compounds captured at this sub-question may tautomerize to a keto-acid, the tautomerization will be ignored for the purposes of the EDT as these keto-acids would also be classified as Class I (at Q1c)). Therefore, only the pre-tautomerization product must satisfy the structural requirements of this question, or



delta-valerolactone

5-hydroxypentanoic acid

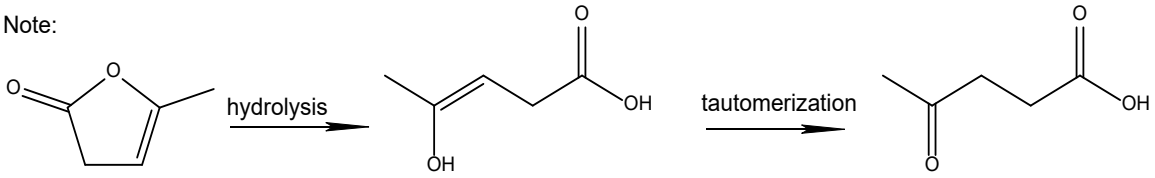


delta-dodecalactone

5-hydroxydodecanoic acid

Note that for the purposes of the EDT, this hydroxycarboxylic acid is considered to be '**linear**' (see bolded chain and the definitions section)

Note:

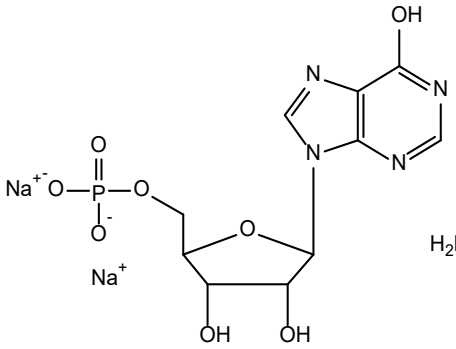


alpha-Angelica lactone

(*Z*)-4-hydroxypent-3-enoic acid

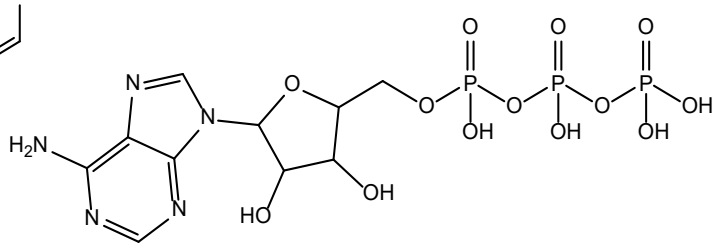
4-oxopentanoic acid

i) Nucleotides (and their phosphate esters with betaine and choline), nucleosides, phospholipids, monophosphates of amino acids, or their **hydrolysis** products, or



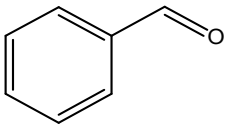
disodium inosinate

(evaluated in the neutral form after disregarding Na^+)



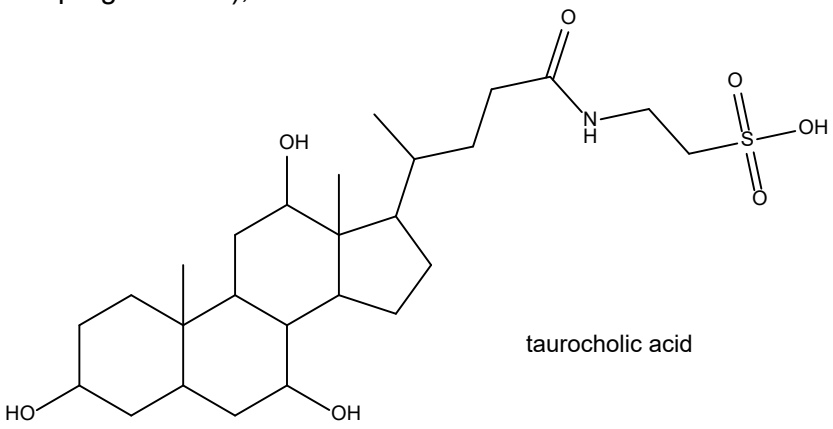
adenosine-5'-triphosphate (ATP)

j) Benzoic acid, its **related** alcohol (benzyl alcohol), aldehyde (benzaldehyde), corresponding alkyl acetals, hemiacetals, the CoA ester and **related** alkyl esters formed from benzyl alcohol or benzoic acid (Note: the benzene ring should not contain ring substituents other that those listed above), or



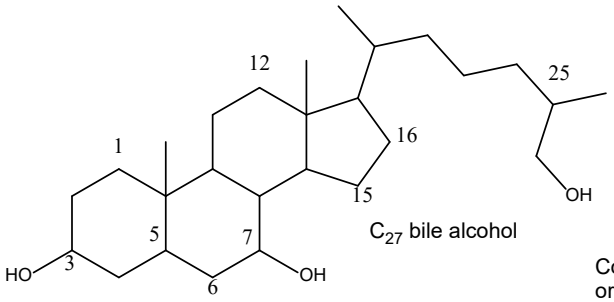
benzaldehyde

k) Bile acids, alkyl esters of bile acids, bile acid conjugates (with the carboxyl group and the amino acids taurine or glycine), bile alcohols, and bile salts, but no other substances containing a steroid **skeletal structure** (e.g., mineralocorticoids, such as aldosterone and progesterone), as these will be dealt with at Q6.



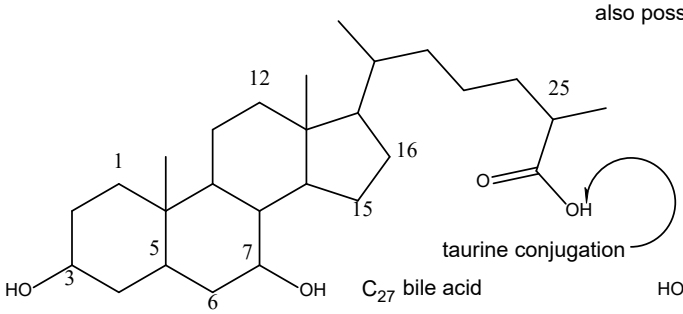
taurocholic acid

Bile acids are family of steroids with a core structure of seventeen carbon atoms arranged in four fused rings (three cyclohexane rings and one cyclopentane ring), together with a five or eight carbon side chain terminating in a carboxylic acid group (or hydroxyl in the bile alcohols). They contain hydroxyl groups at positions C3, C7, and often C12 and hydrophobic methyl groups at positions C18 and C19. Bile acids can form conjugates with the carboxyl group and the amino acids taurine and glycine. Conjugation with glucose or by sulfation, or with N-acetylglucosamine at C7 may also occur. The core structures of bile acids and alcohols are:



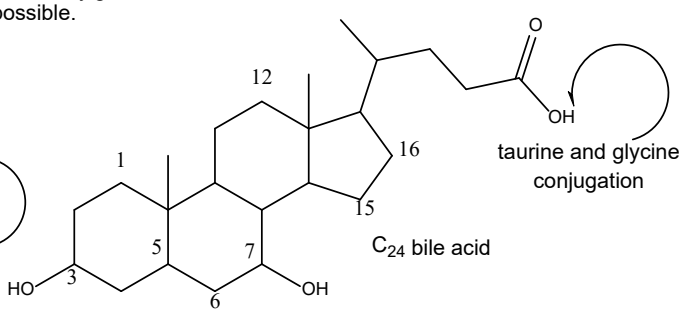
C_{27} bile alcohol

Conjugation with glucose or by sulfation, or with N-acetylglucosamine at C7 is also possible.



taurine conjugation

C_{27} bile acid



taurine and glycine conjugation

C_{24} bile acid

Please note that many structures meet the criteria in more than one sub-question of Q1. However, all structures classified at Q1 are assigned to Class I; therefore, ultimately it does not matter at which sub-question they are captured.

i) If yes to a), b), c), d), e), f), g), h), i), j), or k), assign to Class I.
ii) If no to all, proceed to Q2.

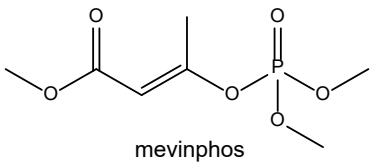
---

2. In Qs 2 and 3 only, disregard sodium, potassium, calcium, magnesium, barium, aluminum, titanium, zinc, manganese, copper, iron, bismuth, ammonium, sulfate, thionosulfate, sulfite, bisulfite, sulfinate, sulfamate, phosphate, fluoride, chloride, or bromide counterions, and evaluate the compound in its neutral form. Does the structure contain

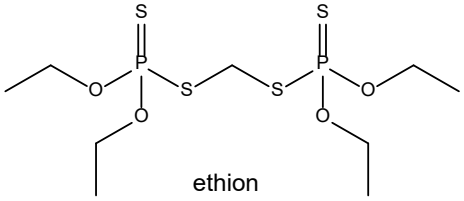
a) covalently bound P (i.e., the P is not simply a phosphate counterion, such as in oseltamivir phosphate or in ethanol, 2,2'-iminobis-, phosphate (salt))

that exists as

b) $O=P(Y)(XR)_2$, $S=P(Y)(XR)_2$, $S=P(OR)_2$-W-$(OR)_2P=S$ and $O=P(ZR)_2$-W-$(ZR)_2P=O$ where X is C, N, O, or S; W is S, N, O, or SC_nS where $n \leq 4$; Z is N or S, and Y is F-, Cl-, Br-, -S- (but not -SH or $-S^-$), CN-, SCN-, OCN-, C=CO- (i.e., potential **leaving groups**) and <8 C, or

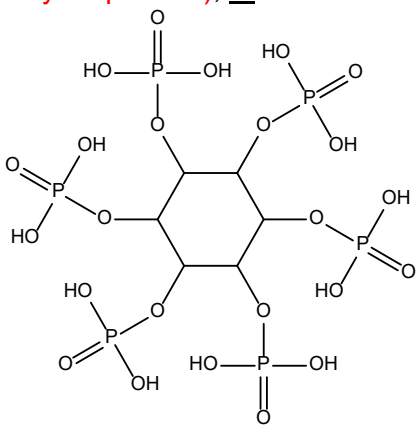


mevinphos

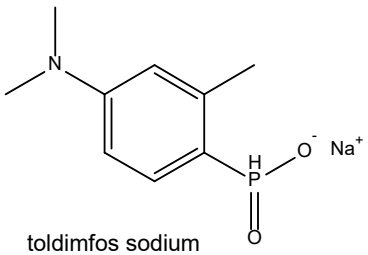


ethion

c) $O=P(OR)_3$, $P(OR)_3$, $PH_n(OR)_n$, $O=P(R)_n(OR)_n$ where (n is 1 or 2) and R is H and/or C containing at least one P-OH or their corresponding salts (no covalently bound halogen may be present), or



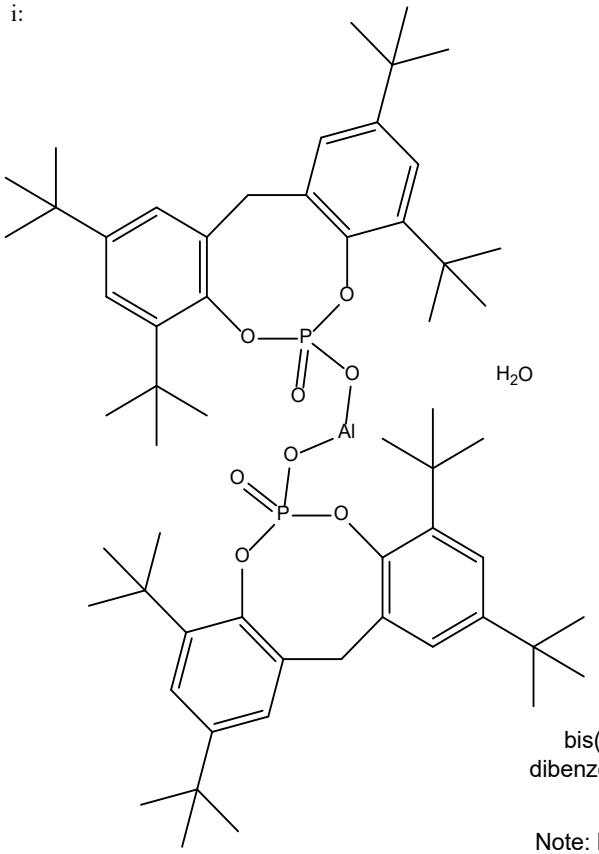
phytic acid



toldimfos sodium

d) i) $O=P(OR)_3$ or **dimer** thereof ($O=P(OR)_2OP(OR)_2=O$) or any other **dimer** that hydrolyses to $O=P(OR)_2OH$ with R is H, alkyl and/or **aryl** but no covalently bound halogen and no N- and/or S-containing functional group may be present (the presence of one or more -O- in the alkyl chain is allowed), or ii) phosphite ($P(OR)_3$ with only R is alkyl and/or **aryl** with or without additional **functional groups** (but no covalently bound halogen may be present)?, or

i:

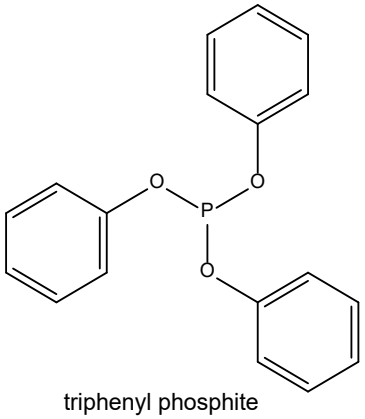


H_2O

bis((2,4,8,10-tetra-*tert*-butyl-6-oxido-12*H*-dibenzo[*d*,*g*][1,3,2]dioxaphosphocin-6-yl)oxy)-l^{2-} alumane hydrate

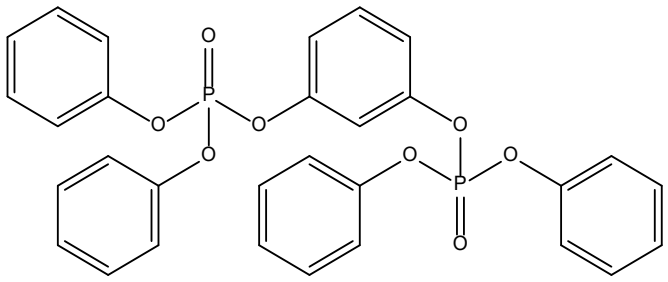
Note: hydrolysis product will satisfy requirement

ii:



triphenyl phosphite

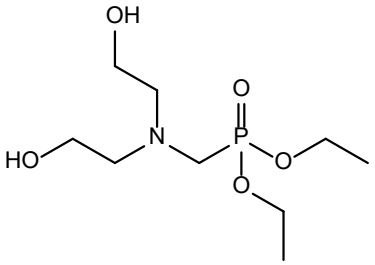
i:



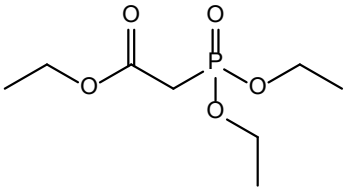
resorcinol bis(diphenyl phosphate)
Note: each phosphate 'subunit' satisfies the requirements in Q2d(i)

e) **i)** alkyl phosphonate ($RP(=O)(OR)_2$) where R is alkyl with or without one or more -OH substitution on the alkyl chain, with or without an ester or N in the alkyl chain (neither directly attached to the P) without any other functional groups or halogens, or **ii)** alkyl and aryl phosphorodithioate ($HSP(=S)(OR)_2$ or $^-SP(=S)(OR)_2$) where R is alkyl or aryl, without the presence of any potential leaving groups (see Q2b)) or halogens?

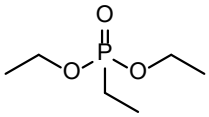
i:



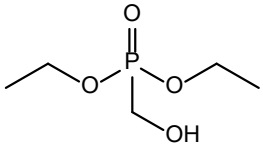
diethyl bis(2-hydroxyethyl)aminomethylphosphonate



triethyl phosphonoacetate

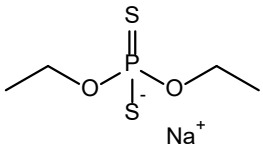


diethyl ethylphosphonate

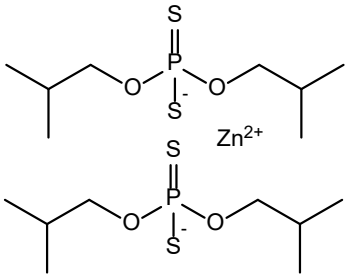


diethyl (hydroxymethyl)phosphonate

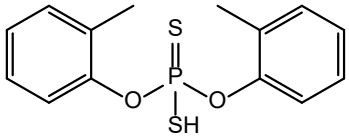
ii:



sodium O,O-diethyl dithiophosphate



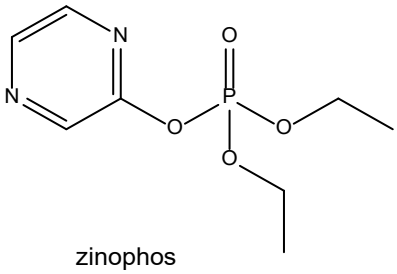
phosphorodithioic acid, mixed O,O-bis(iso-Bu and pentyl) esters, zinc salts



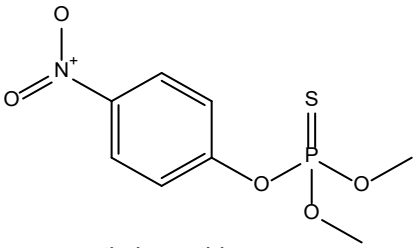
O,O-ditolyl phosphorodithioate

Before assigning the substance to a class at Q2, crosscheck against Qs 5 & 6; that is, if due to the presence of a toxic element addressed at Q5 or a structural moiety of high toxic potential at Q6 the substance would go to a higher class at either Q5 or Q6 than it would at Q2, assign the substance to the highest class it would get at either Q2, Q5, or Q6.

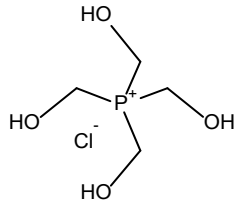
i) If no to a), proceed to Q3.
ii) If yes to a) <u>and</u> b), assign to Class VI.
iii) If yes to a) <u>and</u> either c), d(i)), or d(ii)), assign to Class III. For d(i), evaluate the non-P-containing hydrolysis product starting at Q1, and assign the substance to the highest class any of the hydrolysis products may have.
iv) If yes to a) and e(i)) or e(ii)), assign to Class II.
v) If yes to a) <u>but no</u> to b), c), d), and e), assign to Class V.
Examples for yes to a) but no to b), c), and d):



zinophos



methyl parathion



tetrakis(hydroxymethyl) phosphonium chloride

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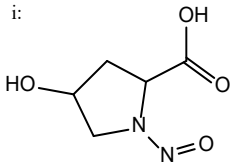
3. For Qs 2 and 3 only, disregard sodium, potassium, calcium, magnesium, barium, aluminum, titanium, zinc, manganese, copper, iron, bismuth, ammonium (or ammonia), sulfate, thionosulfate, sulfite, bisulfite, sulfinate, sulfamate, phosphate, fluoride, chloride, or bromide

counterions, and evaluate the compound in its neutral form. Does the substance contain any of the following **functional groups** or **reactive moieties?**

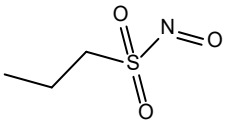
a) **i)** N-N=O (N-nitroso/oxohydrazine), N=O (nitroso), or N-OH (N-hydroxy) (not oxime), and the N is not part of a single sulfonamide function (N-SO_2-), or **ii)** N-N=O (N-nitroso) or N=O (nitroso), and the N is a part of a single sulfonamide function (N-SO_2-), , or

i:



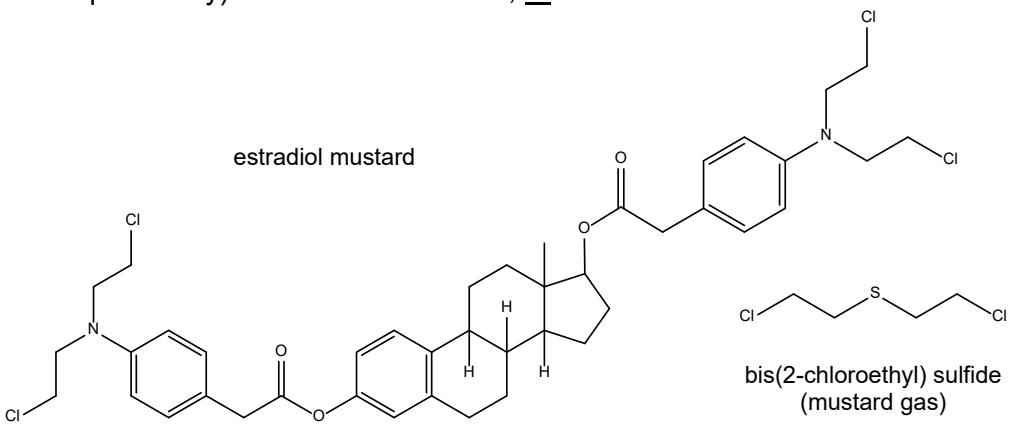
4-hydroxy-1-nitrosopyrrolidine-2-carboxylic acid
(nitrosohydroxyproline)

ii:



1-(nitrososulfonyl)propane

b) one or more **aliphatic** chains of either $(XCC)_2Z$- or (XCC)Z- with Z is S or N (amine only, N not quaternary) and X is Cl and/or Br, or

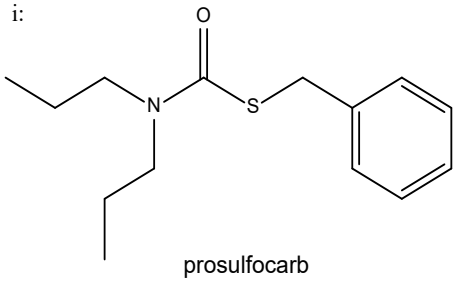


estradiol mustard

bis(2-chloroethyl) sulfide
(mustard gas)

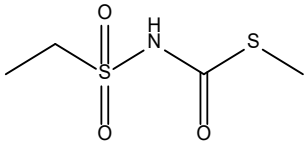
c) **i)** thiocarbamate (both O-**organyl** (OC(=S)N and OC(=S)N=C) and S-**organyl** thiocarbamates (SC(=O)N and SC(=O)N=C)) or dithiocarbamates (SC(=S)N and SC(=S)N=C) (but these cannot be a part of a **heterocyclic** ring itself) and the N is not part of a single sulfonamide function (N-SO_2-), or **ii)** thiocarbamate or dithiocarbamate (but these cannot be a part of a **heterocyclic** ring itself) and the N is a part of a single sulfonamide function (N-SO_2-), or

i:



prosulfocarb

ii:

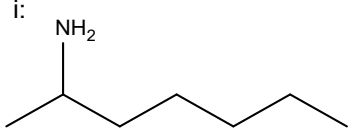


S-methyl (ethylsulfonyl)carbamothioate

d) **i)** an **α**-methyl- or **α**-ethyl-substituted primary **linear aliphatic** amine, or its salt, or **ii)** an **aliphatic linear** or **simply branched** secondary amine or its salt without any other **functional groups** except other primary and/or secondary amines (please note that the

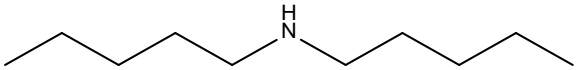
secondary amine cannot be a **connector** or a part of a **connector** between two rings), or

i:



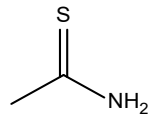
2-aminoheptane

ii:

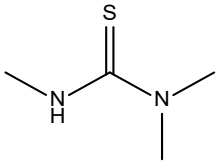


dipentylamine

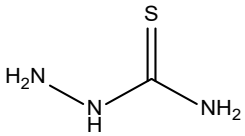
e) Thioamide, thiourea (not thiourea dioxide ($(=O)_2S=C(NR_2)_2$)), or aminothiourea (no atom from these functional groups can be a part of a ring), or



thioacetamide



1,1,3-trimethylthiourea



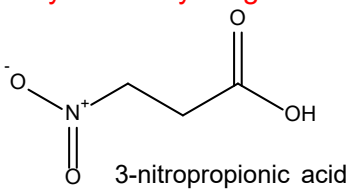
aminothiourea

f) **i)** one or more nitrate esters ($RONO_2$), or

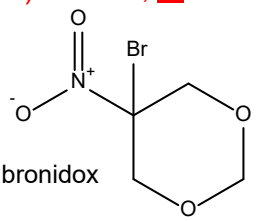
For the sub-sub-questions below (Q3f(ii), (iii), (iv), (v), (vi), (vii), (viii), and (ix)) ignore compounds that contain the following exceptions (that is, if these are present, go to Q3g directly) (R is C or H):

A. iminium ion ($R_2C=N^+R_2$) (note that neither the iminium C or N cannot be a part of a ring)
B. chloride, bromide, sulfonate, or sulfate salt of an **aliphatic** primary or tertiary amine
C. nitrobenzene derivatives (due to the significant toxicity data available for these substances, they are considered at Q43 and Q44) (say no at this sub-sub-question even if other N^+ (other than in nitrobenzene) is present as all nitrobenzenes will be evaluated further along the EDT)
D. choline and choline derivatives ($(CH_3)_3N^+CH_2CH_2O$-) and betaine derivatives ($(CH_3)_3N^+CH_2C(=O)O$-) (note that betaine is captured at Q1)
E. brominated or chlorinated compounds with Ar-$N=N^+(O^-)$-Ar **skeletal structure** (Ar: **aromatic** ring)
F. positively charged N in diazo ($R_2C=N^+=N^-$ or R_2C^--$N^+\equiv N$) and azide (-N^--$N^+\equiv N$ or $RN=N^+=N^-$) as they are addressed at Q3g
G. compounds that fit the structural requirements at Q6e(iii) (as these will be classified at Q6e(iii))
H. compounds with at least two sulfonate and/or sulfamate functional groups.

ii) one or more nitro functional group(s) ($^-ON^+(=O)$-), except in any of the above forms, and the nitro group is neither a heteroaromatic ring substituent nor directly attached to cyclic or acyclic guanidine (-N(=N)N) or a -N-, or

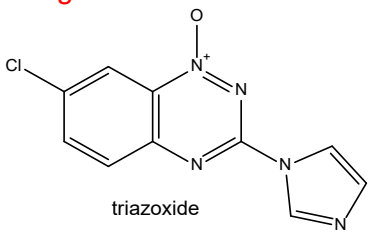


3-nitropropionic acid



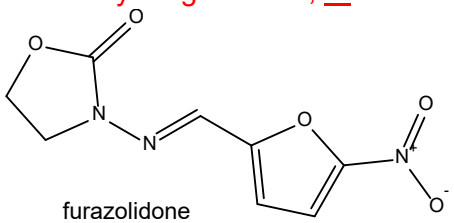
bronidox

iii) a heteroaromatic ring with at least one N^+ as a ring atom and the N^+ is directly substituted by O^- and the compound contains one or more covalently bound halogens and/or one or more additional heteroaromatic rings, or

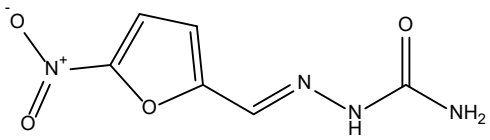


triazoxide

iv) one or more nitro functional group(s), except in any of the above forms, and the nitro group is a substituent on a heteroaromatic ring but the heteroaromatic ring may not have a cyclic guanidine, or

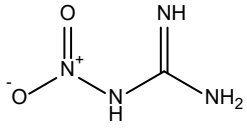


furazolidone

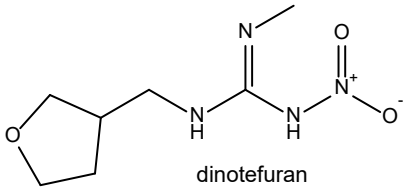


nitrofurazone

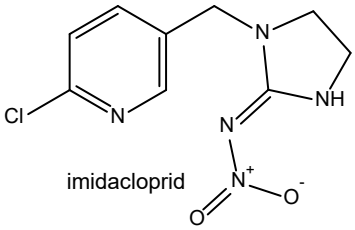
v) one or more nitro functional group(s), except in any of the above forms, where the N^+ from the nitro group is directly attached to cyclic or acyclic guanidine, or



nitroguanidine

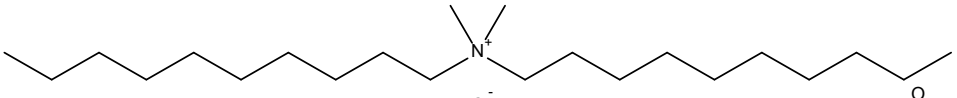


dinotefuran

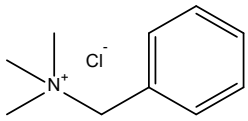


imidacloprid

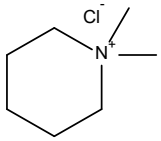
vi) one or more N^+R_4, except in any of the above forms, where the N^+ is attached to 4 Cs and the compound is either acyclic or has a maximum of 2 benzene rings **or** the N^+ is a ring atom in a heterocyclic (not heteroaromatic) ring. The compound may have one or more ether, alcohol, ester, carboxylic acid, amide, sulfoxide, sulfone, sulfonate, sulfamate, and/or a maximum of 3 covalently bound Cl and/or Br (these covalently bound halogens may not be a substituent on a ring), without the presence of any other functional groups. The presence of a single heteroaromatic rings is also allowed, but the N^+ can neither be a ring atom in the heteroaromatic ring nor can it be a direct substituent (i.e., directly attached) to the heteroaromatic ring, or



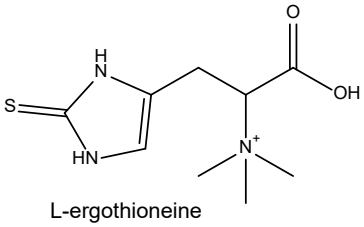
didecyldimonium chloride



benzyltrimethylammonium chloride



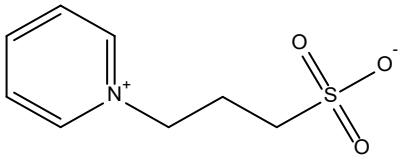
mepiquat chloride



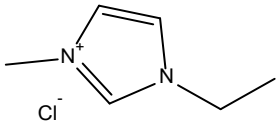
L-ergothioneine

vii) single N^+ as a ring atom in a 5- or 6-membered heteroaromatic ring where the N^+ is directly bonded to Cs only (while more than one ring may be present, only one ring

with a single ring N^+ may be present (the N^+ may not be present at the fusion point of two rings). Note that the N^+ may not be directly substituted by an O^-, it must be directly substituted by a non-ring C), or

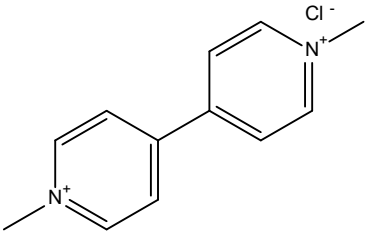


3-(pyridin-1-ium-1-yl)propane-1-sulfonate

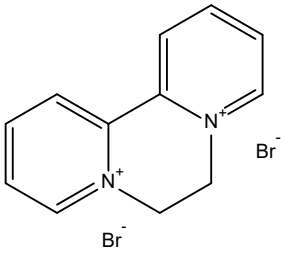


1-ethyl-3-methylimidazolium chloride

viii) at least two rings each with a single N^+ as a ring atom in a 5- or 6-membered heteroaromatic ring where all N^+s are directly bonded to Cs only. (Note that the N^+ may not be substituted by an O^-, it must be directly substituted by a non-ring C), or

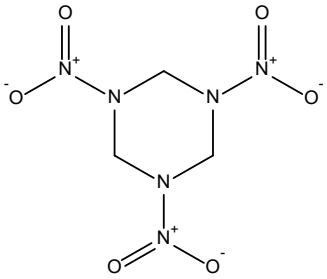


paraquat dichloride

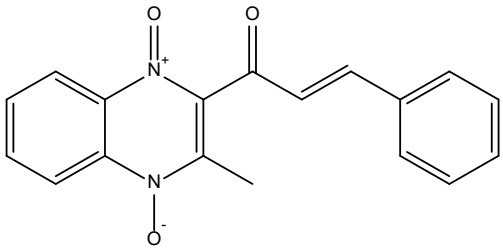


diquat dibromide

ix) all other compounds containing a N^+, except in any of the above forms (i.e., exceptions and Q3f(ii))-Q3f(viii))).



cyclonite

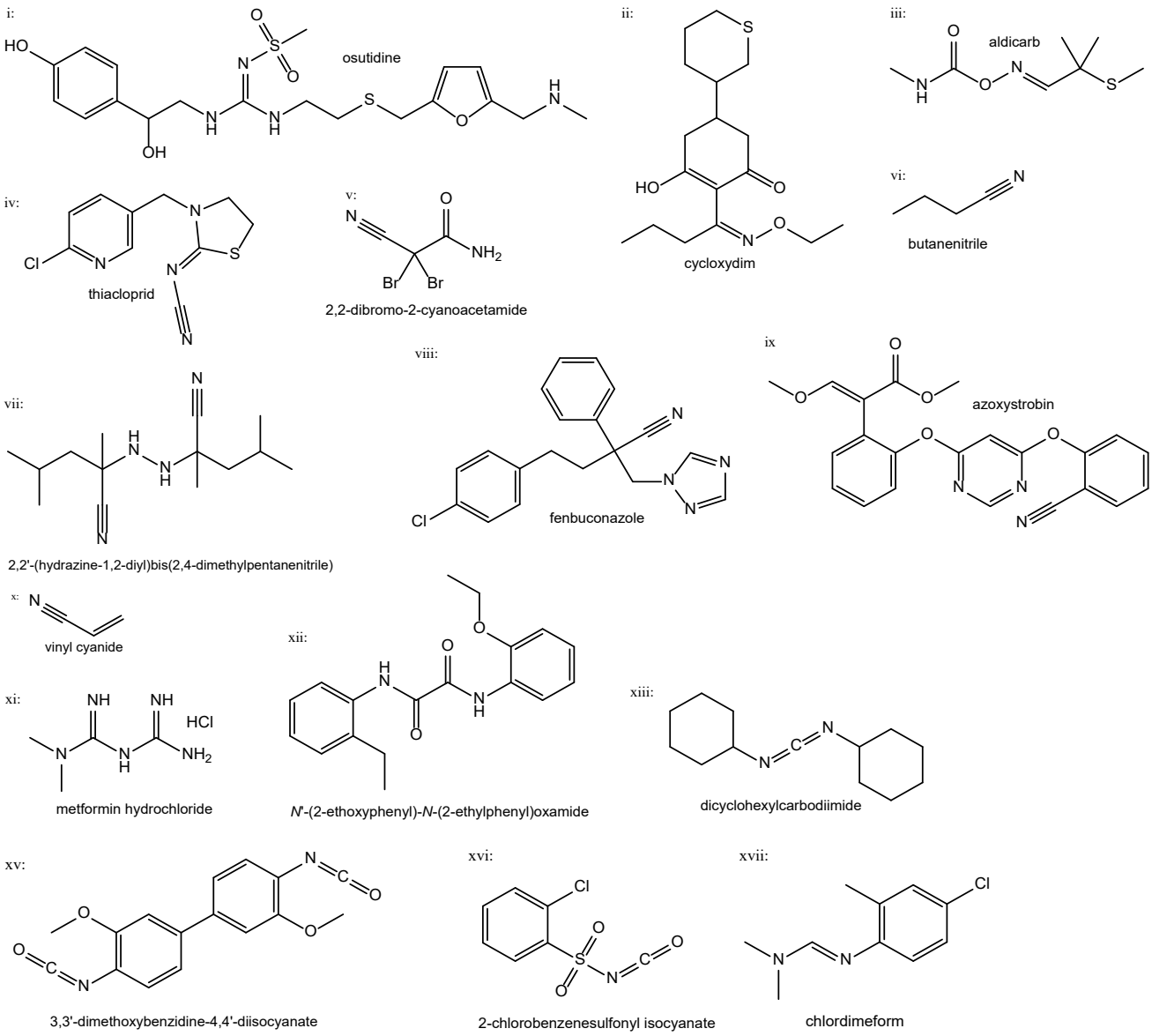


quinocetone

g) **i)** a single sulfonyl carbamate ($S(=O)_2NC(=O)O$), sulfonyl carbohydrazide ($C(=O)N(R)NS(=O)_2$), sulfonyl guanidine ($S(=O)_2NC(=NR)N$), or sulfonylhydrazine ($-S(=O)_2N(H)NH_2$) (no atom from the functional group can be a part of a ring), or

ii) diazo ($C=N^+=N^-$ or $C^--N^+\equiv N$) ((two linked nitrogen atoms (azo) at the terminal position), but not azo ($RN=NR$)), triazeno ($N=N-N$), azide ($^-N-N^+\equiv N$ or $N=N^+=N^-$), hydrazine (R_2N-NR_2) (not when connecting two rings), hydrazide ($-C(=O)NN$ and also $-C(=O)NN(C=O)$ when connecting two benzene rings), hydrazone ($C=N-N$), guanidine ($NC(=NR)N$) (but not biguanidine) (if guanidine is directly attached to a nitro group, ignore the presence of guanidine (i.e., say no at this sub-sub-question)), oxime (C=N-OH) or the corresponding ether (C=N-O-) or the oxime as a product of the **hydrolysis** of the corresponding ester or lactone (C=N-O-C(=O)R), carbamate ($NC(=O)OR$) (but not oxime carbamate ($NC(=O)ONR_2$ or $NC(=O)ON=C$)), or cyanate ($OC\equiv N$). Except for guanidine, and oxime or its derivatives, none of the other **functional groups** may be part of a ring system (i.e., no atom from the functional group can be a part of a ring).

Neither guanidine nor oxime or its derivatives can be fully cyclic (i.e., they cannot be completely contained within a ring), or
iii) at least one oxime carbamate (NC(=O)ON or NC(=O)ON=R), (no atom from the functional group can be a part of a ring), or
iv) one or more cyanamide(s) (N-C≡N) (no atom from the functional group can be a part of a ring), or
v) acyclic substance with one or more nitrile functional group(s) and at least one covalently bound halogen, or
vi) acyclic substance with one or more nitrile functional group(s) either with no other functional group or with only oxygenated functional group(s) and the nitrile triple bond is not **conjugated** with another triple bond or double bond, or
vii) acyclic substance with one or more nitrile functional group(s) and the nitrile triple bond is not **conjugated** with another triple bond or double bond and have either one or more N- and/or S-containing functional group(s) with or without additional oxygenated functional groups (but no other functional groups), or
viii) one or more nitriles with at least one ring and at least one covalently bound halogen present (except substances that fit the structural descriptions at Q6e(iii))). For these substances, say no at Q3g(viii)) as they will be classified at Q6e(iii)), or
ix) one or more nitriles and at least one ring (other than azetidine) without the presence of any covalently bound halogens, or
x) acyclic nitrile with no covalently bound halogens where the nitrile triple bond is **conjugated** with another triple bond or double bond, or
xi) biguanidine (NC(=NH)NC(=NH)N or N=C(N)N=C(N)N) (no atom from the functional group can be a part of a ring), or
xii) oxamide (NC(=O)C(=O)N) (no atom from the functional group can be a part of a ring), or
xiii) carbo diimide (N=C=N) or sulfur diimide (N=S=N) (no atom from the functional group can be a part of a ring), or
xiv) all other nitrile-containing compounds (no azetidine ring may be present), or
xv) one or more isocyanate (N=C=O), or
xvi) sulfonyl isocyanate ($S(=O)_2N=C=O$), or
xvii) amidine (C(=NR)N) (no atom from the functional group can be a part of a ring), or



h) **i)** an isothiocyanate (S=C=N), <u>or</u>

ii) a urea (N(C=O)N) with at least one oxygenated functional group attached to the α carbon (but not a ureide!), a urea with an -O- directly attached to the urea N, or an aminourea where the amine is attached to 2Hs ($H_2NN(C=O)NR$), <u>or</u>

iii) a ureide (C(=O)N(C=O)N) or urea containing at least one aromatic ring with at least one ring halogen substituent or the substance contains at least one heteroaromatic ring (other rings may be present), <u>or</u>

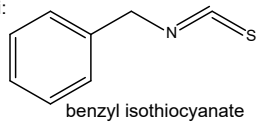
iv) a sulfonylurea ($-S(=O)_2N(=O)N$) containing at least one heteroaromatic ring but without any additional $-S(=O)_2-$, $-NS(=O)_2-$, and/or amide, <u>or</u>

v) sulfonylureas with at least one additional $-S(=O)_2-$, $-NS(=O)_2-$, and/or amide without any covalently bound halogens, <u>or</u>

vi) acyclic aliphatic ureas with no functional groups other than one or more ureas with no covalently bound halogens (and no rings), <u>or</u>

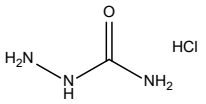
vii) semicarbazones (C=NNC(=O)N), carbohydrazides (NNC(=O)NN, NNC(=O), and C(=O)NNC(=O), guanylureas (NC(=O)N=C(NR)N, and all other ureas and ureides, or
viii) all other sulfonylureas, or
ix) thiocyanate (NCS)? (Note that in i)-ix), none of the functional groups can be a part of the ring itself.)

i:



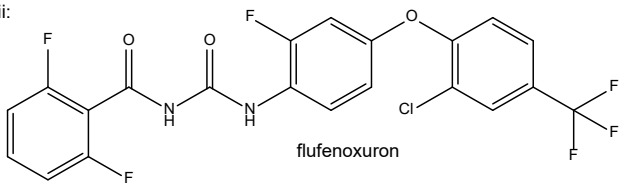
benzyl isothiocyanate

ii:



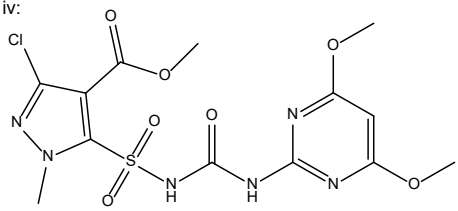
semicarbazide hydrochloride

iii:



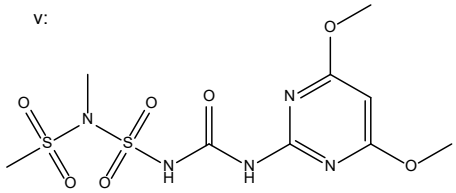
flufenoxuron

iv:



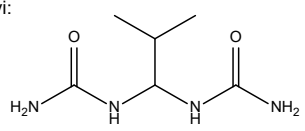
halosulfuron-methyl.

v:



amidosulfuron

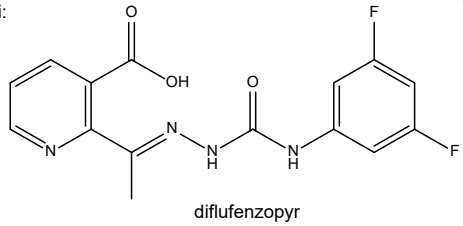
vi:



isobutylidenediurea

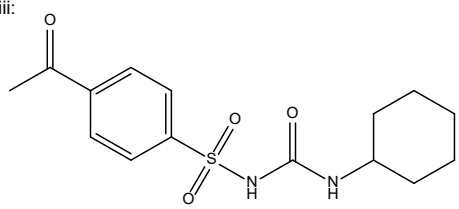
Reminder: SO_2 is not included in the definition of oxygenated functional group.

vii:



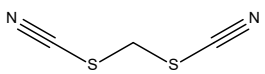
diflufenzopyr

viii:



acetohexamide

ix:



methylene bis(thiocyanate)

Run the substance through all sub-questions (a through h). Do not stop at the first yes to a sub-question. This is done to ensure that the substance gets classified based on its most reactive moiety (i.e., if the answer is yes at multiple sub-questions, assign the substance to a class at the sub-question with the highest class). Before assigning the substance to a final class at Q3, crosscheck against Qs 5 & 6; that is, if due to the presence of a toxic element addressed at Q5 or a structural moiety of high toxic potential at Q6, the substance would go to a higher class at either Q5 or Q6 than it would at Q3, assign the substance to the highest class it would get at either Q3, Q5, or Q6. Moreover, if yes to any of the sub-questions in Q3 and the substance is a diaminobenzene (or its related N-acetyl or N-propionyl derivative), nitroaniline (or its related N-acetyl or N-propionyl derivative (i.e., of amine)), dinitrobenzene, aniline (or its related N-acetyl or N-propionyl derivative), or nitrobenzene, before assigning the compound to a class at Q3, also crosscheck against Q43 and Q44a(ii)), and assign the substance to the highest class it would get at either Q3, Q5, Q6, Q43, or Q44a(ii)).

i) If yes to a(i)), b), c(i)), f(ii)), f(iii)), g(iii)), g(v)), g(viii)), g(xiii)), or g(xvii)), assign to Class V.
ii) If yes to d(i)), e), f(iv)), f(viii)), f(ix)), g(ii)), g(iv)), g(vii)), g(ix)), g(x)), g(xvi)), h(i)), h(ii)), h(iii)), h(iv)), or h(ix)), assign to Class IV.
iii) If yes to a(ii)), c(ii)), d(ii)), f(i)), f(v)), f(vi)), f(vii)), g(i)), g(vi)), g(xii)), g(xiv)), g(xv)), h(v)), or h(vii)), assign to Class III.

iv) If yes to h(vi)), h(viii)), or g(xi)), assign to Class II.
v) If no to all, proceed to Q4.

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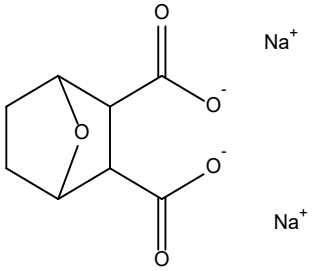
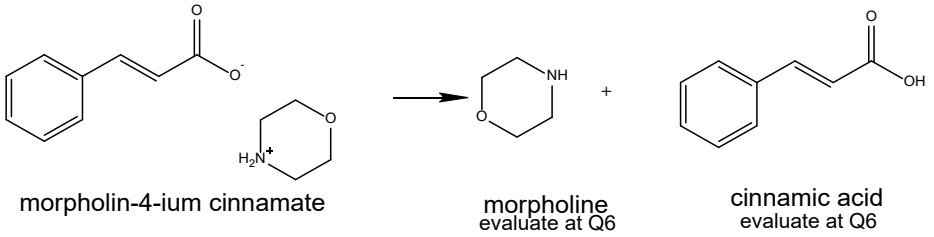
4. Does the structure contain elements <u>other than</u> C, H, O, N (only as trivalent N or tetravalent N^+), S (divalent (sulfide, (-S-)), tetravalent (sulfoxide (-S(=O)-), sulfinate (-S(=O)O-)), or hexavalent S (only as sulfone ($-S(=O)_2-$), sulfamate ($ROS(=O)_2NR_2$ or $^-OS(=O)_2NR_2$), sulfonate ($-S(=O)_2O^-$ or $-S(=O)_2OR$), sulfate ($^-OS(=O)_2OR$ or $ROS(=O)_2OR$), or sulfonamide ($RS(=O)_2NR_2$)), or thionosulfate ($S=S(=O)(O^-)_2$ or $S=S(=O)(OR)_2$), or covalently bound F, Cl, Br, or I (Note: R is H or C)?

i) If yes, proceed to Q5.



disodium endothall

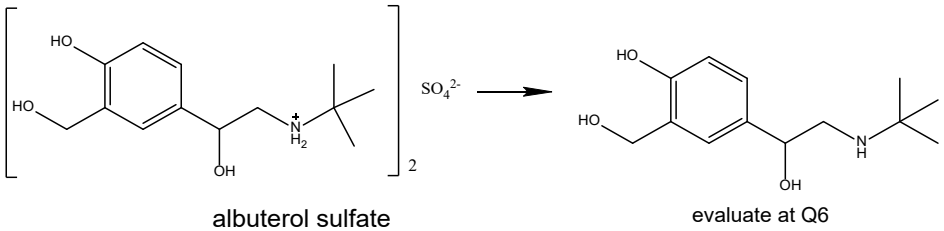
ii) If no, all salts that only contain C, O, H, N, or S (e.g., morpholine-4-ide, mesylate, esylate, or tosylate) should be evaluated considering neutral forms of the counterions going forward and each neutral form should be passed on to Q6 for further evaluation (e.g., the cinnamate salt of morpholine (morpholin-4-ium cinnamate) should be evaluated as morpholine and cinnamic acid at Q6.) As counterions, sulfate, sulfite, bisulfite, and sulfamate in their neutral forms are their corresponding acids and as such are mineral acids not intended to be evaluated by the EDT (i.e., disregard these). If the compound is already in its neutral form, pass it along to Q6.



morpholin-4-ium cinnamate

morpholine
evaluate at Q6

cinnamic acid
evaluate at Q6



albuterol sulfate

evaluate at Q6

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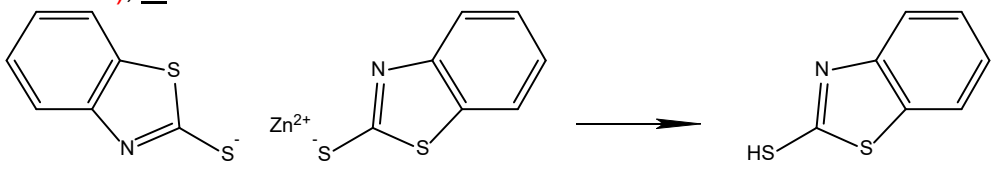
5. Do elements not listed in Q4 occur **<u>only</u>** as

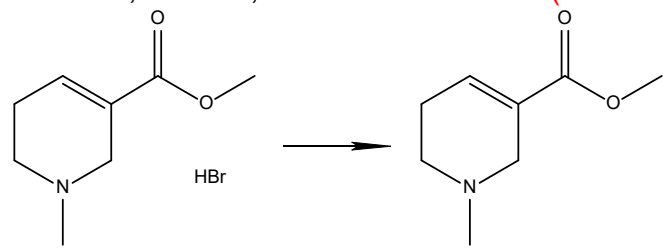
a) a sodium, potassium, calcium, magnesium, barium, aluminum, titanium, zinc, manganese, copper, bismuth, or iron counterion (i.e., not covalently bonded to the 'main' structure), or



2(3H)-benzothiazolethione, zinc salt

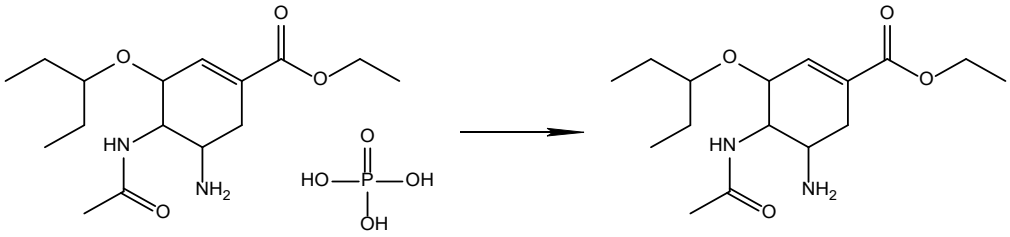
benzo[*d*]thiazole-2-thiol

b) a chloride, bromide, or fluoride counterion (this includes HCl, HBr, and HF), or



arecoline hydrobromide

c) a phosphate counterion, or

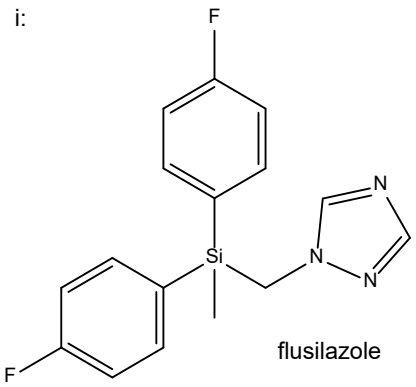


oseltamivir phosphate

d) **i)** a covalently bound silicon (Si) (more than one Si may be present) and the compound has at least one covalently bound halogen and/or at least one heterocyclic ring, or

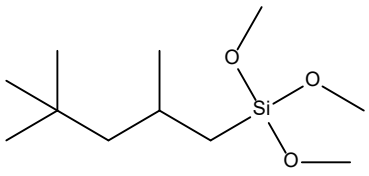
ii) a covalently bound silicon (Si) (more than one Si may be present) and the compound contains neither covalently bound halogen(s) nor heterocyclic ring(s)?

i:



flusilazole

ii:



trimethoxy(2,4,4-trimethylpentyl)silane

e) **i)** tin (Sn) as Sn^{2+} counterion(s) to an organic molecule, or

ii) organotin compounds containing one or more covalently bound halogen(s) where the halogen is directly bonded to the tin, or

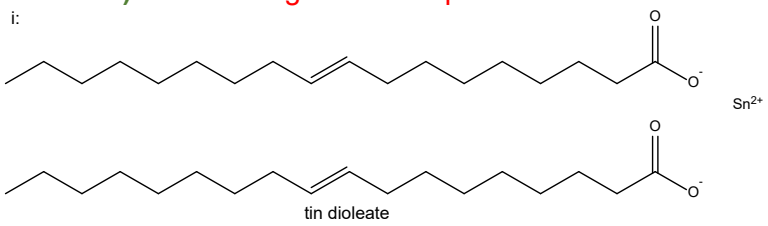
iii) mono-, di-, or tri-alkyltin (these 1-3 alkyl chains must be directly attached to the Sn) with each alkyl chain having a chain length of maximum 4 carbons. In addition to the direct alkyl substitution(s), the tin may have any number of direct hydroxyl, alkyl ester, alkyl ether, -S-, and/or sulfate substitution along with a single benzoic acid ester (i.e., benzoate) substitution, but no other substitutions. If two or more direct Sn-S substitutions are present and the chain length of these -S- linked substitutions exceeds 4 atoms, say no at this sub-sub-question (see example), or

iv) mono-, di-, tri-, or tetra-alkyltin (these 1-4 alkyl chains must be directly attached to the Sn) with each alkyl chain having a chain length of 5 or more carbons. The tin may have any number of direct hydroxyl, alkyl ester, alkyl ether, -S-, and/or sulfate substitution along with a single benzoic acid ester substitution, but no other substitutions, or

v) phenyltin or cyclohexyltin with the phenyl or cyclohexyl ring(s) directly attached to the tin, with or without the tin having direct hydroxyl, ether, -S-, and/or sulfate substitution, but no other functional groups or halogens, or

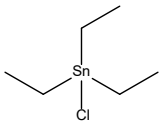
vi) all other organotin compounds.

i:



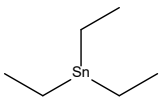
tin dioleate

ii:



triethyltin chloride

iii:

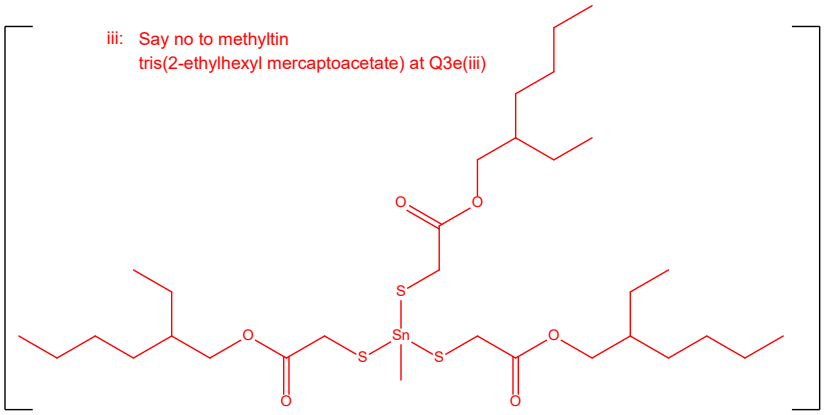


triethyltin

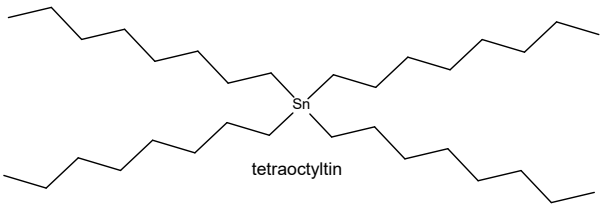
iii:

tributyltin benzoate

iii: Say no to methyltin tris(2-ethylhexyl mercaptoacetate) at Q3e(iii)

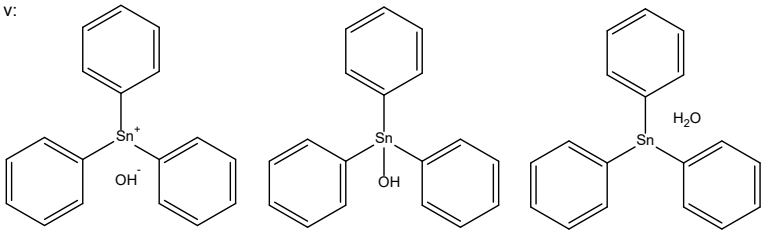


iv:



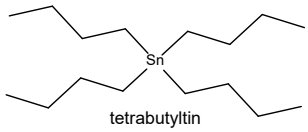
tetraoctyltin

v:



triphenyltin hydroxide
note: tin hydroxides are depicted various ways in the literature

vi:



tetrabutyltin

Depending on your response to the above sub-questions and sub-sub-questions, please choose from the following options:

i) Regardless of a yes or no response to a), b), c), and d), if the substance contains Hg, Tl, Pb, Os, Po, a Lanthanide, an Actinide, or an element in the 7th period from Group 4 to Group 18, assign to Class VI.

ii) Regardless of a yes or no response to a), b), c), and d), if the substance contains As, Be, Cd, or Cr(VI), assign to Class V.

iii) If yes to a), b), and/or c), and the compound has no covalently bound Si, Bi, and/or Sn, disregard the above-listed counterions, treat the compound as the neutral substance, and proceed to Q6.

iv) If yes to a), b), and/or c), and the compound has at least one covalently bound Si, disregard the above-listed counterions, treat the substance as the neutral substance, and evaluate the compound at Q5d. If yes to Q5d(i)), proceed to Q6.

v) If no to a), b), and c), but yes to d(i)), proceed to Q6.

vi) If yes to d(ii)), assign to Class II.

vii) If yes to e(i)), run the organic molecule through the EDT. If the organic counterion is Class II to VI, assign the compound to the class the organic counterion would receive. If the organic counterion would be placed into Class I, assign the compound to Class II due to the presence of Sn^{2+}.

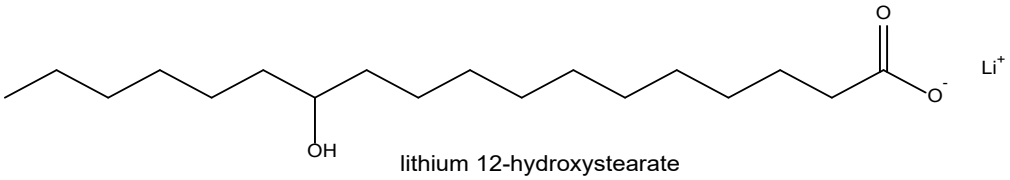
viii) If yes to e(ii)), assign to Class VI.

ix) If yes to e(iii)) or e(v)), assign to Class V.

x) If yes to e(iv)) or e(vi)), assign to Class IV.

xi) If the organic compound contains covalently bound bismuth but does not contain covalently bound Sn or Si or any of the elements listed in i) and ii), assign to Class III.

xii) In all other cases when the answer is no to a), b), c), d), and e), assign to Class IV. Example for no:



lithium 12-hydroxystearate

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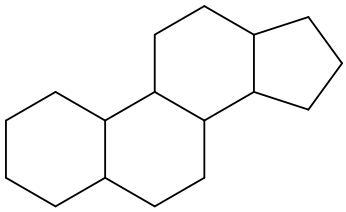
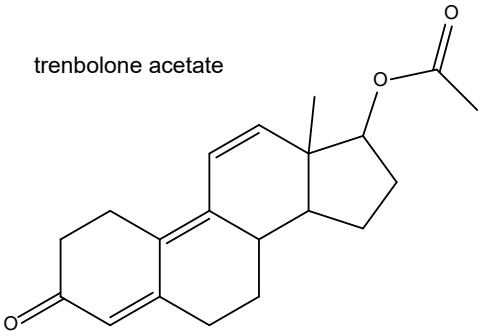
6. Does the substance contain only the elements C, Si, H, O, N, S, P, F, Br, Cl, or I and exhibit any of the following structural features?

a) a steroidal nucleus with or without additional rings or substituents (note that bile acids are dealt with in Q1). The additional rings, if present, cannot be fused (except for the cyclopropyl ring), spiro fused, or bridged to the steroidal nucleus or

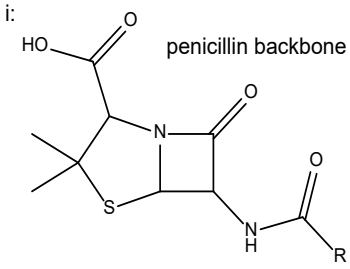


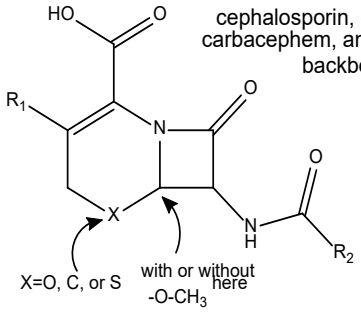
gonane, the simplest steroid, consisting only of the common steroid nucleus

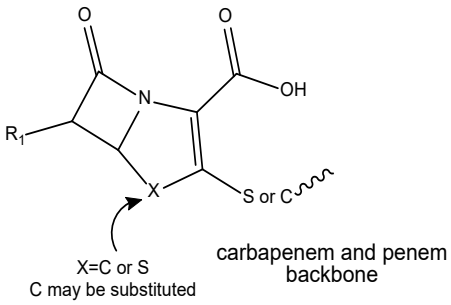


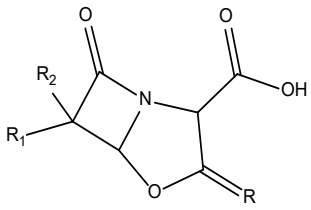
b) **i)** an amine or amide N located at the fusion point of two or more ring systems and the substance has a penicillin, cephalosporin, cephamycin, carbapenem, penem, carbacephem, oxacephem, or clavam **skeleton**, or **ii)** an amidine, amine, or amide N (not N^+) located at the fusion point of two or more ring systems and the compound does not have any of the skeletal structures listed in sub-sub-question i), or **iii)** a substance with a monobactam skeleton, or **iv)** substance containing one or more azetidine ring(s) (but not captured at another sub-sub-question at Q6b)), or

i:

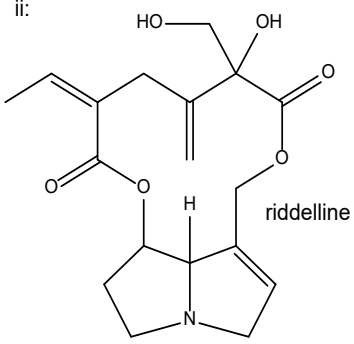




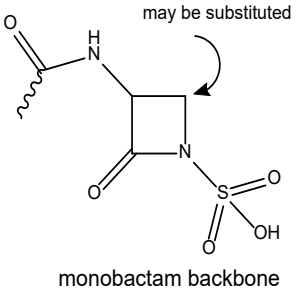




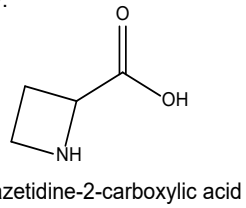
ii:



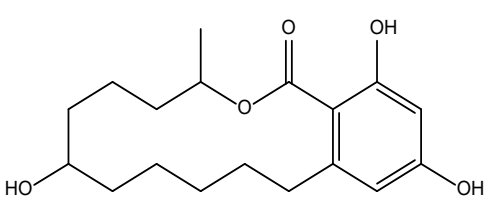
iii:



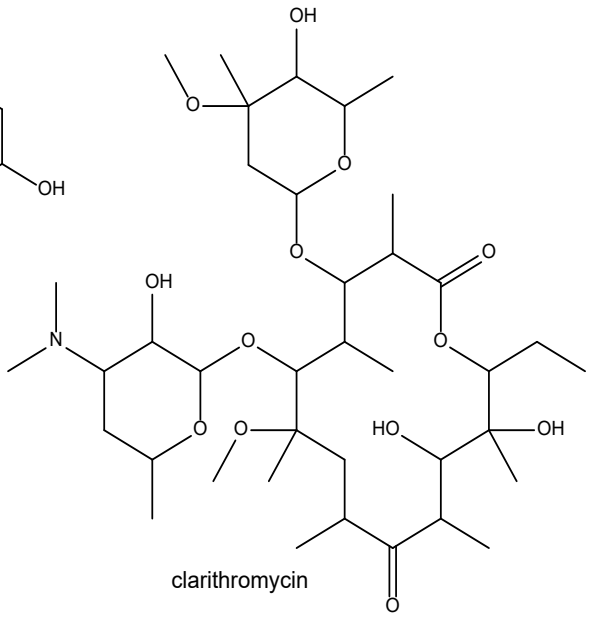
iv:



c) a **macrocyclic** ring (either **alicyclic** or **heterocyclic** (only O and/or N may be present as a heteroatom)) of ≥11 atoms, **fused**, **spiro-fused, singly bonded**, or connected by an -O- to one or more additional ring systems (additional to the above **macrocyclic** ring) with ≥2 **oxygenated functional groups** and/or one or more lactone, or

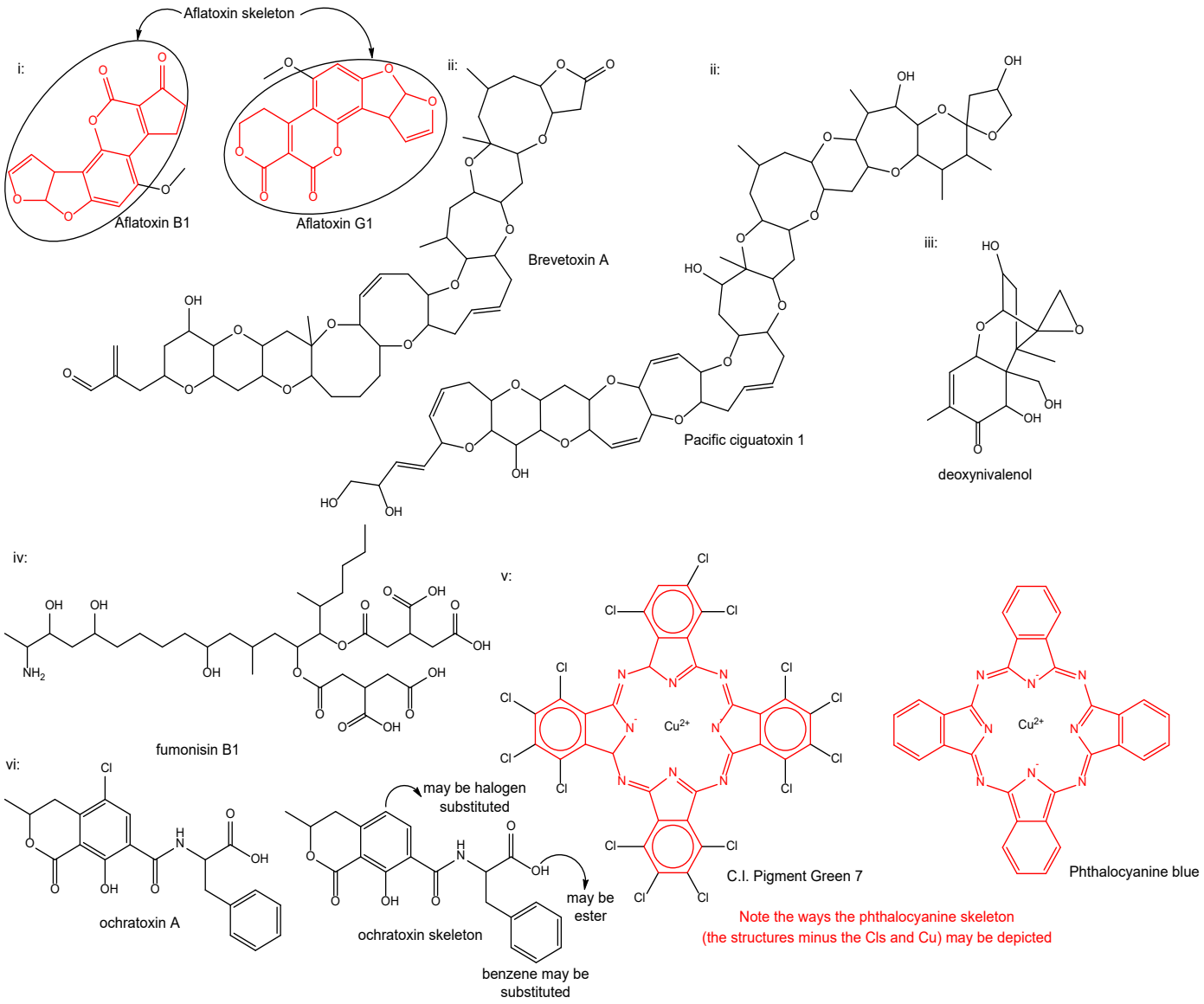


zearalanol

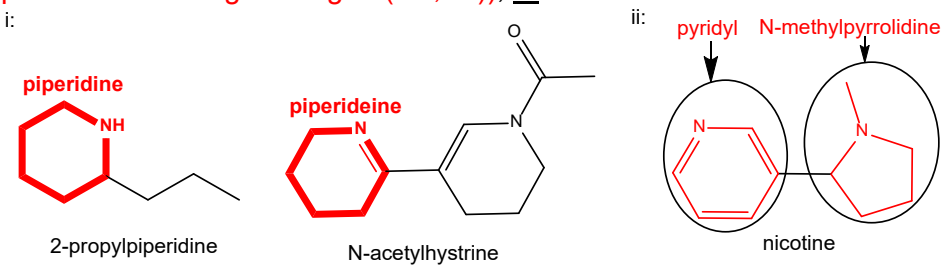


clarithromycin

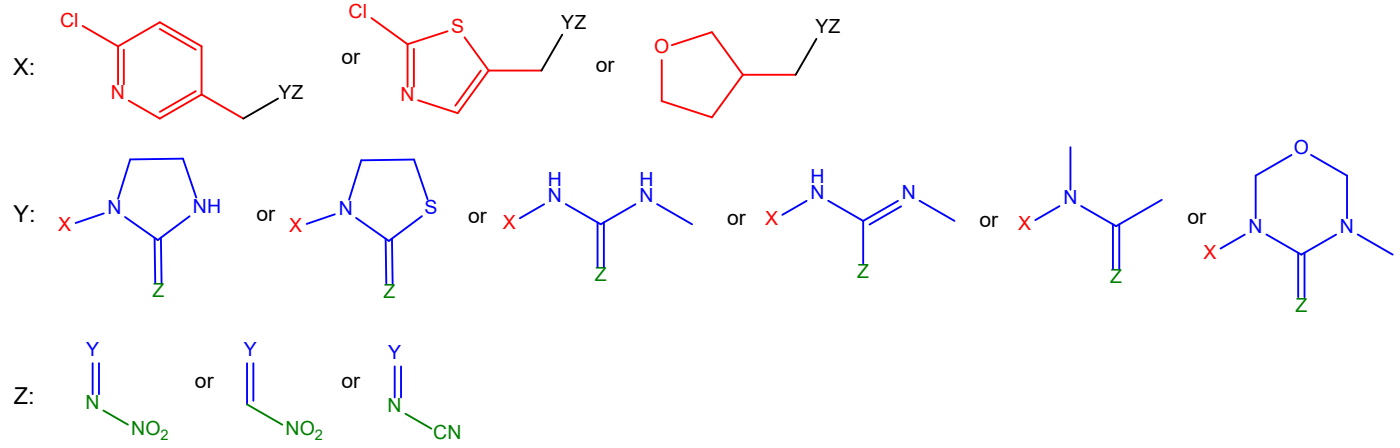
d) **i)** compounds with a difurocoumarolactone skeleton (a bifuran ring fused to a coumarin nucleus with a pentenone ring or a six-membered lactone ring), or
ii) compounds containing at least five fused cyclic ether rings (but no epoxide ring) and at least one fused lactone ring or compounds with over ten fused cyclic ether rings (but no epoxide ring), or
iii) at least four **fused** and/or **spiro-fused** and/or **bridged alicyclic**, **heterocyclic**, **aromatic** or **heteroaromatic** rings at least one of which is an epoxide, tetrahydrofuran, dihydrofuran, furan, pyrrole, dihydropyrrole, pyrrolidine, quinone, or semiquinone except compounds with a phthalocyanine skeleton and those captured at i) and ii); or
iv) a **linear, simply branched,** or **branched chain** of ≥20 Cs, containing at least six **electron pair donors** (except brominated triglycerides) or two lactone rings as substituents with or without additional electron pair donors, or
v) compounds with a phthalocyanine skeleton (the skeleton is highlighted red in the examples below), or
vi) compounds with ochratoxin skeleton; or



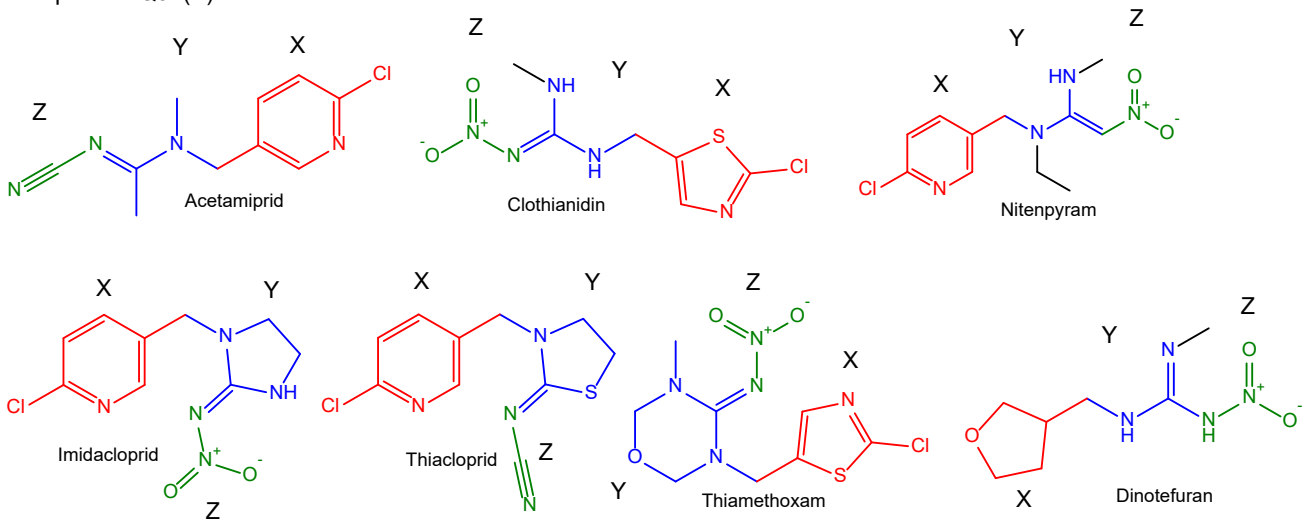
e) **i)** a piperidine or 1-piperideine ring substituted at the 2-position by a hydrocarbon chain of ≥3 Cs, a 3-pyridyl ring, or a 3-(N-acetyl-2-piperideinyl) ring, or **ii)** a N-methylpyrrolidine ring substituted at the 2-position by a 3-pyridyl ring, or **iii)** compounds with the neonicotinoid skeletal structures provided below (note that this Q only allows for the presence of a single halogen (i.e., Cl)), or



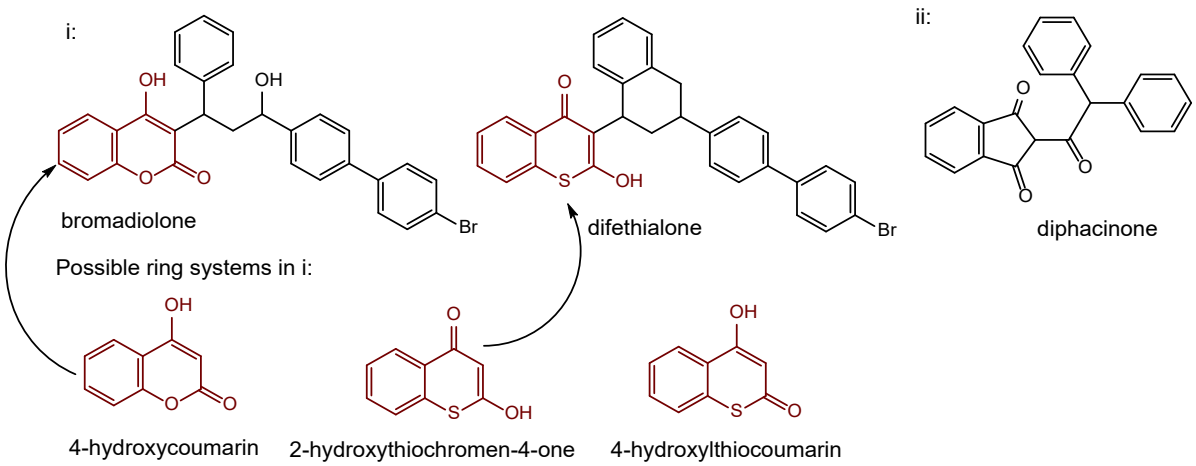
Neonicotinoid skeletal structures:



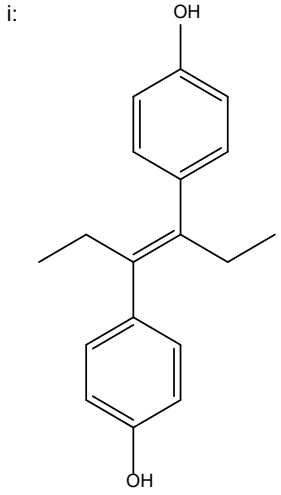
Examples for Q3e(iii):



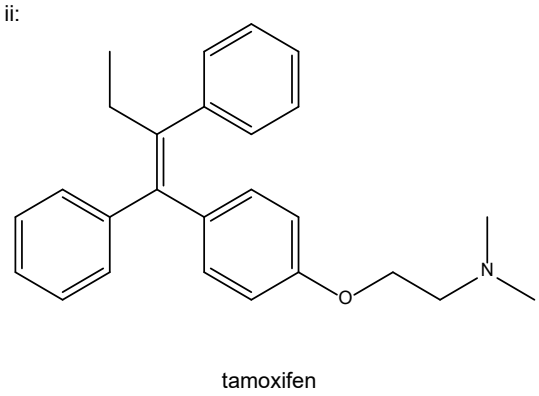
f) **i)** a 4-hydroxycoumarin, 4-hydroxyltiocoumarin, or 2-hydroxythiochromen-4-one ring system substituted at the 3-position either by an alkyl chain (the chain can be a part of an alicyclic ring) containing 1-phenyl or 1-phenyl-3-keto (or hydroxy) substituent or **ii)** a 1,3-diketoindane or 1-keto-3-hydroxyindene containing a 2-phenyl-1-keto substituent at the 2-position, or



g) **i)** two benzene rings connected by a 2- or 3-carbon chain (**connector**, with or without unsaturation) (no additional rings may be present) and a hydroxy, corresponding ester, methoxy, and/or ether in the para position on each ring with or without methyl, ethyl, and/or ethylidene substitution on one or more **connector** carbons (not more than one per carbon). One or more halogen(s) are allowed anywhere on the molecule along with methyl group(s) in the meta position on the benzene ring(s) or **ii)** two benzene rings connected by a -C=C- and one **connector** carbon is substituted by a benzene ring (a total of three benzene rings) and the other **connector** carbon is either unsubstituted or substituted by a methyl or ethyl group or a halogen. Any or all of the benzene rings may be substituted by a hydroxy, corresponding ester, methoxy, and/or ether in the para position, but this is not required, or



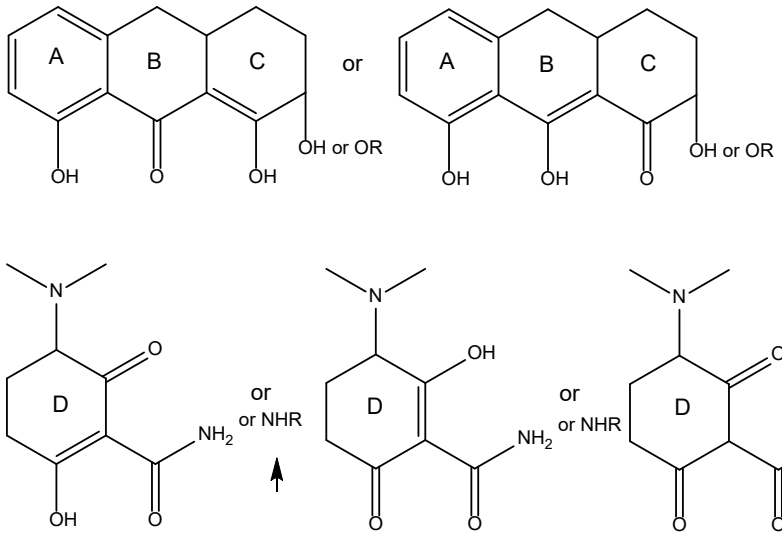
diethylstilbestrol



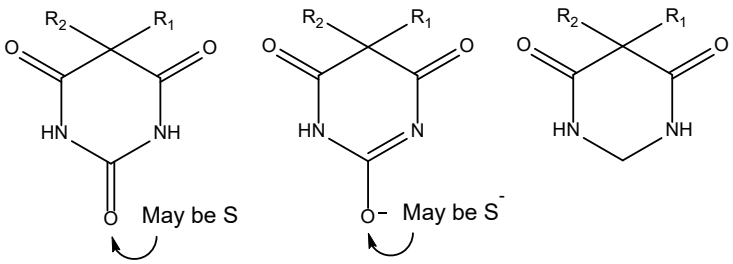
tamoxifen

h) **i)** a tetracycline **skeletal structure** consisting of four (A, B, C, and D) fused rings (D is fused to C) where rings A, B, C, and D are depicted below (note: rings A, B, and C can have additional substituents), or **ii)** a barbiturate skeleton, or iii) a compound having a prostaglandin or prostacyclin skeleton?

i:

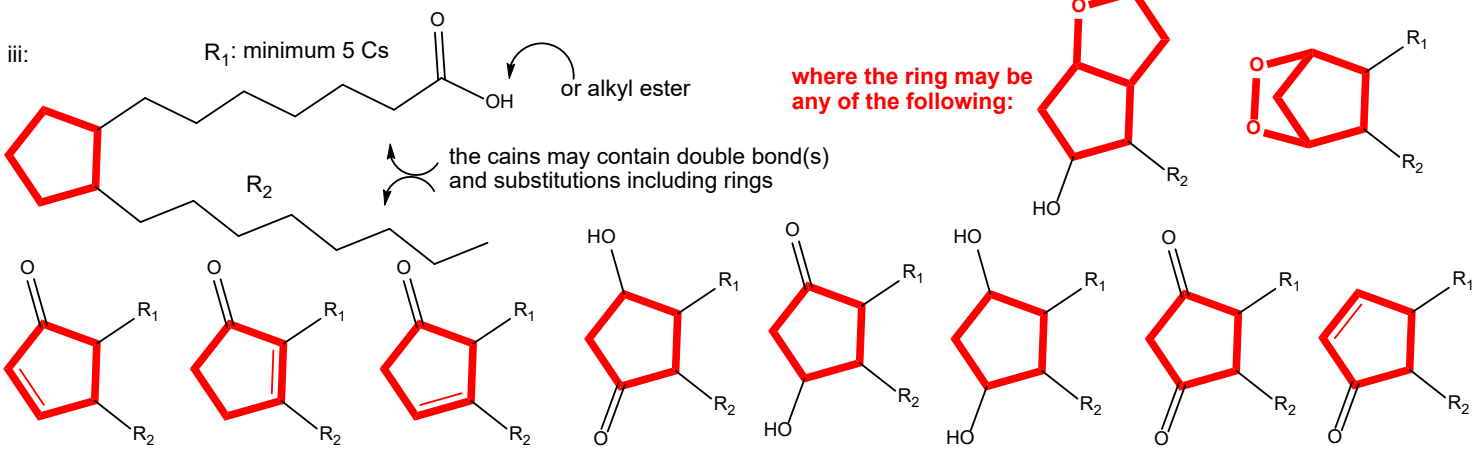


ii:



R1: a single benzene ring or an alkyl chain
with or without a terminal double bond
R2: an alkyl chain with or without a terminal double bond
One of the alkyl chains may be substituted with a single Br.

iii:



i) If yes to a, d(i)), d(ii)), f(i)), f(ii)), or h(iii)), assign to Class VI.

ii) If yes to b(ii)), b(iv)), c), d(iii)), d(iv)), d(vi)), e(i)), e(ii)), g(i)), or g(ii)), assign to Class V.

iii) If yes to e(iii)), assign to Class IV. Note that at Q3, the user is asked to cross-check against Q6 and assign the substance to the highest class it would get either at Q3 or Q6. For Q6e(iii)), ignore this instruction and assign the substance according to the instructions at Q6e(iii)) (i.e., Class IV).

iv) If yes to h(ii)), assign to Class IV.
v) If yes to b(i)), b(iii)), or h(i)), assign to Class II. Note that at Q3 the user is asked to cross-check against Q6 and assign the substance to the highest class it would get either at Q3 or Q6. For Q6b(i)) and Q6b(iii)), ignore this instruction and assign the substance according to the instructions at Q6b(i)) and Q6b(iii)) (i.e., Class II).
vi) If yes to d(v)), assign to Class I.
vii) If no to all, proceed to Q7.

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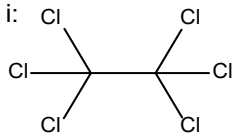
7. Is the substance

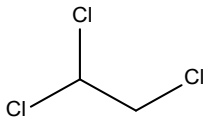
a) a compound in which carbon is covalently bonded to one or more of the following elements: Cl, Br, F, and/or I

and

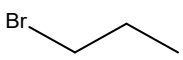
b) a saturated **acyclic** or **alicyclic** hydrocarbon with **i)** fully saturated with F, Cl, Br, and/or I, or **ii)** a **vicinal** halide of any combination of Cl, Br, and/or I, or **iii)** ≤2 F, Cl, Br, or CF_3 (**CF_3 = 1 halogen**) in any combination except the **vicinal** position, or **iv)** ≥3 F, Cl, Br, and/or CF_3 in any combination, (Note that the presence of I in iii) and iv) are not allowed and these compounds will be sorted into Class IV at the end of Q8), or

i: 

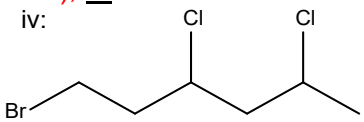
perchloroethane

ii: 

1,1,2-trichloroethane

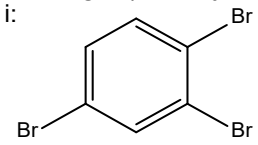
iii: 

1-bromopropane

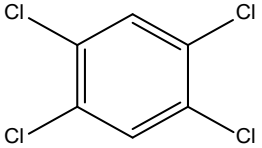
iv: 

1-bromo-3,5-dichlorohexane

c) benzene (i.e., a single benzene molecule, no other rings may be present) substituted only by any combination of **i)** ≤3 F, Cl, Br, and/or CF_3 (CF_3 is 1 halogen) in any arrangement without any additional substituents or **ii)** ≥ 4 F, Cl, Br, I, and/or CF_3 (CF_3 is 1 halogen) in any arrangement without any additional substituents, or

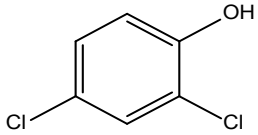
i: 

1,2,4-tribromobenzene

ii: 

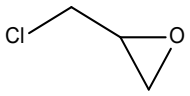
1,2,4,5-tetrachlorobenzene

d) benzene (i.e., a single benzene molecule, no other rings may be present) substituted by ≥1 Cl and/or Br in any combination, one of which must be ***ortho*** or ***para*** to an O substituent (with O directly bonded to the benzene ring), or



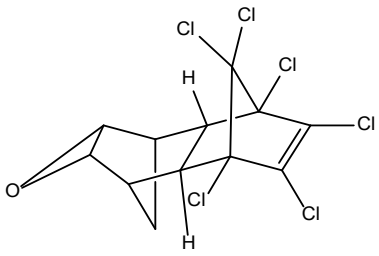
2,4-dichlorophenol

e) one or more Cl, Br, and/or I bonded to an epoxide ring or as the only substituent(s) of an epoxide carbon side chain of ≤2 Cs, or

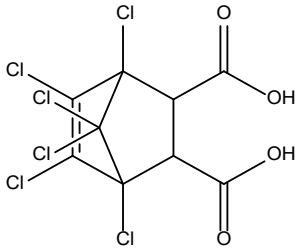


2-(chloromethyl)oxirane

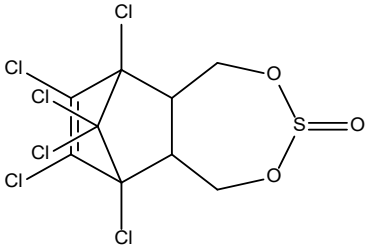
f) a mono- or poly-**alicyclic** ring system (**fused**, **spiro-fused**, or **bridged**) with ≥5 ring carbons and with ≥6 Cl, Br, and/or I with or without additional **oxygenated functional groups** and/or a maximum of one (nonaromatic) heterocyclic ring (only S and/or O as ring heteroatoms may be present), or



dieldrin



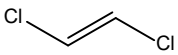
chlorendic acid



endosulfan

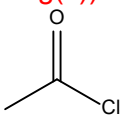
g) **i)** ≥1Cl and/or Br bonded directly to the double bonded carbon(s) of an alkene (the alkene is not cyclic), or
ii) an aliphatic acyl halide (F, Cl, and/or Br), or
iii) a halogen (F, Cl, Br, and/or I) on a carbon adjacent to a carbon bearing an aliphatic primary or secondary alcohol oxygen or corresponding ether or ester oxygen, or
iv) a halogen (F, Cl, Br, and/or I) on a carbon bearing an ether oxygen (the whole molecule must be aliphatic), or
v) at least one halogen (F, Cl, Br, and/or I) on the alpha carbon next to an aldehyde, ketone, carboxylic acid, ester or amide (note that the halogen and either the aldehyde, ketone, carboxylic acid, ester, or amide must be in the acyclic portion of the molecule if the compound also contains ring(s))?

i:



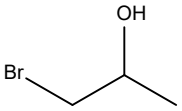
(*E*)-1,2-dichloroethene

ii:



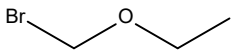
acetyl chloride

iii:



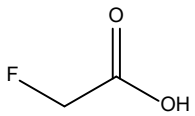
1-bromopropan-2-ol

iv:

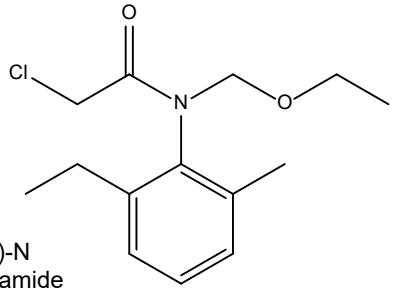


(bromomethoxy)ethane

v:



2-fluoroacetic acid



2-chloro-N-(ethoxymethyl)-N
(2-ethyl-6-methylphenyl)acetamide

Run the substance through all sub-questions (a through g). Do not stop at the first yes to a sub-question. This is done to ensure that the substance gets classified based on its

most reactive moiety (i.e., if the answer is yes at multiple sub-questions, assign the substance to a class at the sub-question with the highest class).

i) If yes to a) and b(ii)), c(ii)), e), f), or g(iv)), assign to Class V.
ii) If yes to a) and b(i)), d), or g(i, ii, iii, or v)), assign to Class IV.
iii) If yes to a) and b(iv)) or c(i)), assign to Class III.
iv) If yes to a) and b(iii)), assign to Class II.
v) If yes to a), but no to b), c), d), e), f), and g), proceed to Q8.
vi) If no to a), proceed to Q9.

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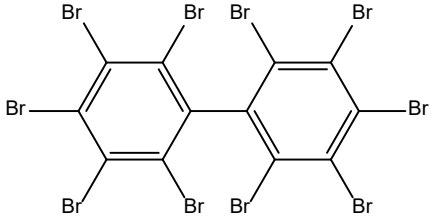
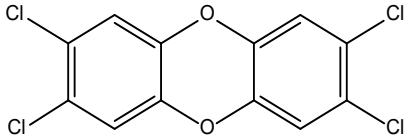
8. Is the halogenated substance

a) a dibenzodioxin, dibenzofuran, biphenyl, diphenyl ether, diphenylthio ether, or naphthalene **skeleton** fully substituted with only Cl and/or Br, or



perbromo-1,1'-biphenyl

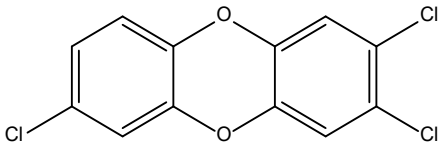
b) a dibenzodioxin, dibenzofuran, or naphthalene substituted with only Cl and/or Br in all positions that are ***para*** to the ring fusion points, and no more than 2 Cl and/or Br ***ortho*** to ring fusion points, or



2,3,7,8-tetrachlorodibenzo[*b*,*e*][1,4]dioxin

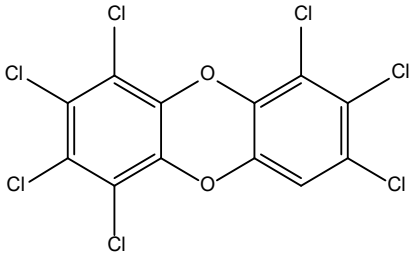
c) a dibenzodioxin, dibenzofuran, or naphthalene substituted with only Cl and/or Br **i)** at three of the four ***para*** positions or **ii)** at all (4) ***para*** positions and three ***ortho*** positions, or

i:



2,3,7-trichlorodibenzo[b,e][1,4]dioxin

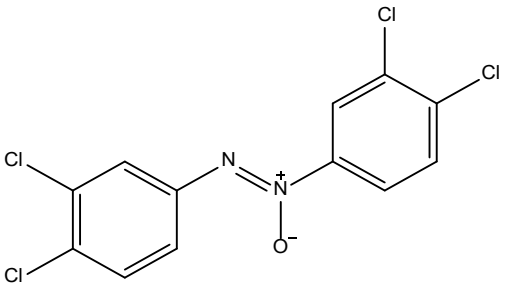
ii:



1,2,3,4,6,7,8-heptachlorodibenzo[*b*,*e*][1,4]dioxin

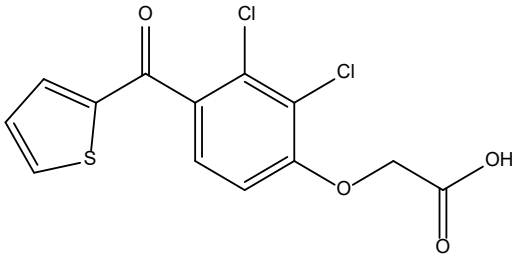
d) biphenyl, diphenylether, diphenylthioether, or azobenzene (Ar-N=N-Ar) and its N-oxide (Ar-N=N^{+}(O^{-})-Ar) only substituted with 3, 4, 5, or 6 Cl located only at ***meta*** or ***para*** positions or 3, 4, 5, 6, 7, or 8 Br atoms at any position or a biphenyl substituted with 4, 5, 6, or 7 Cl with at least one Cl located at the ortho, meta, and para positions each (does

not have to be on the same ring), and each ring must be substituted by at least one Cl (i.e., no unsubstituted ring)?



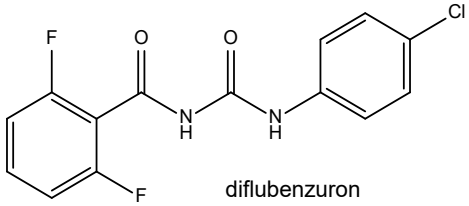
(*Z*)-1,2-bis(3,4-dichlorophenyl)diazene 1-oxide

i) If yes to a), assign to Class III.
ii) If yes to b), assign to Class VI.
iii) If yes to c) or d), assign to Class V.
iv) If no to a), b), c), and d), and the compound has at least one **heterocyclic** (for clarity: this includes both aromatic and non-aromatic **heterocyclic**) ring, proceed to Q11.
Example:



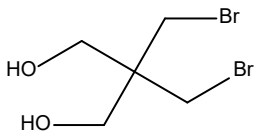
2-[2,3-dichloro-4-(thiophene-2-carbonyl)phenoxy]acetic acid

v) If no to a), b), c), and d), and the compound is **aromatic** (no heterocyclic ring should be present, those are addressed by the previous directions (i.e., if both an aromatic and a heterocyclic ring are present, the presence of the heterocyclic ring takes priority)), proceed to Q33.
Example:



diflubenzuron

vi) If no to a), b), c), and d), and the compound is neither **heterocyclic** nor **aromatic**, assign to Class IV.
Example:



dibromoneopentyl glycol

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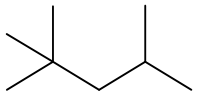
9. Is the substance a **linear** or **simply branched-chain aliphatic acyclic** hydrocarbon, except hexane and substances with a terminal double bond that is further **conjugated** with another double bond (i.e., terminal dienes)?

i) If yes, assign to Class I.



2,2,4-trimethylpentane

ii) If no, proceed to Q10.

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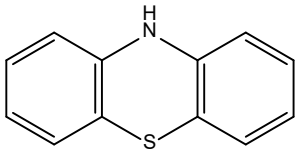
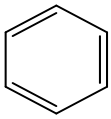
10. Is the substance **heterocyclic**?

i) If yes, proceed to Q11.



phenothiazine

ii) If no, proceed to Q23.

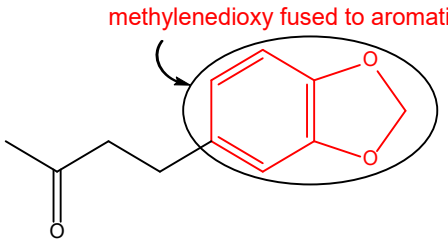


benzene

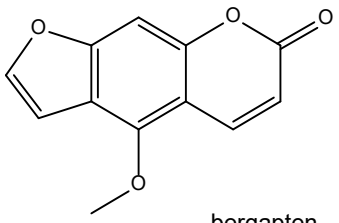
---

11. Does the substance contain one or more of the following: ester (but not cyclic diester and lactone; these **functional groups** together with lactams are dealt with in Q12), thioester, hemiacetal, acetal (other than cyclic methylenedioxy fused to an aromatic ring), hemiketal, ketal, sulfate, non-halogenated mono- or poly-glycoside (i.e., glycone), carbonate (including dicarbonate), anhydride, and/or polysulfide (including disulfide)?

i) If no to Q11 and the compound is a cyclic methylenedioxy **fused** to an **aromatic** ring, proceed to Q33. For all other compounds, if no to Q11, proceed to Q12.



piperonyl acetone
Proceed to Q33



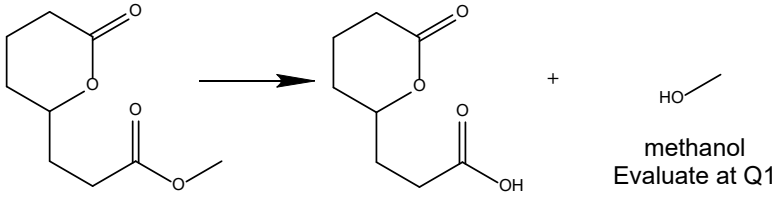
bergapten
Proceed to Q12

ii) If yes to Q11 and the compound is a cyclic methylenedioxy fused to an aromatic ring, hydrolyze or reduce the functional groups listed in the question (but not the methylenedioxy fused to the aromatic ring). Send the cyclic methylenedioxy fused to the aromatic ring to Q33 and all other hydrolysis products to Q1. Assign the compound to the highest class any of its hydrolysis and/or reduction products may have.

iii) If yes to Q11 and the compound is an epoxidized triglyceride, send the compound to Q14 without performing any hydrolysis (i.e., do not hydrolyze the triglyceride).

iv) If yes to Q11, and the compound is a lactone, lactam, or cyclic diester, hydrolyze or reduce the functional groups listed in the question, but do not hydrolyze the lactone, lactam, and cyclic diester moieties. After hydrolysis and/or reduction, send the lactone, lactam, and cyclic diester to Q12 and all other hydrolysis products to Q1. Assign the compound to the highest class any of its hydrolysis and/or reduction products may have.

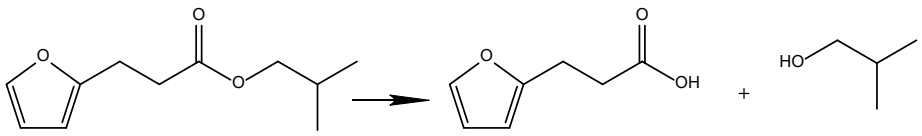
v) If yes to Q11 and the compound is not a lactone, lactam, or cyclic diester, assume the **heterocyclic** substance is **hydrolyzed** and/or **reduced** (exclusively for di- and poly-sulfide linkages), and evaluate any heterocyclic products at Q13 and all other product(s) at Q1. Assign the compound to the highest class any of its hydrolysis and/or reduction products may have.



methyl 3-(6-oxotetrahydro-2*H*-pyran-2-yl)propanoate

3-(6-oxotetrahydro-2*H*-pyran-2-yl)propanoic acid
Evaluate lactone at Q12

methanol
Evaluate at Q1



isobutyl furylpropionate

3-(furan-2-yl)propanoic acid
Evaluate at Q13

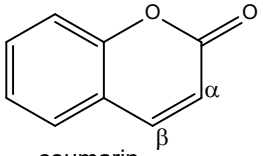
2-methylpropan-1-ol
Evaluate at Q1

---

12. Is the **heterocyclic** substance

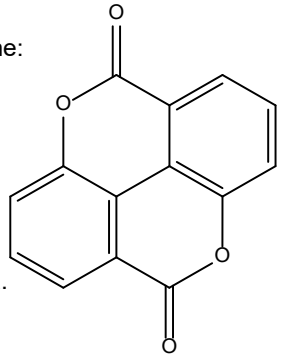
a) an **α,β**-unsaturated lactone **fused** to an **alicyclic**, **aromatic,** or **heteroaromatic** ring such that the lactone ring can attain a completed cyclic array of 4n+2 π electrons assuming **enolization** of the lactone carbonyl (aka **pseudoaromaticity**) (exception:

compounds with an ellagic acid **skeletal structure**. If ellagic acid **skeleton** is present, proceed to Q12e)), <u>or</u>



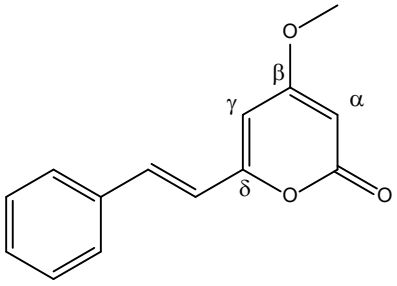
coumarin

Exception: ellagic acid backbone:

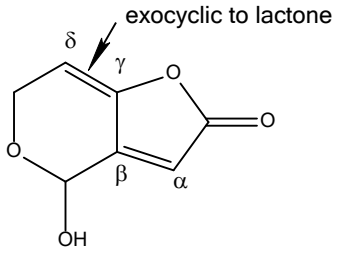


Note: regardless of the substitution pattern on the ellagic acid backbone, if the lactones are present as a part of the ellagic acid backbone, respond no at Q12a. These compounds are evaluated at Q12e (see example at Q12e).

b) an **α,β**- and **γ,δ-conjugated** δ-lactone <u>or</u> an **α,β**-unsaturated-γ-lactone containing an **exocyclic** (to the lactone) alkene at the **γ**-position (the γ-lactone cannot be fused to a benzene ring), <u>or</u>

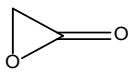


5,6-dehydrokawain
(δ–lactone)

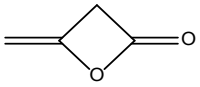


4-hydroxy-4,6-dihydrofuro[3,2-c]pyran-2-one
(γ-lactone)

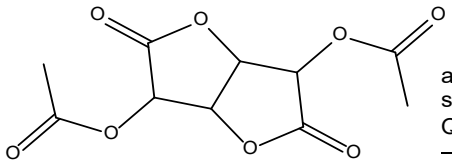
c) an α- or β-lactone or substance containing two or more lactone rings, <u>or</u>



oxiran-2-one
(α-lactone)

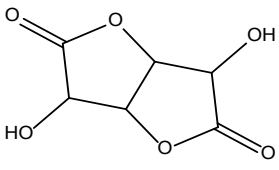


diketene
(β-lactone)



glucaron

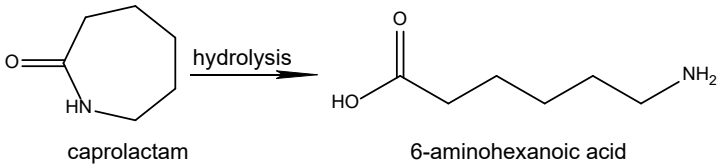
after hydrolysis of side chain esters at Q11, evaluated as



3,6-dihydroxytetrahydrofuro[3,2-*b*]furan-2,5-dione

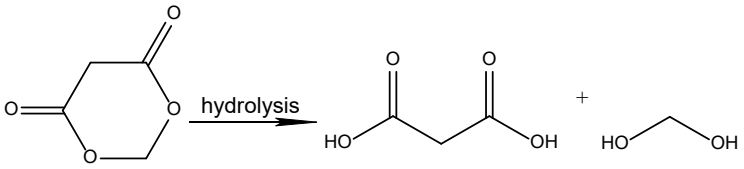
d) a cyclic diester or lactone that **hydrolyzes** to a **linear aliphatic** or simply **branched-chain** hydroxycarboxylic acid, dicarboxylic acid, and/or diol or a secondary lactam (γ,δ,

ε, …) that **hydrolyzes** to a **linear or simply branched aliphatic** aminocarboxylic acid not bonded to any other ring system, or

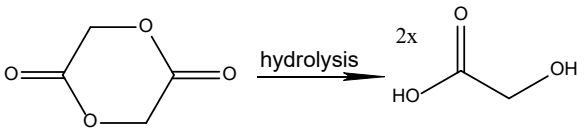


caprolactam 6-aminohexanoic acid

cyclic diesters

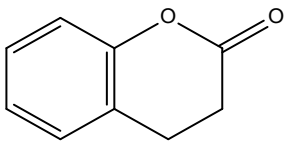


1,3-dioxane-4,6-dione malonic acid methanediol

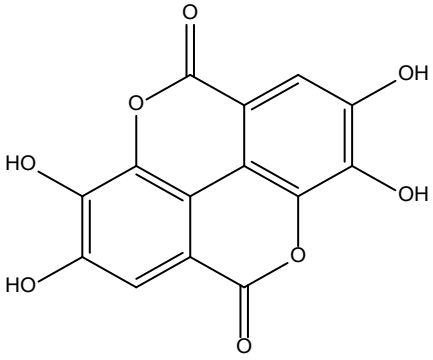


1,4-dioxane-2,5-dione 2-hydroxyacetic acid

e) a lactone (γ, δ, ε, …) **fused**, **singly bonded**, or connected by a carbon chain of 4≤ Cs to an **alicyclic**, **aromatic**, and/or **heterocyclic** ring(s) without containing a continuous cyclic array of 4n+2 π electrons within the lactone (no other rings may be present)?

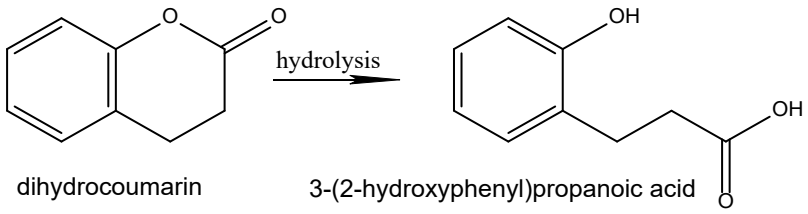


dihydrocoumarin



ellagic acid

i) If yes to a), b), or c), assign to Class IV.
ii) If yes to d), assume **hydrolysis** and proceed to Q1 to evaluate all hydrolysis products. See examples for lactone, lactam, and cyclic diester hydrolysis provided after sub-question d).
iii) If yes to e), consider that the lactone is **hydrolyzed** to an **alicyclic**-, **aromatic**-, or **heterocyclic**-ring substituted hydroxycarboxylic acid derivative. Proceed to Q30, Q33, or Q10 to evaluate the **alicyclic**, **aromatic**, or **heterocyclic hydrolysis** products, respectively. Note: if the compound contains a mixed ring system (such as a combination of alicyclic and heterocyclic rings), go to Q10.
Example:



dihydrocoumarin 3-(2-hydroxyphenyl)propanoic acid

iv) If no to a), b), c), d), and e), proceed to Q13.

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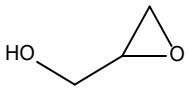
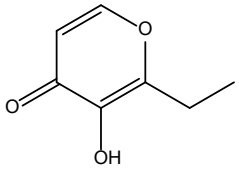
13. Does the substance contain one or more three-membered **heterocyclic** rings containing either a single N, O, or S?

i) If yes, proceed to Q14.



glycidol

ii) If no, proceed to Q15.



ethyl maltol

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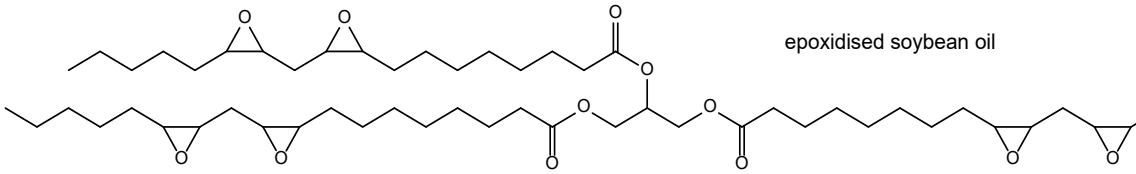
14. Is the substance

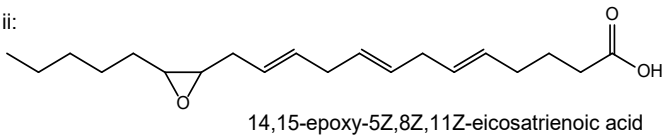
a) i) a mono- or poly-epoxidized triglyceride or ii) an epoxy fatty acid or fatty acid ester other than those in i), or

i:

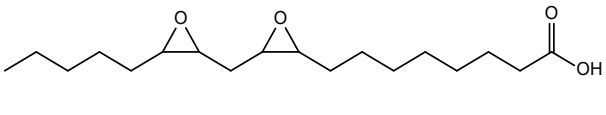


epoxidised soybean oil

ii:



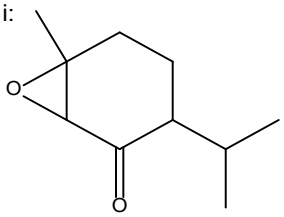
14,15-epoxy-5Z,8Z,11Z-eicosatrienoic acid



9,10-12,13-diepoxyoctadecanoate

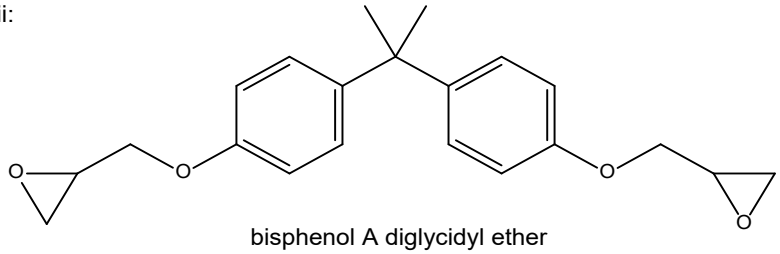
b) i) a monoepoxide containing a total number of ≥6 Cs or ii) polyepoxides (≥2 epoxide rings; other than those addressed in 14a))?

i:



piperitone oxide

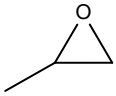
ii:



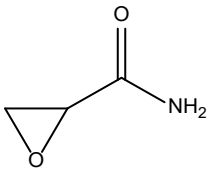
bisphenol A diglycidyl ether

i) If yes to 14a(i)), assign to Class II.
ii) If yes to 14a(ii)), assign to Class III.
iii) If yes to 14b(i)), and the epoxide is substituted by or **fused** to a poly**aromatic** ring system, proceed to Q33. In all other cases, if yes to b(i)), assign to Class III.
iv) If yes to 14b(ii)), and the epoxides are substituted by or fused to a polyaromatic ring system, proceed to Q33. In all other cases, if yes to 14b(ii)), assign to Class V.
v) If no to a) and b), assign to Class IV.
Examples for no reply:

Class IV:

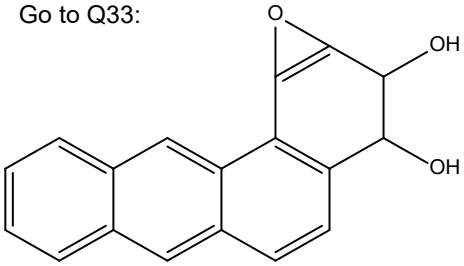


propylene oxide



glycidamide

Go to Q33:



2,3-dihydrotetrapheno[1,2-*b*]oxirene-2,3-diol

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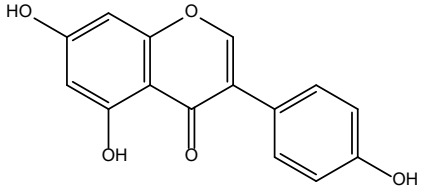
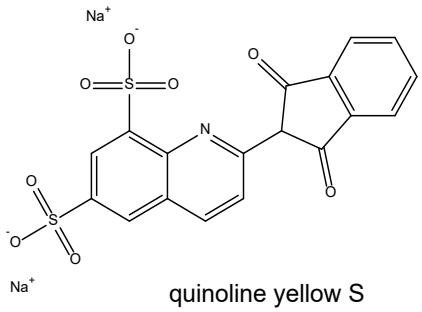
15. Is the **heterocycle** a six-membered ring containing only a single ring O with or without a ketone or alcohol ring substituent at the 4 position (no other substitutions are allowed at this position) and the **heterocyclic** ring is [2.3]-**fused** to one benzene ring and connected at the 5 or 6 position by a single bond (i.e., **singly bonded**) to a second benzene ring (i.e., commonly recognized as the flavonoid carbon **skeleton**)? The benzene rings should be substituted by more than 2 phenolic hydroxy and/or methoxy substituents with each benzene ring having at least one phenolic hydroxy or methoxy substituent.

i) If yes, proceed to Q28.



genistein

ii) If no, proceed to Q16.



quinoline yellow S

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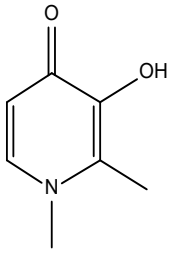
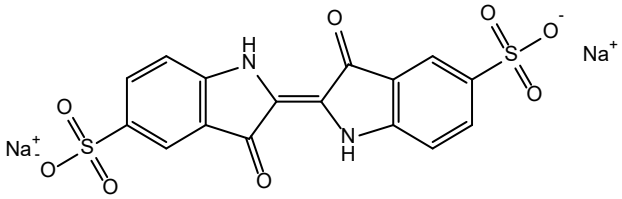
16. Does the **heterocyclic** ring contain an **α**-ketoenol moiety (C=C(OH)C=O) in which the enolic double bond is further **conjugated** with a heteroatom (O or N) possessing a non-bonding electron pair or another double bond?

i) If yes, assign to Class III.



deferiprone

ii) If no, proceed to Q17.



FD & C Blue No. 2

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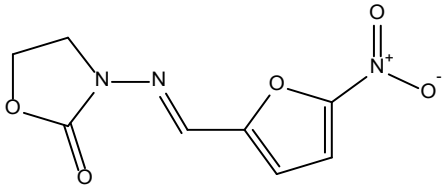
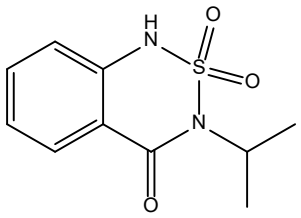
17. Does the substance contain one or more **heteroaromatic** rings or may have one or more **tautomer**s that is/are heteroaromatic?

i) If yes, proceed to Q19.



furazolidone

ii) If no, proceed to Q18.



bentazon

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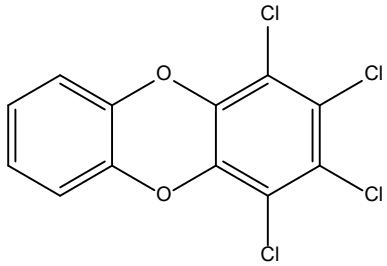
18. Does the **heterocyclic** ring(s) contain

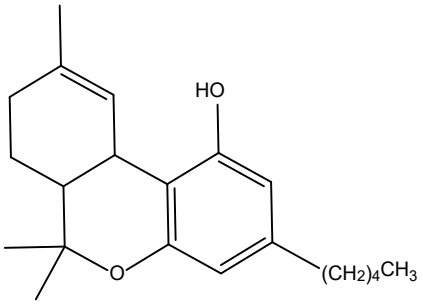
a) **i)** a dibenzo-p-dioxin **skeletal structure** or **ii)** at least three rings that are fused, bridged, spiro-fused, and/or singly bonded (other than dibenzo-p-dioxins as they are captured at 18a(i)) (additional rings connected in any way may be present, but at least 3 rings need to be fused, bridged, spiro-fused, and/or singly bonded), or

i:



1,2,3,4-tetrachlorodibenzo-p-dioxin

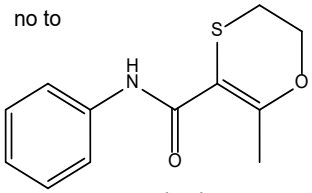
ii:



delta-9-tetrahydrocannabinol

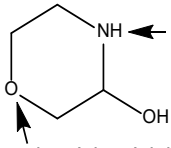
b) substituents (note: the heteroatoms contained within the ring (i.e., ring atoms) are not considered substituents*) *other than* **linear** or **simply branched aliphatic** chains of ≤6 Cs (each), **alicyclic** ring (≤6 Cs), **bridged** chain (≤6 Cs) (i.e., a chain on a ring producing a bridged ring), only one **aromatic** ring (**fused**, **singly bonded**, or connected by an aliphatic carbon chain of ≤4 Cs or connected by an -O-), with or without primary alcohols, secondary alcohols, aldehydes, ketones, carboxylic acids, lactone or lactam (if the amide is cyclic, more than one cyclic amide may be present; moreover, the presence of cyclic urea and ureide are also allowed), peroxide, hydroperoxide, peroxyester, peracid, diacylperoxide, primary amines (cannot be bonded to a ring nitrogen), thiols, thioesters, polysulfides, sulfides, sulfoxides, single sulfonate, sulfonamide, or sulfone as a substituent or part of the ring or a single ring sulfamate, methoxy, ethoxy, or polyoxyethylene ($-OCH_2-CH_2-)_x$ with x is 2, 3, or 4, (*while ring heteroatoms 'alone/by themselves' are not considered substituents, if they are a part of a functional group, the functional group must be considered in its entirety (see dazomet below)), or

no to



carboxin

also no to

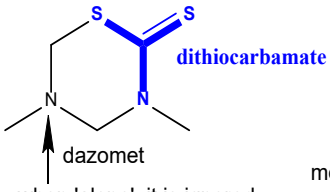


when 'alone', it is ignored
it is not considered
a cyclic secondary **amine**

morpholin-3-ol

when 'alone', it is ignored
it is not considered to be a cyclic ether

yes to

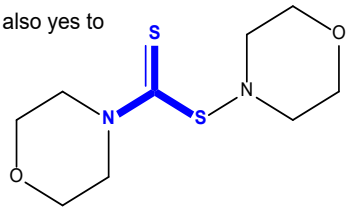


dithiocarbamate

dazomet

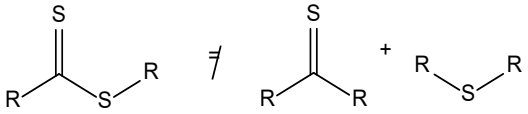
when 'alone', it is ignored
it is not considered to be
a cyclic tertiary amine

also yes to



morpholino morpholine-4-carbodithioate

Reminder:



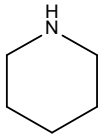
dithiocarboxylic acid ester/
dithioate/carbothioate

thione

sulfide

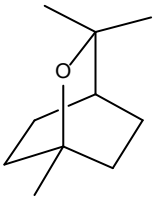
dithiocarboxylic acid ester is a distinct functional group and should not be treated as the combination of thione & sulfide functional groups

c) no substituents on the ring (i.e., it is an unsubstituted ring)?

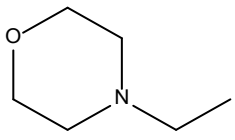


piperidine

i) If yes to a(i)), assign to class V.
ii) If yes to a(ii)) and/or b) or for ≥2 sulfur moieties in b), proceed to Q28. Assign the substance to a class it would get at Q28a) through Q28s). If no at Q28, go to Q47 for final class assignment (that is, if Q28N, do not assign the substance to Class II or III at Q28).
iii) If yes to c), assign to Class III.
iv) If no to a), b), and c), proceed to Q28.
Examples for no:



eucalyptol



ethylmorpholine

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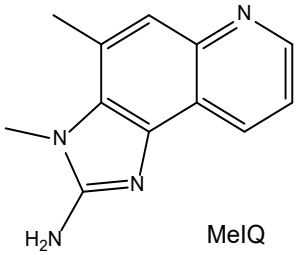
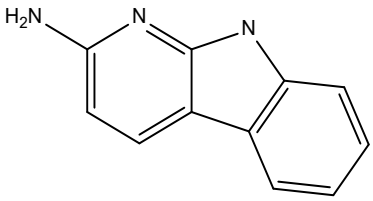
19. Does the **heteroaromatic** substance contain

a) ≥3 **fused** and/or **singly bonded aromatic** or **heteroaromatic** rings in which one of the rings is a 2-aminoimidazolyl or 2-aminopyridyl ring, or

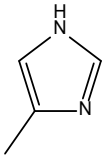


MeIQ



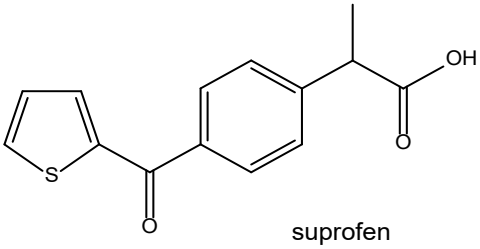
Aα C

b) a 5- or 4- methyl- or ethyl- imidazole ring (with no other substitutions in the 4 or 5 position), or



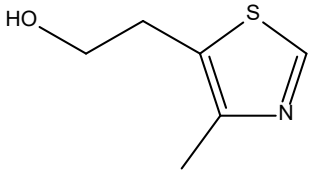
4-methylimidazole

c) a thiophene ring, or



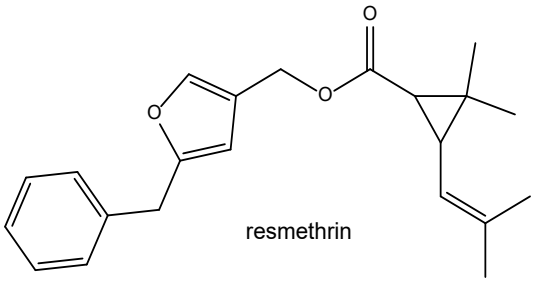
suprofen

d) a thiazole ring substituted at the 2, 4 and/or 5-position(s) by alkyl or **aryl** substituents (the **aryl** ring cannot be **fused** to the thiazole ring) with or without **oxygenated functional group**s (the ester should be an alkyl ester)?

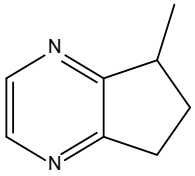


4-methyl-5-thiazoleethanol

i) If yes to a), assign to Class V.
i) If yes b) or c), assign to Class IV.
ii) If yes to d), assign to Class III.
iii) If no to a), b), c), and d), proceed to Q20.
Examples for no:



resmethrin



5H-5-methyl-6,7-dihydrocyclopentapyrazine

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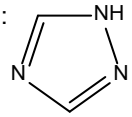
20. Does the **heteroaromatic** compound contain

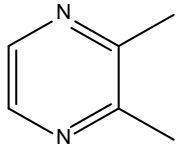
a) only one **heteroaromatic** ring that is **i)** unsubstituted, **ii)** substituted, but not by a ring(s), or **iii)** substituted by one or more cyclopropylamine ring, or

i:



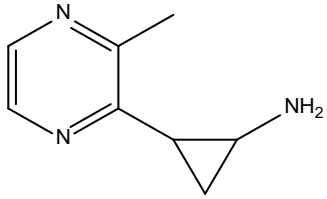
1,2,4-triazole

ii:



2,3-dimethylpyrazine

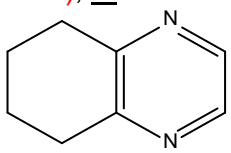
iii:



2-(3-methylpyrazin-2-yl)cyclopropan-1-amine

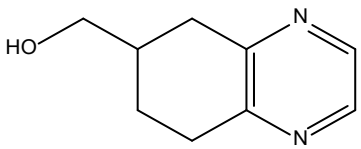
b) only one **heteroaromatic** ring **fused** or **singly bonded** to an **alicyclic** ring and the rings are **i)** unsubstituted or **ii)** substituted (note: substitution by additional rings is allowed, but these additional rings should not be fused, spiro fused, bridged, singly bonded, or connected by an -C(=O)-, -O-, -N-, or -S- to the two rings specified in this sub-sub-question), or

i:



5,6,7,8-tetrahydroquinoxaline

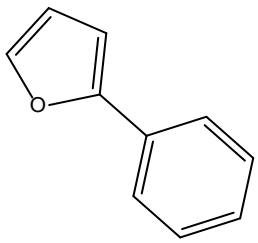
ii:



(5,6,7,8-tetrahydroquinoxalin-6-yl)methanol

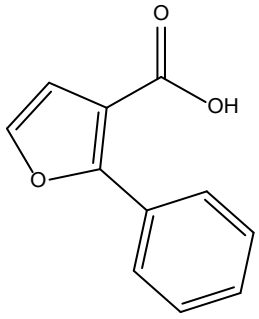
c) only one **heteroaromatic** ring **singly bonded** to an **aryl** ring and the rings are i) unsubstituted or ii) substituted (note: substitution by additional rings is allowed, but these additional rings should not be fused, spiro fused, bridged, singly bonded, or connected by an -C(=O)-, -O-, -N-, or -S- to the two rings specified in this sub-sub-question), or

i:



2-phenylfuran

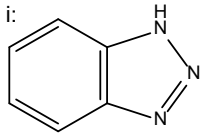
ii:



2-phenylfuran-3-carboxylic acid

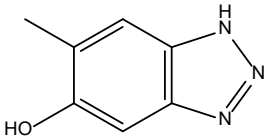
d) only one **heteroaromatic** ring **fused** to an **aromatic** ring and the rings are **i)** unsubstituted or **ii)** substituted (note: substitution by additional rings is allowed, but these additional rings should not be fused, spiro fused, bridged, singly bonded, or connected by an -C(=O), -O-, -N-, or -S- to the two rings specified in this sub-sub-question), or

i:



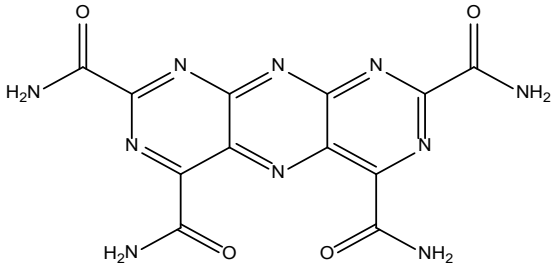
1,2,3-benzotriazole

ii:



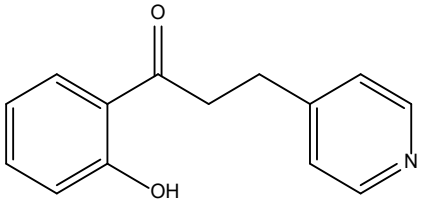
6-methyl-1*H*-benzo[*d*][1,2,3]triazol-5-ol

e) substituted or unsubstituted ring system composed of any combination of at least three **aromatic** and **heteroaromatic** rings or at least 3 **heteroaromatic** rings if no **aromatic** rings are present, (note that these three rings may only be fused, spirofused, bridged, singly bonded, or connected by an -C=C-, -O-, -C(=O)-, -N- (but not an amide **connector**), or -S-)), or



pyrimido[5,4-*g*]pteridine-2,4,6,8-tetracarboxamide

f) **heteroaromatic** ring or ring systems other than in a), b), c), d), and e)?



1-(2-hydroxyphenyl)-3-(4-pyridyl)propan-1-one

i) If yes to a(iii)), assign to Class V.
ii) If yes to a(i)) or d(i)), assign to Class IV.
iii) If yes to e) or f), and one or more sulfonate, sulfamate, or carboxylate substituents are present or the compound has any of the skeletons at Q47g), proceed to Q47. If yes to e) or f) and one or more aniline (also diaminobenzene), nitroaniline, and/or (di)nitrobenzene moiety is present, before proceeding to Q47, crosscheck against Q43c). Assign the substance to the highest class it would get at either Q47 or Q43c). If yes to e) or f) and no sulfonate, sulfamate, carboxylate, aniline, and/or nitrobenzene is present and the compound does not contain any of the skeletons at Q47g), assign the substance to Class IV.
iv) If yes to b(i)) or c(i)), assign to Class III.
v) If yes to a(ii)), b(ii)), c(ii)), or d(ii)), proceed to Q21.
Note: If no to a) through e), f) must be yes.

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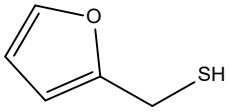
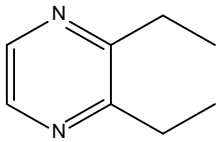
21. Does the **heteroaromatic** substance contain any of the **reactive moieties** listed in Q28 c), e), g), m), n), q), or r)?

i) If yes, proceed to Q28 and assign to Class III, IV, or V as appropriate.

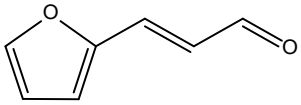


furfuryl mercaptan

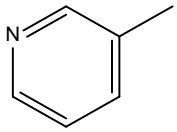


2,3-diethylpyrazine

ii) If no, proceed to Q22.



furylacrolein



beta-picoline

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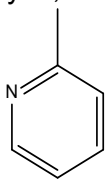
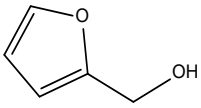
22. Is/Are the ring(s) substituted only by one or more **aliphatic** chains with or without one or more ring hydroxy, carboxylic acid, methoxy, ethoxy. The aliphatic side chain(s) may be substituted by or contain one or more primary alcohol, secondary alcohol, aldehyde, ketone, carboxylic acid, ether, primary amide, methoxy or ethoxy, monosulfide, or sulfoxide?

i) If yes, assign to Class III.

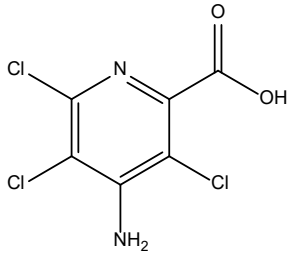


2-methylpyridine



furfuryl alcohol

ii) If no to Q22 and the substance does not contain an aniline, diaminobenzene, nitroaniline, and/or (di)nitrobenzene moiety, proceed to Q47. If no to Q22 but the substance contains at least one aniline (also diaminobenzene), nitroaniline, and/or (di)nitrobenzene moiety, before sending the substance to Q47, crosscheck against Q43c). Assign the substance to the highest class it would get at either Q47 or Q43c).



picloram

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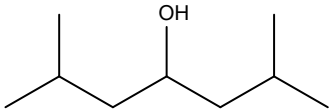
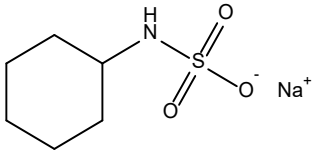
23. Is the structure **acyclic**?

i) If yes, proceed to Q24.



2,6-dimethylheptan-4-ol

ii) If no, proceed to Q29.



sodium cyclamate

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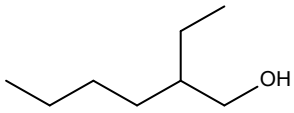
24. Is the **acyclic** substance

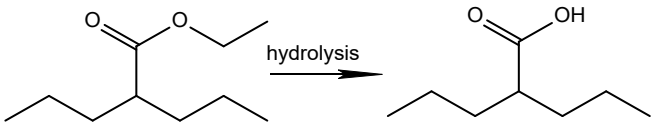
a) **i)** a primary alcohol, the primary alcohol's **corresponding** aldehyde or carboxylic acid, with no other **functional group**s, and a main chain (containing the alcohol, aldehyde, or carboxylic acid) length of 5-8 Cs containing only one 2-alkyl substituent (2-4 Cs) or **ii)** an ester, acetal, hemi-acetal, carbonate, peroxide, peroxyester, peroxyacid, diacylperoxide, hydroperoxide, peroxycarbonate, peroxydicarbonate, or any other peroxides for which at least one of the **hydrolysis** or **reduction** products satisfies the structural requirements in i), or

i:



2-ethyl-1-hexanol

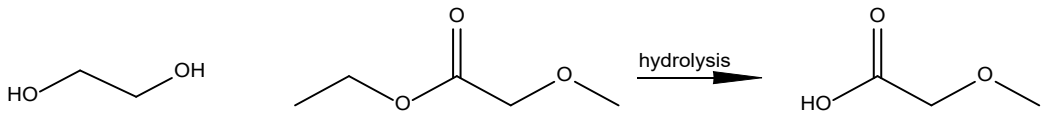
ii:



ethyl 2-propylpentanoate

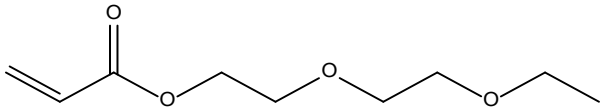
2-propyl-pentanoic acid
(hydrolysis product satisfies requirements in i)

b) an **α**-hydroxy- or **α**-alkoxy-ethanoic acid (no alkyl substituents), its corresponding alcohol or aldehyde, or an ester, acetal, or hemi-acetal that **hydrolyses** to an α-hydroxy- or α-alkoxy-ethanoic acid, its **corresponding** alcohol or aldehyde where the alkoxy substituent adjacent to the above **oxygenated functional groups** has ≤4 Cs (note that no repeating units of polyoxyethylene [-CH_2CH_2O-] should be present), or



ethylene glycol ethyl 2-methoxyacetate 2-methoxyacetic acid

say no to:



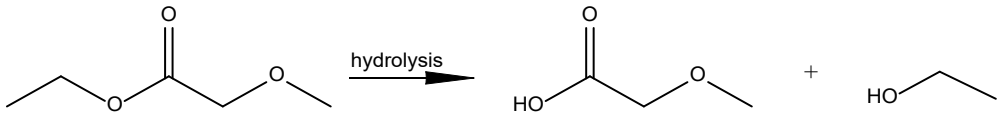
2-(2-ethoxyethoxy)ethyl acrylate

c) an ester*, hemiacetal*, acetal*, or carbonate (including dicarbonate) (*other than those listed in 24a) and 24b))? (Do not hydrolyze peroxyesters, peroxycarbonate, peroxydicarbonates, or any other peroxides at this sub-question).

i) If yes to a) or b) and the substance has no hydrolyzable functional group, assign the substance to Class III.

ii) If yes to a) or b) and the substance has one or more hydrolyzable functional group(s), hydrolyze the substance and run all fragments through the EDT starting at Q1. Assign the substance to the highest class any of its hydrolysis products may have.

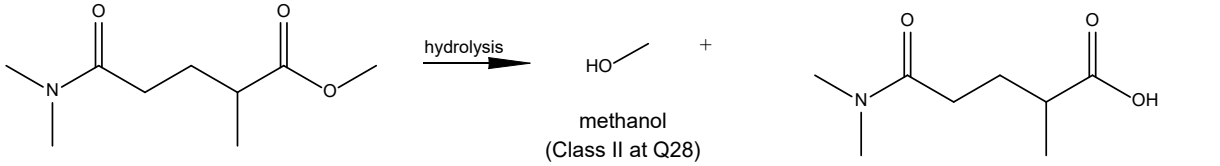
Example:



ethyl 2-methoxyacetate (overall Class III) | 2-methoxyacetic acid (Class III at Q24b) | ethanol (Class I at Q1a)

iii) If yes to c), hydrolyze the listed functional groups. Any fragments that may result in a yes at Q1 can be disregarded. Evaluate all other fragments starting at Q25. If all fragments would be classified as Class I at Q1, assign the substance to Class I.

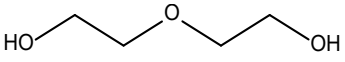
Example:



methyl 5-(dimethylamino)-2-methyl-5-oxopentanoate (overall Class IV) | methanol (Class II at Q28) | 5-(dimethylamino)-2-methyl-5-oxopentanoic acid (Class IV at Q47)

iv) If no to a), b), and c), proceed to Q25.

Example for no:



diethylene glycol

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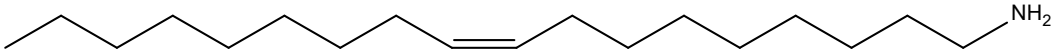
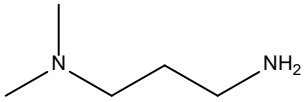
25. Is the substance a a) primary and/or tertiary **aliphatic** amine (if tertiary, only one tertiary amine may be present) or b) primary, secondary, and tertiary amide and both a) and b) of a chain length ≥12 Cs or a combination of carbons, oxygens, and nitrogens (the chain length requirement applies to the longest continuous chain that contains the amine or amide, the side-chain atoms (branching) should not be counted toward the 12 Cs/atoms) with or without **oxygenated functional groups** but no other **functional groups** (note that the presence of secondary amine is not allowed)?

i) If yes to a) or b), assign to Class III.



oleylamine

ii) If no to a) and b), and more than one tertiary amine substituents are present, go to Q47. In all other cases, if no to Q25, proceed to Q26.



N',N'-dimethylpropane-1,3-diamine

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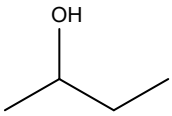
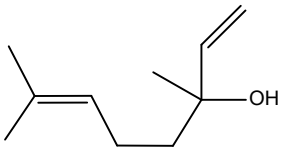
26. Is the structure a **linear** or **simply branched aliphatic** substance (methylene branching is allowed as well) or a linear or simply branched alkyne, either unsubstituted or containing any one or a combination of only the following **functional groups**:

a) any combination of six or fewer of primary alcohols, secondary alcohols, aldehydes, carboxylic acids, sulfate esters, or alkynes. In addition to or instead of the above functional groups, four or fewer ethers may also be present and/or a total of two or fewer peroxide, hydroperoxide, peroxycarbonate, peroxydicarbonate, peroxyester, peracid, and/or diacylperoxide, and/or



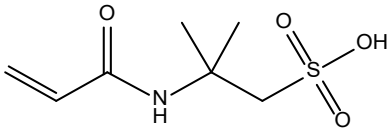
2-butanol

b) one each of one or more of the following: hemiketal, ketal, tertiary amine, sulfoxide, thiol, dithiol, monosulfide, polysulfide (includes disulfide), thioester, tertiary alcohol, primary or secondary amide, polyoxyethylene $(-OCH_2-CH_2-)_x$ or polyoxypropylene $(-OCH_2-CH_2-CH_2-)_x$ with x>1 but ≤4, a trimethyl ammonium moiety $((CH3)_3N^+-)$, N^+ in betaine and choline derivatives only, a secondary amine but only when monosulfide, polysulfide, sulfoxide, sulfone or primary alcohol, aldehyde, or carboxylic acid **functional group** is also present, and/or a maximum of two primary amines and up to two ketones, and/or



linalool

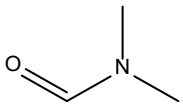
c) one sulfone, sulfonate, sulfate, sulfonamide, sulfamate, sulfinate, or thionosulfate group (note that some of these may have been 'neutralized' at Q4)?



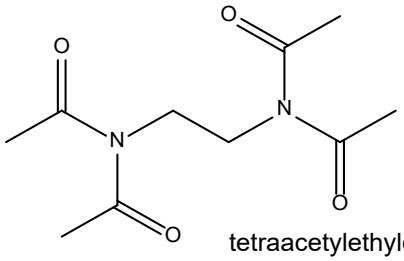
2-acrylamido-2-methyl-1-propane sulfonic acid

i) If the substance is a linear or simply branched aliphatic substance (methylene branching is allowed as well) or linear or simply branched alkyne that is unsubstituted or if yes to a), b), and/or c), proceed to Q27.

ii) If the compound contains one or more functional group(s) (including halogens or Si) not mentioned in a), b), and/or c) (i.e., no to a), b), and c)), proceed to Q47. Before assigning the substance to a class at Q47, crosscheck against Q28. Assign the substance to the highest class it would receive at either Q47 or Q28.
Examples for no:



dimethylformamide



tetraacetylethylenediamine

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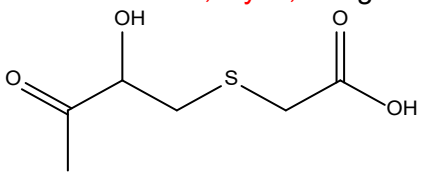
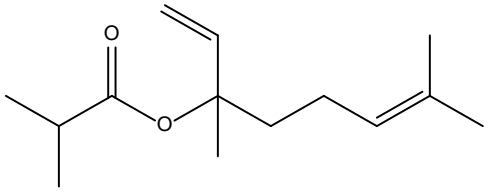
27. Does the structure contain more than three different **functional groups**? The following metabolically **related functional groups** count as one: i) ester, orthoester, and carboxylic acid; ii) hemiketal, ketal, and ketone; iii) hemiacetal, acetal, and aldehyde; iv) primary alcohol and methoxy; and v) thioester and thiol.

i) If yes, and the substance meets the structural requirements in 28c(i)), assign to Class V. In all other cases, if yes, assign to Class IV.



2-((2-hydroxy-3-oxobutyl)thio)acetic acid
Classified at Q27

ii) If no, proceed to Q28.



linalyl isobutyrate

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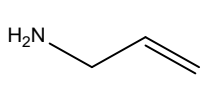
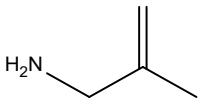
28. Does the substance contain any one or more of the following moieties or is the substance an

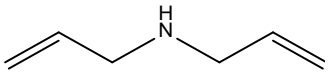
a) allyl amine, β-methylallylamine, or their corresponding secondary amide or the corresponding tertiary amide of diallylamine, and di(β-methyl-allyl)amine, or



allyl amine

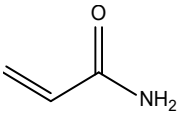


beta-methylallylamine



diallylamine

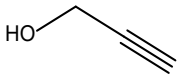
b) acrylamide or N-alkyl or **aryl**-substituted acrylamide without any other **functional groups**, or



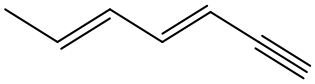
acrylamide

c) alkyne **i) conjugated** with one or more alkyne, alkene, carbonyl group/s or adjacent to the corresponding alcohols (e.g., 2-butyn-1-ol) or a terminal alkyne regardless of conjugation, or **ii)** unconjugated and not a terminal alkyne, or

i:

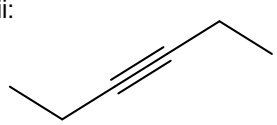


propargyl alcohol



(3*E*,5*E*)-hepta-3,5-dien-1-yne

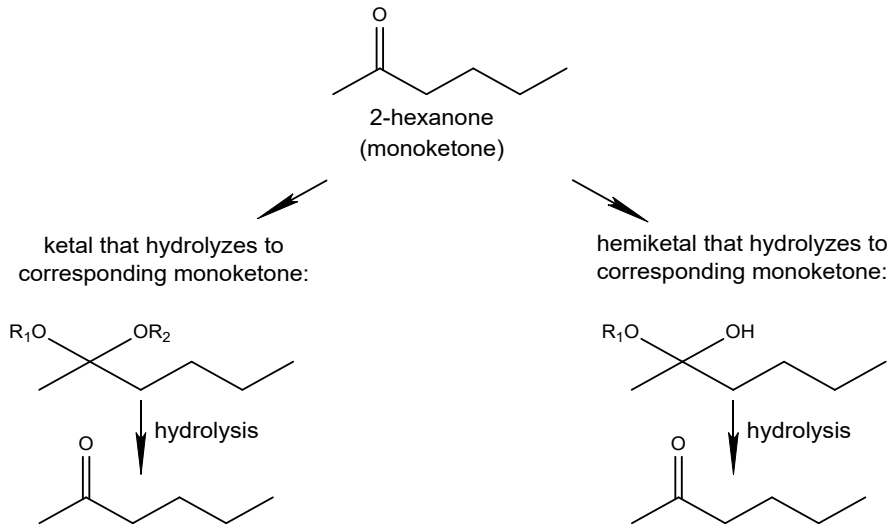
ii:



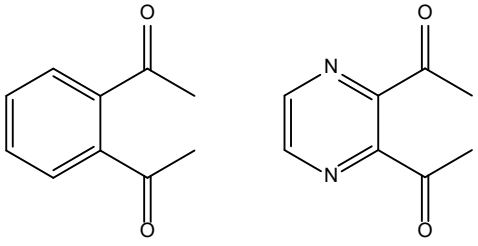
hex-3-yne

d) **i)** hexane; or 2-hexanone, 3-heptanone, or 5-nonanone or their corresponding hemiketals or ketals with or without methyl or methoxy substituents in position(s) 3 and/or 4 for 2-hexanone and 5-nonanone and position(s)) 4 and/or 5 for 3-heptanone (no other substituents in these positions are allowed), or **ii)** 2,5-hexanedione, 2,5-heptanedione, or 2,5-nonanedione with or without methyl and/or methoxy substituents between the ketone functions (normally position(s) 3 and/or 4), or their corresponding hemiketals or ketals, or

Example:

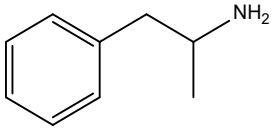


e) **aromatic** or **heteroaromatic** substance with *o*-diacetyl substituents (e.g., 1,2-diacetylbenzene (aromatic) and 2,3-diacetylpyrazine (heteroaromatic)), its corresponding alcohols, hemiketals, ketals, or diethyl precursor (e.g., 1,2-diethylbenzene and 2,3-diethylpyrazine, respectively), or



1,2-diacetylbenzene 2,3-diacetylpyrazine

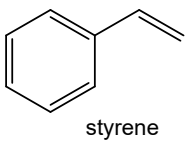
f) β-phenylethylamine (primary or secondary but not tertiary amine) moiety with or without additional alkyl, hydroxy, methoxy, or ethoxy substitution, or



amphetamine

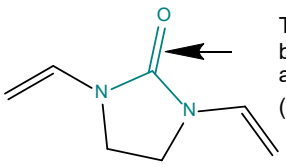
g) **aromatic**, **heteroaromatic**, or mono**heterocyclic** substance with one or more terminal vinyl (i.e., $RHC=CH_2$) group(s) as the only ring substituent(s), or

Yes to:



styrene

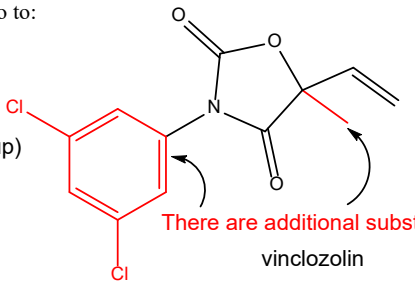
Yes to:



1,3-divinylimidazolidin-2-one

The =O is not considered to be a ring substitution as it is a part of a cyclic functional group (the urea (N(=O)N) functional group)

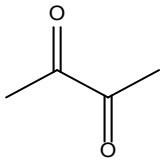
No to:



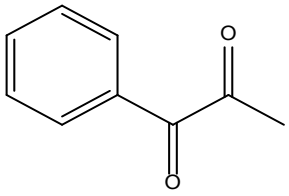
vinclozolin

h) **i)** **acyclic** or **aromatic** ring substituted **α**-diketone or its corresponding hemiketal or ketal or **ii)** an aliphatic dialdehyde without α,β-unsaturation as the only **functional group**s, or

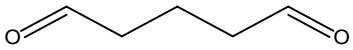
i: ii:



diacetyl

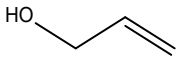


1-phenyl-1,2-propanedione

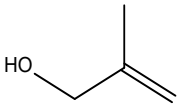


pentane dialdehyde

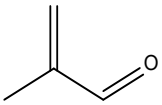
i) allyl alcohol, methallyl alcohol, methacrolein, crotonyl alcohol, crotonaldehyde, or corresponding ester (e.g., allyl hexanoate or crotonyl acetate), carbonate, orthoester, acetal, hemiacetal, ketal, or hemiketal or



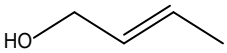
allyl alcohol



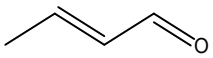
methallyl alcohol



methacrolein

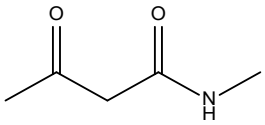


crotonyl alcohol

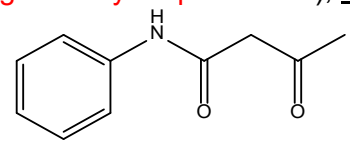


crotonaldehyde

j) aliphatic **β**-diketone or **β**-ketoamide moiety (may be a substituent on a ring but not a **connector** between two rings or a cyclic β-diketone), or

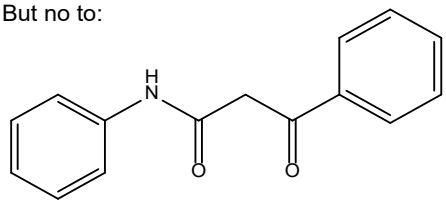


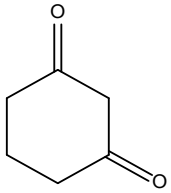
N-methylacetoacetamide



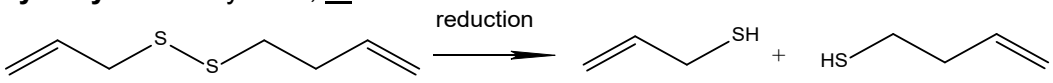
acetoacetanilide

But no to:





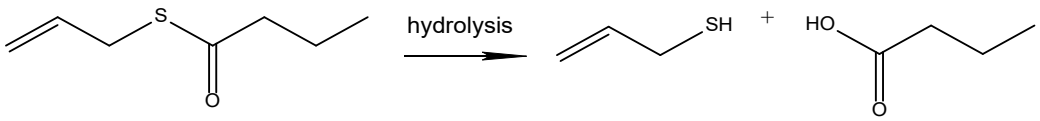
k) allyl thiol, mono- or di-allyl disulfide that is **reduced** to allyl thiol, or allyl thioester that is **hydrolyzed** to allyl thiol, or



1-allyl-2-(but-3-en-1-yl)disulfane

prop-2-ene-1-thiol

but-3-ene-1-thiol

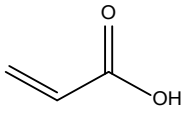


S-allyl butanethioate

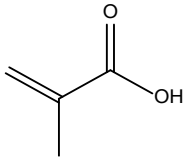
prop-2-ene-1-thiol

butyric acid

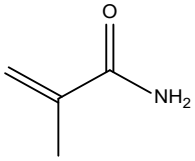
l) acrylic acid, methacrylic acid, methacrylamide, or crotonic acid and corresponding esters of the acids (e.g., ethyl acrylate), or



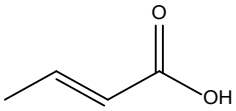
acrylic acid



methacrylic acid

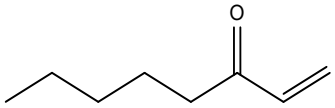


methacrylamide



crotonic acid

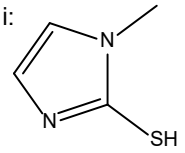
m) ketone, a ketal, a hemiketal, or secondary alcohol (or corresponding ester) directly bonded to a terminal alkene, or



vinyl amyl ketone

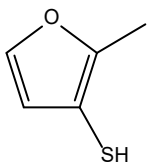
n) a mono- or di-thiol, thioester, thiocarbonate, or disulfide **i)** in which S is connected by a single bond to the 2-position of an imidazole or pyrimidine ring, **ii)** in which S is connected by a single bond to a **heteroaromatic** ring (other than those captured at Q28n(i))), **iii)** as a substituent of an **alicyclic** ring, **aromatic** or **heterocyclic** ring, or contained within a heterocyclic ring (e.g., cyclic disulfide), or as a substituent (or part) of a linear or branched aliphatic chain (either an acyclic compound or a substituent on an **alicyclic**, **aromatic**, **heterocyclic**, or **heteroaromatic** ring), or **iv)** a polysulfide with S_n where n≥3, or

i:



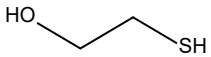
2-mercapto-1-methylimidazole

ii:



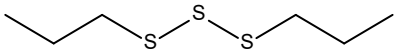
2-methyl-3-furanthiol

iii:



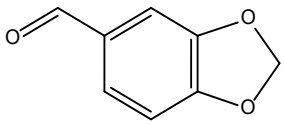
2-mercaptoethanol

iv:



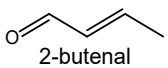
dipropyl trisulfide

o) a methylenedioxy ring **fused** to an **aromatic** ring, or

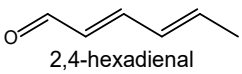


piperonal

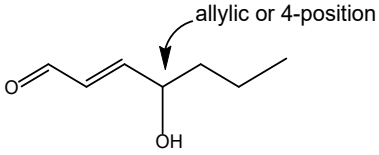
p) **linear aliphatic α,β**-unsaturated aldehyde or dialdehydes of <10 Cs, or their corresponding acetals or hemiacetal, or their corresponding continuously **conjugated** di- or tri-enal with or without a hydroxy or hydroperoxy substituent(s) at the allylic (e.g., 4-) position of mono **α,β**-unsaturated compounds (the aldehydes matching this description should not be ring substituents), or



2-butenal

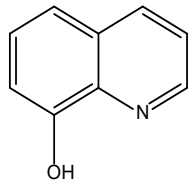


2,4-hexadienal



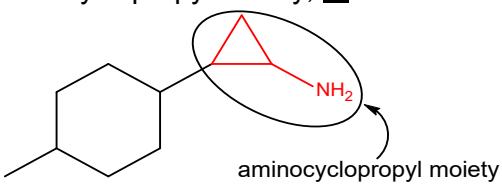
(*E*)-4-hydroxyhept-2-enal

q) **aromatic** or **heteroaromatic** substance containing a heteroaromatic or aromatic ring substituent hydroxyl, ether, aldehyde, or ketone that is separated from a ring or substituent N by two ring carbons (note: the ring carbons can be on the same or on different rings), ($HOC{=}CNH_2$ or NC-C=O) (e.g., *o*-aminophenol, 8-hydroxyquinoline, or 2-acetylpyrrole), or



8-hydroxyquinoline

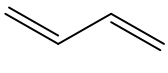
r) aminocyclopropyl moiety, or



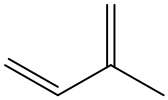
2-(4-methylcyclohexyl)cyclopropan-1-amine

s) **linear** or **simply branched-chain aliphatic** acyclic hydrocarbon with or without one or more $=CH_2$ branches that has a terminal diene and i) ≤ 6 Cs or ii) > 6 Cs ((i) and (ii) may also be a substituent on a ring)? Please note that the restriction on carbon number applies to the chain containing the terminal diene and not the whole molecule.

i:

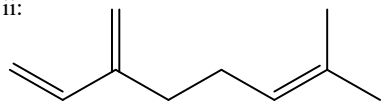


1,3-butadiene



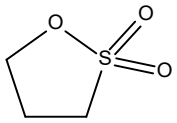
isoprene

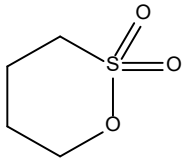
ii:



beta-myrcene

t) aliphatic sultones (cyclic sulfonic esters) (note that the sultone ring cannot be fused to an aromatic ring)



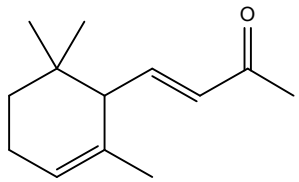


1,3-propane sultone 1,4-butane sultone

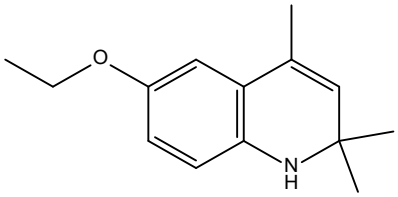
i) If yes to c(i)), n(i)), r), or t), assign to class V.
ii) If yes to a), b), c(ii)), d), e), f), n(ii)), n(iv)), p, or s(i)) assign to Class IV.
iii) If yes to g), h(i)), h(ii)), j), m), n(iii)), o), q), or s(ii)) assign to Class III.
iv) If yes to i), k) or l), before assigning the compound to Class III, run all potential ester and thioester hydrolysis products and/or disulfide reduction products through the EDT starting at Q1. Assign the compound to the highest class any of its products may have. If no ester, thioester, or disulfide is present, assign to Class III.
v) If no to a) through s) and the compound is not heterocyclic, assign to Class II.

vi) If no to a) through s) and the compound has at least one heterocyclic ring, assign to Class II only if at least one of the heterocyclic rings contains either a cyclic anhydride, one or more cyclic ester, one or more cyclic amide (N can be connected to another N or S), an imidazolidinone, and/or a 5- or 6-membered ring with 1, 2, or 3 ring oxygen atoms (but not cyclic peroxides (i.e., the peroxide is a part of the ring and not a ring substituent) or cyclic ozonides) with or without a single ring double bond with or without additional ring N and/or S atoms. Also assign the heterocyclic ring to Class II if it contains one or more sulfonate (but not as a sultone) and/or sulfamate. Finally, assign the heterocycle to Class II if it contains at least two hydroxy, methoxy, aldehyde, and/or carboxylic acid functional groups. Note: in all cases, heterocyclic rings may be substituted. In the case of cyclic peroxides, ozonides, and all other heterocycles, if no to a) through s), assign to Class III.

vii) Reminder for substances that were sent here from Q18: If no at Q28, go to Q47 (that is if Q28N, do not assign the substance to Class II or III at Q28).
Examples for no to a) through s):



alpha-Ionone



ethoxyquin

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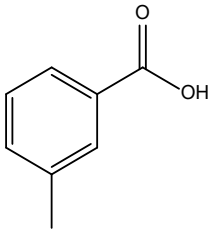
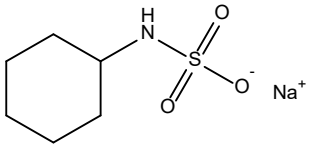
29. Does the substance contain one or more **aromatic** rings?

i) If yes, proceed to Q33.



3-methylbenzoic acid

ii) If no, proceed to Q30.



sodium cyclamate

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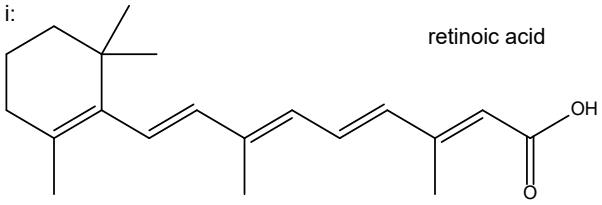
30. Does the **alicyclic** substance contain

a) **i)** one cycloalkane ring containing a single ring double bond, substituted with exactly 3 methyl groups and a **linear** or **simply branched** chain of ≥6 Cs but ≤12 Cs (in total or if

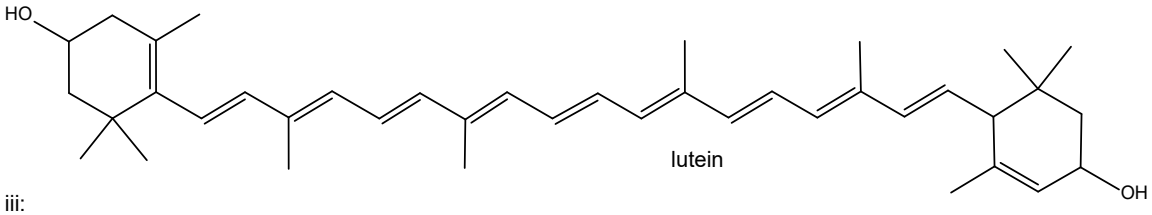
an ester is present, then between the ester -O- and the ring) with alternating double bonds (extended conjugation) and either a terminal carboxylic acid substituent or its corresponding alkyl ester, or **ii)** two cycloalkane rings containing a single ring double bond per each ring, substituted with exactly 3 methyl groups per ring with or without one ketone and/or one hydroxy substitution per ring, and connected by a **linear** or **simply branched** hydrocarbon chain with alternating double bonds. These alternating double bonds must be further **conjugated** with the ring double bond, which must be further **conjugated** with the ring ketone substitution, or **iii)** one, two, or three **alicyclic** rings containing ≤30 ring Cs (with or without a single ring double bond per each ring), unsubstituted or substituted with or without **linear** or **simply branched aliphatic chains** each of ≤12* Cs with or without one or more of only the following **functional groups**: alcohol, aldehyde (except for **vicinal** dialdehydes), acetal, carboxylic acid, ester, ether, ketone (including ring ketone), ketal, peroxide, hydroperoxide, peroxyester, peracid, peroxycarbonate, peroxydicarbonate, diacylperoxide, thiol, sulfide (mono-di- or poly-sulfide), sulfoxide, primary or tertiary amine, or primary or secondary amide (*If one long chain connects two rings, the linker chain can contain up to 24Cs.) (Note that no additional rings, substituents, or functional groups other than those listed in i), ii), and iii) are allowed to be present), or

i:



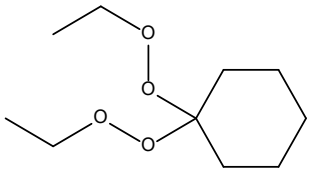
retinoic acid

ii:



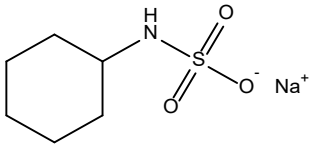
lutein

iii:



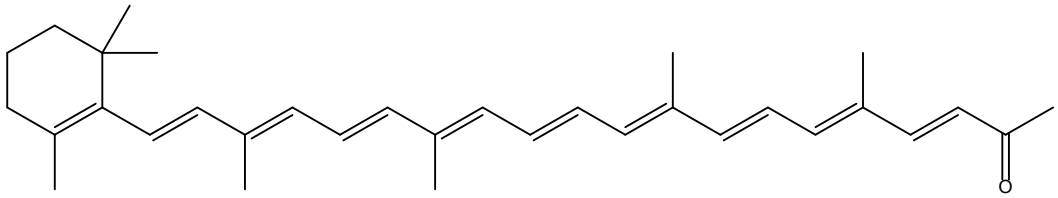
1,1-bis(ethylperoxy)cyclohexane

b) a sulfonate, sulfonamide, or sulfamate?



sodium cyclamate

i) If yes to a(i)), assign to Class IV.
ii) If yes to a(ii)), assign to Class III.
iii) If yes at a(iii)), proceed to Q31.
iv) If yes to b), assign to Class I.
v) If no to a) and b), proceed to Q47. Before assigning the substance to a class, also crosscheck against Q28. Assign the substance to the highest class it would receive either at Q28 or Q47.
Example for no:



5,9,14,18-tetramethyl-20-(2,6,6-trimethylcyclohexen-1-yl)icosa-3,5,7,9,11,13,15,17,19-nonaen-2-one
(Citranaxanthin)

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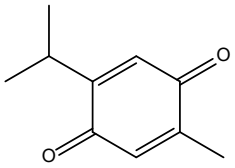
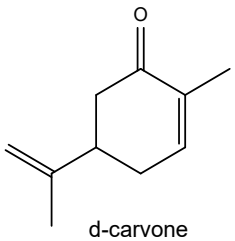
31. Is the **alicyclic** substance an *o*- or *p*-quinone with or without substitution by one or more alkyl substituent of ≤6 Cs with no additional **functional groups**?

i) If yes, assign to Class III.



thymoquinone

ii) If no, proceed to Q32.



d-carvone

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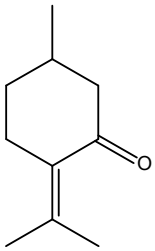
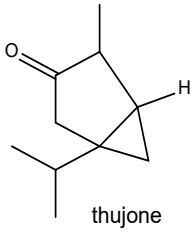
32. Is the substance a mono or bicyclic ring that contains

a) a cyclohexane or cyclohexene ring with ketone or ketal and an isopropylidene or isobutylidene side chain adjacent to the ketone function, or



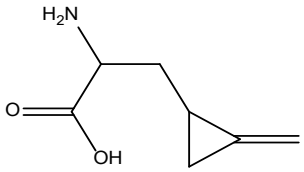
pulegone

b) a ring ketone or ketal with a 4-methyl-1-isopropyl bicyclo[3.1.0]-2- or 3-cyclohexanone carbon **skeleton**, or



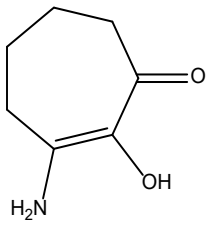
thujone

c) a cyclopropyl ring with an **exocyclic** or **endocyclic** alkene, or

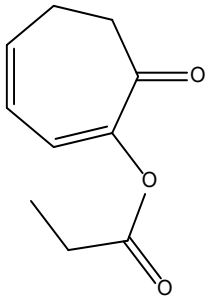


hypoglycin A

d) Does the **alicyclic** ring contain an **α**-ketoenol moiety (C=C(OH)C=O), or the corresponding **α**-ketoester (C=C(OC(=O)R)C=O) in which the enolic double bond is further **conjugated** with an O or N atom possessing a non-bonding electron pair or another double bond?



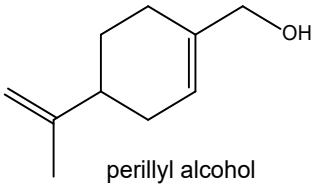
3-amino-2-hydroxycyclohept-2-en-1-one



7-oxocyclohepta-1,3-dien-1-yl propionate

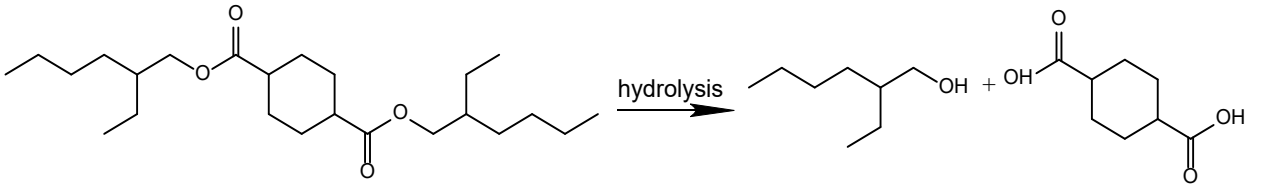
i) If yes to a) or d), assign to Class III.
ii) If yes to b) or c), assign to Class IV.
iii) If no to a), b), c), and d), and the substance does not contain a hydrolyzable functional group, proceed to Q28.

iv) If no to a), b), c), and d) and the substance contains a hydrolyzable functional group, before assigning the substance to a class at Q28, crosscheck whether any of the hydrolysis products satisfy the structural requirements at Q24a) or Q24b). Assign the substance to the highest class it would get at either Q24a), Q24b), or Q28.
Example for no:



perillyl alcohol

Example for cross check:



triethylene glycol bis(2-ethylhexanoate): Class III ← Yes at Q24a(i) (Class III) No at Q28 (Class II)

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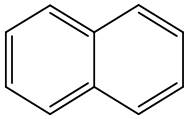
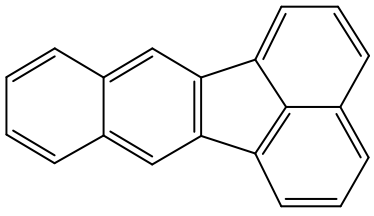
33. Is the substance

a) an unsubstituted benzene ring (i.e., benzene) or composed of 2 or 3 unsubstituted **fused aromatic** rings, or



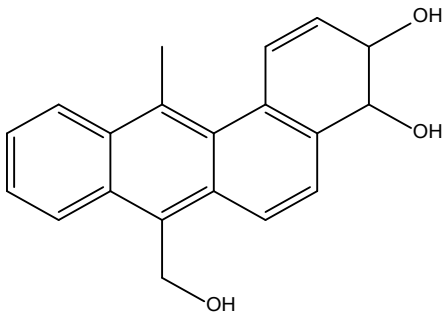
naphthalene

b) unsubstituted and composed of >3 **fused aromatic** rings, or



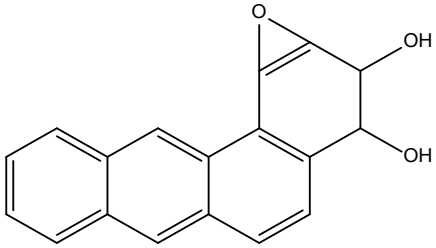
benzo[k]fluoranthene

c) a poly**aromatic** ring system of three or more **fused** rings containing either one or more -CH_3, -CH_2CH_3, -CH_2F, -CH_2Cl, -CH_2Br, -CH_2I, -C(=O)H, -CH_2OH, -CH_2OCH_3, -CH_2-O-$S(=O)_2OH$, -CH_2-O-$S(=O)_2CH_3$, -CH_2-O-$S(=O)_2CF_3$, -CH_2-O-C(=O)R, -CH_2-O-CH_2-Ar (Ar is benzene), and/or -CH_2-O-gluc substituents (no other substituents should be present). If ≥2 sulfonate (-$S(=O)_2OH$) substituents where there is at least one sulfonate for every ≤10 Cs are present, say no at Q33c), or

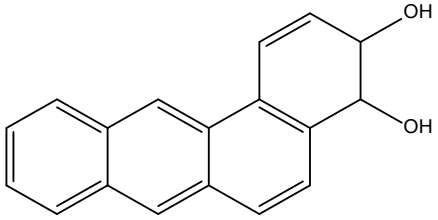


7-(hydroxymethyl)-12-methyl-3,4-dihydrotetraphene-3,4-diol

d) a poly**aromatic** ring system of three or more **fused** rings substituted by any combination of diol(s) and/or epoxide(s) in the K-region and/or on **bay or fjord region trio**(s) **and/or quartet**(s)?

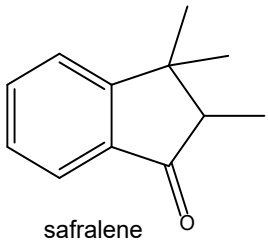


2,3-dihydrotetrapheno[1,2-*b*]oxirene-2,3-diol



3,4-dihydrotetraphene-3,4-diol

i) If yes to a), assign Class IV.
ii) If yes to b), c), or d), assign Class V.
iii) If no to a), b), c), and d), proceed to Q34.
Example for no:



safralene

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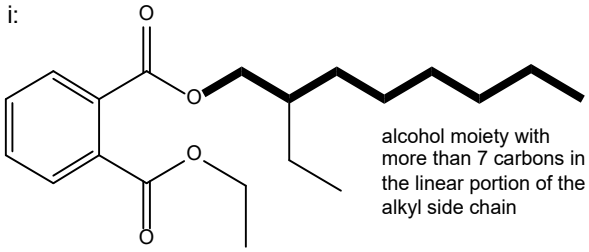
34. Is the substance

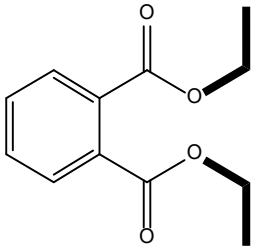
a) **i)** an *o*-phthalate diester that either contains at least one alcohol moiety with more than 7 carbons in the linear portion of the alkyl side chain or contain two alcohol moieties each containing a maximum of 2 carbons in the linear portion of the alkyl side chains, or **ii)** a benzoic acid ester substituted at the *o*-position by a moiety bearing a non-bonding pair of electrons (e.g., -OR, -OH, $-NH_2$, or $-CO_2H$), or **iii)** all *o*-phthalate diesters that are not captured at Q34a(i)).

i:



alcohol moiety with more than 7 carbons in the linear portion of the alkyl side chain

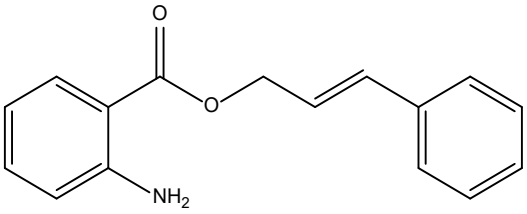
(*R*)-ethyl (2-ethyloctyl) phthalate



alcohol moieties with not more than 2 carbons each in the linear portion of the alkyl side chains

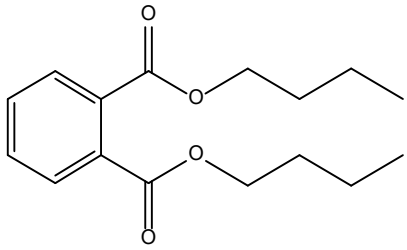
diethyl phthalate

ii:



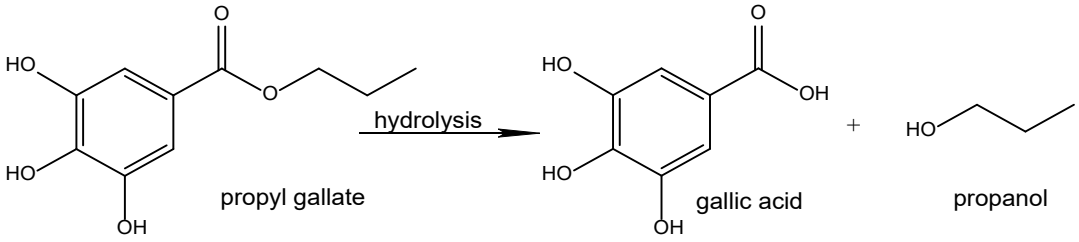
cinnamyl anthranilate

iii:



dibutyl phthalate

b) an ester, orthoester, thioester, sulfite ester, acetal, ketal, hemiacetal, hemiketal, sulfate ester, carbonate (including dicarbonate), peroxide, hydroperoxide, peroxyester, peracid, diacylperoxide, peroxycarbonate, peroxydicarbonate, or anhydride that would be anticipated to be completely **hydrolyzed** or **reduced**?



propyl gallate

gallic acid

proceed to Q1

propanol

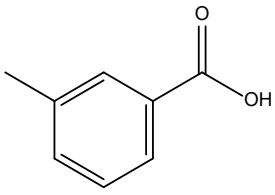
proceed to Q1

i) If yes to a(i)) or a(ii)), assign to Class III.

ii) If yes to a(iii)), assign to Class IV.

iii) If yes to b), assume **hydrolysis** and start the evaluation of the **hydrolysis** products at Q1. The compound will be assigned to the highest class any of its hydrolysis products may have.

iv) If no to a(i)), a(ii)), and b), proceed to Q35.
Example for no:



3-methylbenzoic acid

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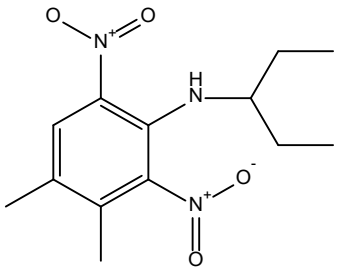
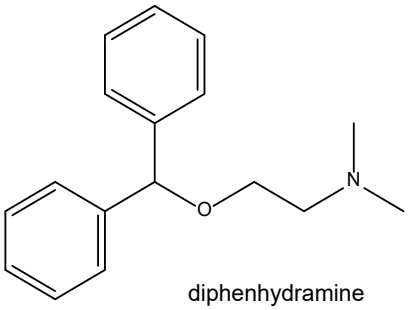
35. Does the substance contain

a) only one **aromatic** ring (additional ring(s) that are not aromatic are allowed) <u>or</u>



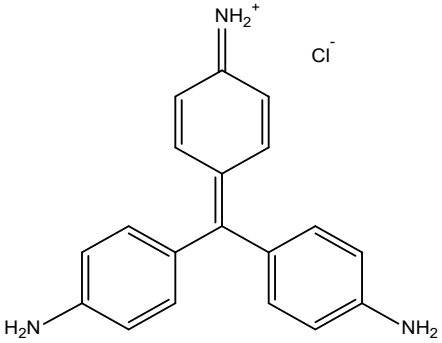
pendimethalin

b) only two **aromatic** rings (additional ring(s) that are not aromatic are allowed)?



diphenhydramine

i) If yes to a), proceed to Q38.
ii) If yes to b), proceed to Q36.
iii) If no to a) and b), proceed to Q47.
Example for no:



C.I. Basic Red 9

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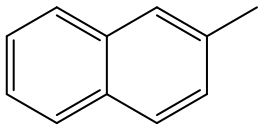
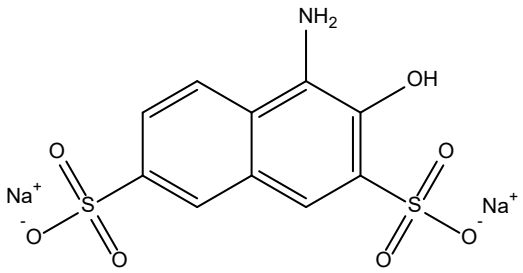
36. Is the binuclear substance

a) **fused** (e.g., naphthalene or azulene), <u>or</u>

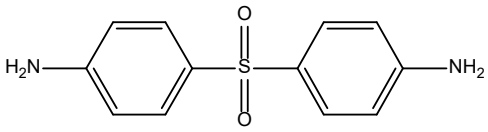


2-methylnaphthalene

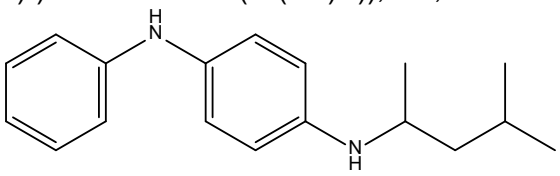


disodium 4-amino-3-hydroxy-2,7-naphthalenedisulfonate

b) unfused with benzene rings either **singly bonded** or connected by an -O- or one or more -S- (divalent (-S-), tetravalent (-S(=O)-) or hexavalent (-$S(=O)_2$-)), -N-, or -N=N-), <u>or</u>

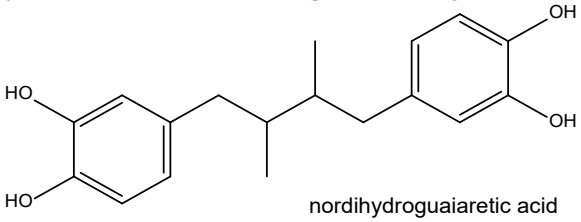


dapsone

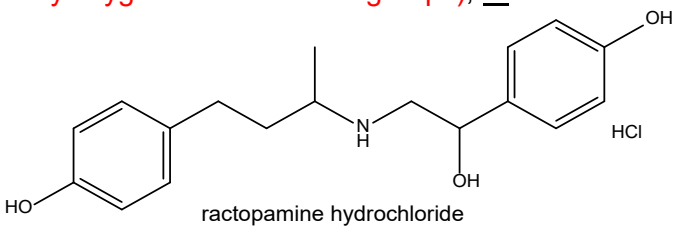


4-N-(4-methylpentan-2-yl)-1-N-phenylbenzene-1,4-diamine

c) unfused but linked by either one **linear aliphatic** chain of ≤8 Cs or a **simply branched aliphatic chain** with ≤2 branches of ≤2 Cs each and a total of ≤8 Cs. The connecting chain may contain -O-, -C(=O)-, and one or more -S- (divalent, tetravalent, or hexavalent), -N-, -N=, -N=N-, -NC(=O)-), -$S(=O)_2N(H)$-, or not more than 3 amino acids (note that the connecting chain may be substituted by oxygenated functional groups), <u>or</u>

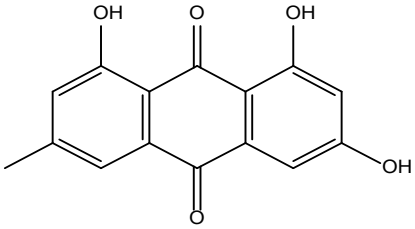


nordihydroguaiaretic acid

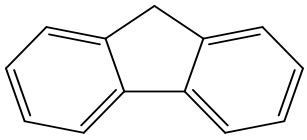


ractopamine hydrochloride

d) linked at ***ortho*** positions by a single bond (i.e., **singly bonded**) and a **linear** or **simply branched** chain of ≤4 Cs <u>or</u> two **linear** or **simply branched aliphatic chains** of ≤4 Cs each?

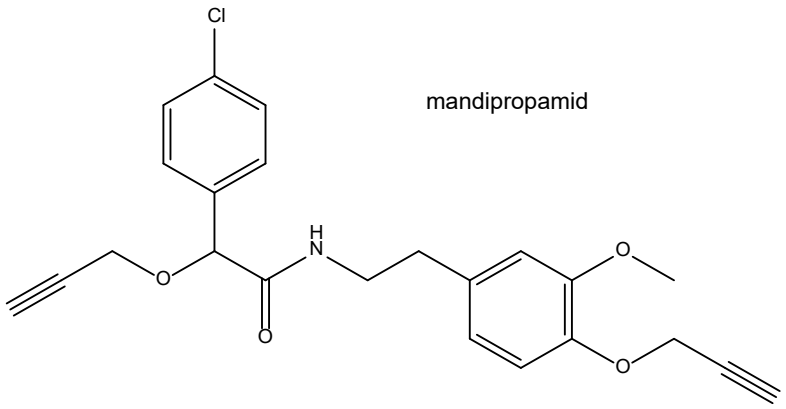


emodin



fluorene

i) If yes to a), proceed to Q37.
ii) If yes to b), c), or d), proceed to Q41.
iii) If no to a), b), c), and d), proceed to Q47.
Example for no:



mandipropamid

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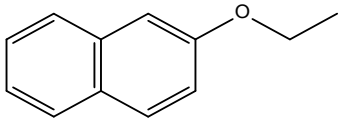
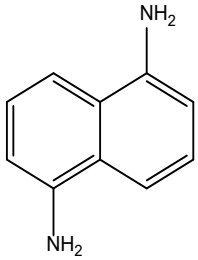
37. Does the **fused** ring system contain only the following substituent(s)

a) one or more alkyl substituent(s) (may be cycloalkyl) each of ≤4 Cs and/or at least one hydroxy, methoxy, ethoxy, primary or secondary alcohol, aldehyde, ether, ketone, carboxylic acid, or ester, or



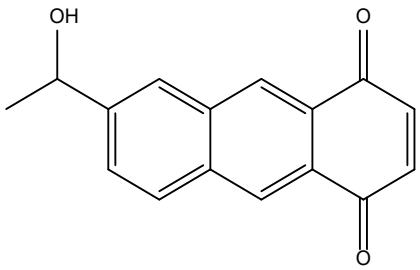
2-ethoxynaphthalene

b) either one primary amine (or its N-acetyl amide) and/or one nitro group or two primary amines (or their N-acetyl amide) or two nitro groups at any position with or without one alkyl substituent of ≤2 Cs and no other **functional groups**, or



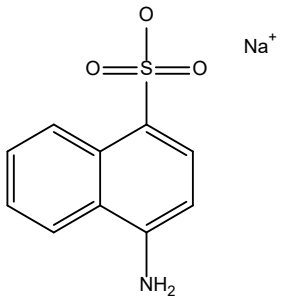
1,5-naphthalenediamine

c) an *o*- or *p*-quinone with or without additional alkyl chains of ≤4 Cs and/or the following **oxygenated functional groups**: hydroxy, methoxy, ethoxy, primary or secondary alcohol, aldehyde, carboxylic acid, or ester?



6-(1-hydroxyethyl)anthracene-1,4-dione

i) If yes to a) or c), assign to Class III.
ii) If yes to b), assign to Class V.
iii) If no to a), b), and c), proceed to Q47.
Example for no:



sodium naphthionate

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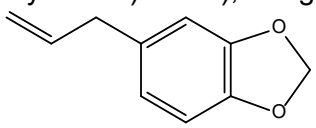
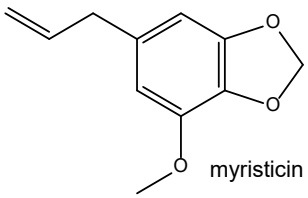
38. Is the substance a single benzene ring that consists only of
a) 2'-alkene or a 1'-hydroxy or 1'-ester of the 2'-alkene and
b) one or more alkoxy groups, one of which must be ***para*** to the hydrocarbon chain? (Note: The *p*-alkoxy includes the alkoxy of a 3,4-methylenedioxy substituent.)

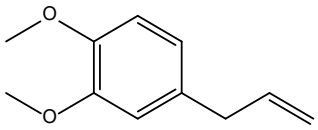
i) If yes to a) and b), assign to Class IV.



safrol

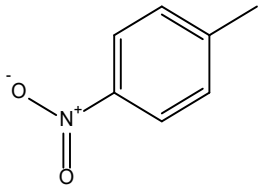


myristicin



methyleugenol

ii) If no to a) and/or b), proceed to Q39.



4-nitrotoluene

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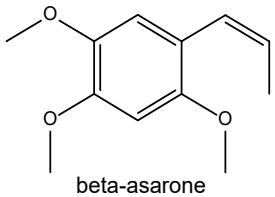
39. Is the substance a single benzene ring substituted only by
a) a hydrocarbon chain of 2 or 3 Cs containing a 1'-alkene with or without a terminal **oxygenated functional group** (i.e., hydroxy, aldehyde, carboxylic acid, or **corresponding** hemiacetals, acetal, or alkyl ester) and

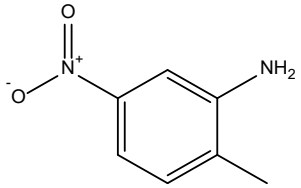
b) one *o*-hydroxy or one or more methoxy groups one of which is *o*- to the hydrocarbon chain?

i) If yes to a) and b), assign to Class III.



beta-asarone

ii) If no to a) and/or b), proceed to Q40.



2-methyl-5-nitroaniline

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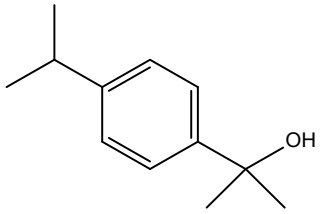
40. Is the substance

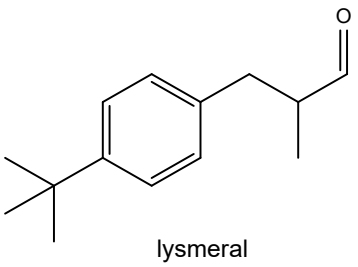
a) **i)** benzoic acid or its precursors (i.e., toluene, benzyl alcohol, or benzaldehyde) or **ii)** 3-phenylpropanoic or 3-phenylpropenoic acid or their corresponding alcohol or aldehyde with or without side chain alkyl substituents of ≤6 Cs, and i) or ii) is substituted at the ***para*** position by a tertiary butyl, isopropyl, or isobutyl group with no other substituents directly bonded to the ring?, or

i:



2-(4-isopropylphenyl)propan-2-ol

ii:

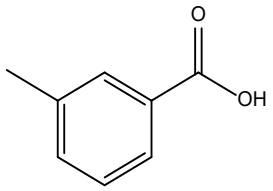


lysmeral

b) benzoic acid, benzaldehyde, or benzyl alcohol that is ring substituted by

i) any combination of one or more hydroxy (except for *o*-hydroxybenzoic acid derivatives) and/or ether of ≤4 Cs (note this hydroxy substitution on benzyl alcohol is in addition to the alcohol that makes/defines benzyl alcohol and unlike the hydroxyl group in benzyl alcohol, this hydroxy group is a direct aromatic ring substituent) and/or

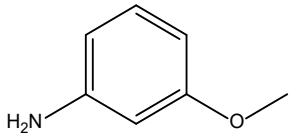
ii) a single **linear** alkyl substituent of ≤4 Cs with or without hydroxy or ether present (but no other functional groups)?



3-methylbenzoic acid

Depending on the response at 40a) and b):

i) If yes to a(i)) or a(ii)), assign to Class III.
ii) If yes to b), assign to Class I.
iii) If no to a) and b), proceed to Q41.
Example for no:



m-anisidine

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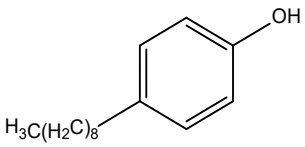
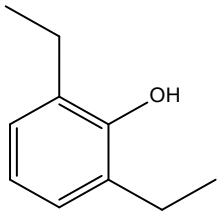
41. Does the substance have only one or a maximum of two aromatic ring(s) and is substituted by at least one phenolic -OH but not more than one phenolic -OH per **aromatic** ring and

a) one or more *o*- and/or *p*- (to the phenolic -OH) alkyl substituents, one of which must have ≥ 4 Cs (no N and/or S containing functional groups and no halogens may be present), or



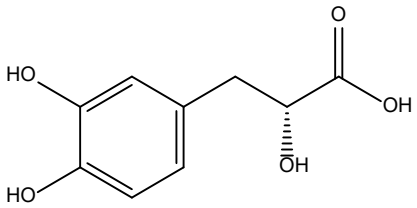
4-n-nonylphenol

b) two *o*-alkyl substituents of ≥1 C but ≤8 Cs (no N and/or S containing functional groups and no halogens may be present)?



2,6-diethylphenol

i) If yes a) or b), assign to Class III.
ii) If no to a) and b), proceed to Q42.
Example for no:

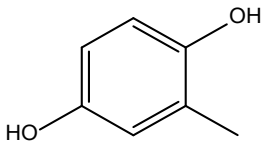


(2R)-3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid

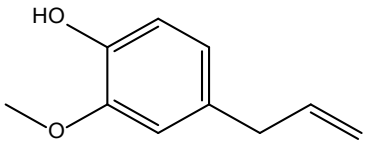
---

42. Is the substance an

a) *o*- or *p*-hydroquinone, or its methoxy or ethoxy derivative with no additional **oxygenated functional** group and with no N- and/or S-containing functional groups or halogens, or

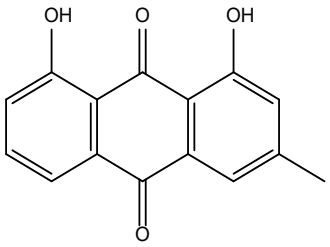


2-methylhydroquinone



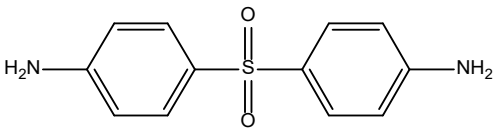
eugenol

b) *o*- or *p*-quinone with **fused aryl** ring(s) (e.g., naphthoquinone), with or without additional alkyl chains (≤4 Cs), and/or one **alicyclic** or an additional **heterocyclic** ring and/or containing the following **oxygenated functional groups**: hydroxy, methoxy, ethoxy, primary or secondary alcohol, aldehyde, ketone, and/or carboxylic acid but no other functional groups or halogens?



chrysophanol

i) If yes to a), assign to Class II.
ii) If yes to b), assign to Class III.
iii) If no to a) and b), proceed to Q43.
Example for no:



dapsone

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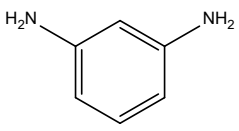
43. Is the substance (note: an -N- **connector** between two aromatic rings (Ar-N-Ar) should be disregarded when determining whether the compound is a (di)aminobenzene)

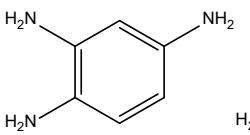
a) a diaminobenzene (or its **related** N-acetyl or N-propionyl derivative), nitroaniline (or its **related** N-acetyl or N-propionyl derivative (i.e., of amine)), or dinitrobenzene either **i)** unsubstituted (except for additional $-NH_2$ or $-N^+O_2$) or substituted with one or more halogens and/or $-CF_3$ moieties directly bonded to the benzene ring (note: other substituents may also be present in addition to the halogens and $-CF_3$ moiety). The presence of only a single benzene ring bearing the amino- and/or nitro- substitution is allowed, or **ii)** substituted with one or more ring alkyl substituents of ≤4 Cs only (i.e., no other functional groups, halogens, or atoms other than C, H, O, and N should be

present) and the presence of only a single benzene ring bearing the amino- and/or nitro-substitutions is allowed, or **iii)** a compound with two unfused benzene rings and one of the rings is a dinitrobenzene, and both rings are substituted by one or more halogens and/or -CF_3 moieties directly bonded to the benzene ring, or

i:

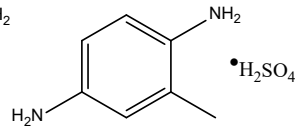


m-phenylenediamine



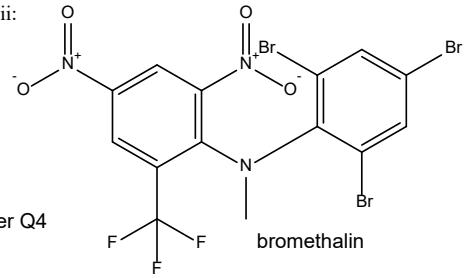
benzene-1,2,4-triamine

ii:



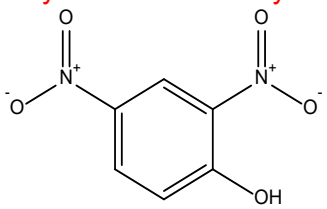
2,5-toluenediamine sulfate
Reminder: the sulfate is disregarded after Q4

iii:



bromethalin

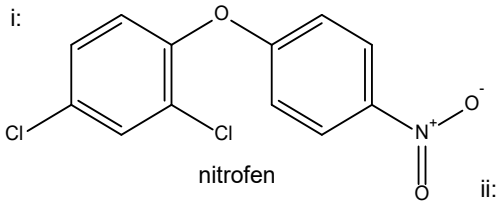
b) a diaminobenzene (or its **related** N-acetyl or N-propionyl substituent), nitroaniline (or its **related** N-acetyl or N-propionyl substituent), or dinitrobenzene (i.e., only a single benzene ring is present) with a ring hydroxy, N-(2-hydroxyethyl)-, carboxy, methoxy, ethoxy, with or without additional ring alkyl substituents of ≤4 Cs except those adjacent (*o*-position) to the above listed **oxygenated groups**. Alkyl substituents may be unsubstituted or substituted by primary or secondary alcohol, aldehyde, ketone, carboxylic acid, ether, or ester substituents and the methoxy and/or ethoxy substituent may be substituted by alcohol, aldehyde, or carboxylic acid, or



2,4-dinitrophenol

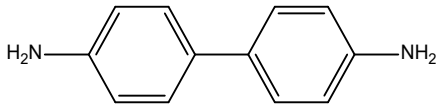
c) a biphenyl, methylenebis(phenyl) and homologues with linkages of ≤4 Cs (a maximum of 4 Cs linking the two phenyl rings, the presence of -C(=O)- and/or -N- in the link is allowed), diphenyl ether, diphenylthioether, or diphenylsulfoxide (note that one of the rings may be a heteroaromatic ring instead of a benzene ring) containing **i)** a single primary amine, N-acetyl derivative, or nitro group at the 4-position (i.e., *para* to the **connector**) or **ii)** diamine (or N-acetyl derivative), nitroamine (or N-acetyl derivative), or dinitro groups at the 4,4'- positions and both i) and ii) with or without additional alkyl, methoxy, and/or halogen substituents but not substituted by any other **functional group** (note that the -C(=O)-, -N-, -O-, -S-, and -$S(=O)_2$- in the **connector** between the rings do not count as functional groups for the purpose of this question)?

i:



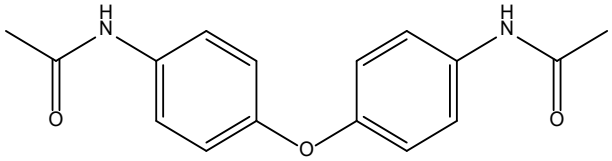
nitrofen

ii:



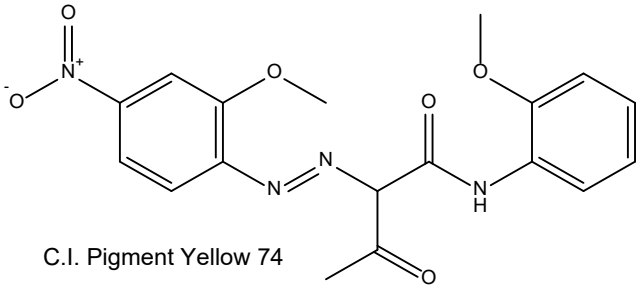
benzidine

ii:



bis(p-acetylaminophenyl) ether

i) If yes to a(iii)), c(i)), or c(ii)), assign to Class V.
ii) If yes to a(i)) or a(ii)), assign to Class IV.
iii) If yes to b), assign to Class III.
iv) If no to a), b), and c), proceed to Q44.
Example for no:



C.I. Pigment Yellow 74

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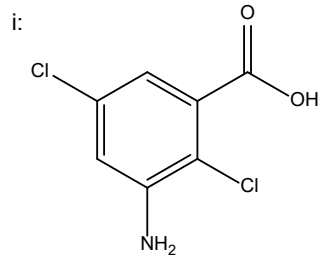
44. Is the substance aniline (or its **related** N-acetyl or N-propionyl derivative) or nitrobenzene (i.e., only a single benzene ring is present) with only the following substituents

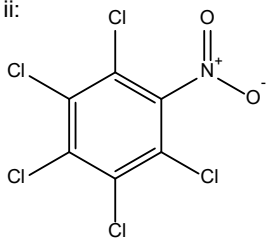
a) **i)** one or more Cl and/or Br substituents with at least one halogen at the *o*- or *p*-position with one or more alkyl substituents of ≤ 4 Cs, hydroxy, carboxy, methoxy, or ethoxy in any of the remaining positions or **ii)** one or more Cl and/or Br substituents with at least one halogen at the *o*- or *p*-position, or

i:



3-amino-2,5-dichlorobenzoic acid

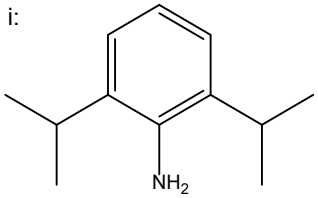
ii:



pentachloronitrobenzene

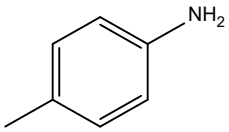
b) **i)** with two alkyl substituents of ≤5 Cs at the *o*-positions or ii) alkyl group(s) of ≤4 Cs at any other position (other than at the two *o*-alkyl position) and the compounds described in i) and ii) of this sub-question cannot have any additional functional groups (including no halogens), or

i:



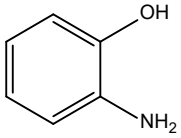
2,6-diisopropyl-aniline

ii:



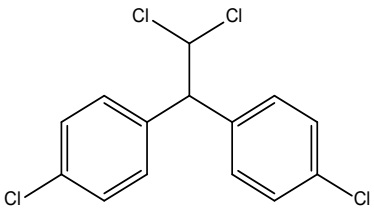
p-toluidine

c) one or more hydroxy, N-(2-hydroxyethyl), N-bis(2-hydroxyethyl), carboxy, methoxy, or ethoxy with or without additional alkyl substituents of ≤4 Cs with or without additional **oxygenated functional groups**, but no other functional groups (including no halogens)?



o-aminophenol

i) If yes to a(ii)), assign to Class V.
ii) If yes to b(ii)), assign to Class IV.
iii) If yes to a(i)), b(i)), or c), assign to Class III.
iv) If no to a), b), and c), proceed to Q45.
Example for no:

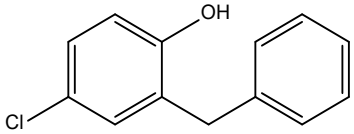


4,4'-(2,2-dichloroethane-1,1-diyl)bis(chlorobenzene)

---

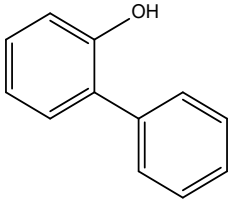
45. Disregarding any combination of **aromatic** ring hydroxy, methoxy, ethoxy, aldehyde, carboxylic acid, or thiol, does the mono- or binuclear system (i.e., maximum 2 aromatic rings) contain substituents *other than* **linear aliphatic chain**(s), **simply branched aliphatic chain(s)**, and/or **alicyclic** ring(s) each of ≤8 Cs total (the aliphatic chain(s) may contain up to one -O- each), an alkyne, a β-ethylamine, a methylenedioxy group fused to a benzene ring, a cyclopropylamine substituent, together with or without one or more side chain (or **connector** or alicyclic ring) substituent alcohol, methoxy, ethoxy, ketone, aldehyde, carboxylic acid, a mercaptan, thioester, polysulfide, or monosulfide (or it's S-oxide (sulfoxide) or S-dioxide (sulfone)), primary amide, β-ketoamide, secondary amides (but only for simple peptides connecting ≤5 amino acids or their N-acyl derivative), sulfonamide (but only when the sulfonamide or the sulfonate is either a **connector** between two rings or when they are not terminal (not terminal: $-S(=O)_2N(H)C-$ or $-S(=O)_2N(C)C-$; terminal: $-S(=O)_2NH_2$), a sulfonate but only when it is a **connector** between two rings, or esters (or sulfate ester), ketals, or acetals that can be **hydrolyzed** to ring substituents of ≤8 Cs?

i) If yes, proceed to Q46.



chlorophene

ii) If no, proceed to Q28.



2-phenylphenol

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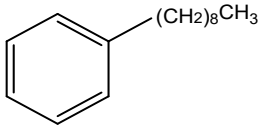
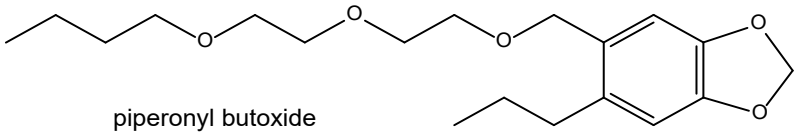
46. Does the substance contain the following moieties with or without those identified in Q45 but no other functional groups or moieties

a) **aliphatic** hydrocarbon chains of 9 or 10 Cs <u>or</u>



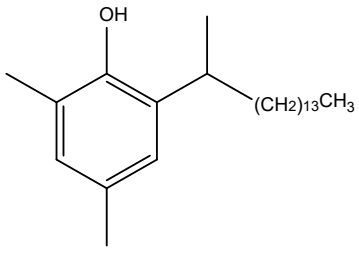
nonylbenzene

b) one or more polyoxyethylene or polyoxypropylene chain(s) ((-O-CH_2-CH_2-)$_n$ and/or (-O-CH_2(CH_3)CH_2-)$_n$) with n≤4 (in total) bonded either to the **aromatic** ring or **aliphatic** side chain?



piperonyl butoxide

i) If yes to a), assign to Class III.
ii) If yes to b), assign to Class II.
iii) If no to a) and b), proceed to Q47.
Example for no:



2-(hexadecan-2-yl)-4,6-dimethylphenol

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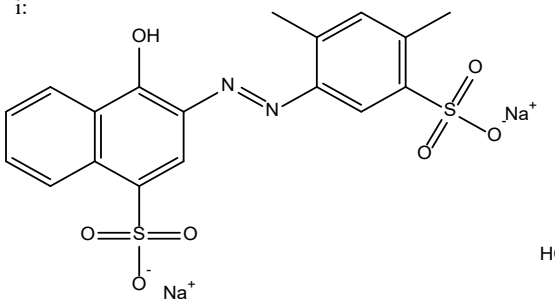
47. Does the substance contain

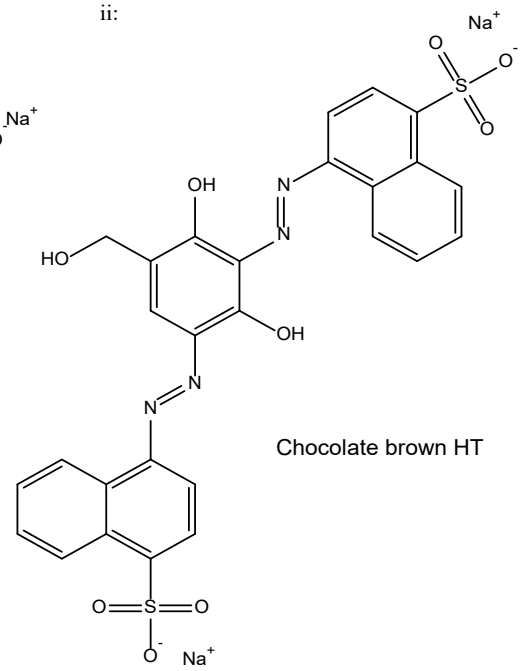
a) **i)** one or more azo (-N=N-) or N-N=C-C=O ↔ -N=N-C=C-O **functional groups** in which each N is bonded to a structural fragment bearing at least one sulfonate, sulfamate, or carboxylate per each fragment and for each sulfonate, sulfamate, or carboxylate ≤20 Cs per structural fragment without any primary amines except those adjacent to a sulfonate, sulfamate, or carboxylate substituent, or **ii)** one or more azo groups and one or more sulfonate, sulfamate, or carboxylate, but not on each fragment (except substances also fitting the structural requirements at Q47g) (i.e., if yes at Q47g), classify the substance based on the instructions at Q47g), or

i:



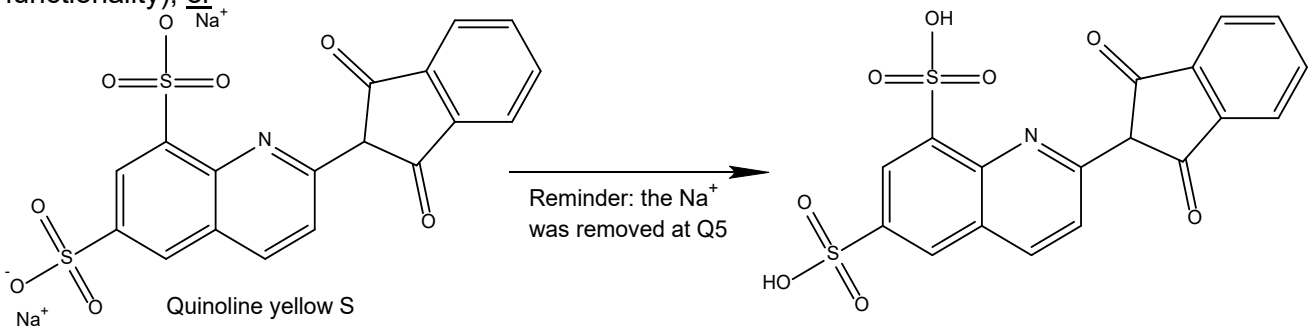
FD&C Red No. 4

ii:



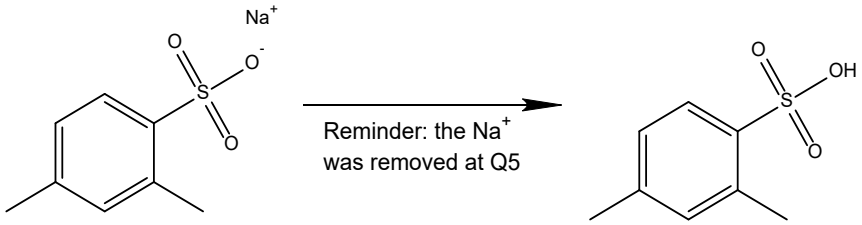
Chocolate brown HT

b) ≥2 sulfonate ($-S(=O)_2OH$) or sulfamate ($-OS(=O)_2NH_2$ or $R_2NS(=O)_2OH$) substituents where there is at least one sulfonate or sulfamate for every ≤10 Cs (note: but no azo functionality), or



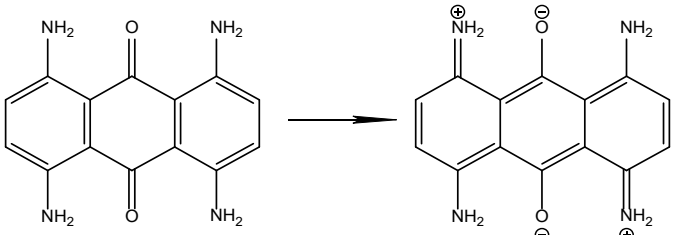
Quinoline yellow S

c) one sulfonate ($-S(=O)_2OH$) or sulfamate ($-OS(=O)_2NH_2$ or $R_2NS(=O)_2OH$) for every ≤20 Cs (but no azo functionality) (or two sulfonate for 21-40 Cs, etc.), or



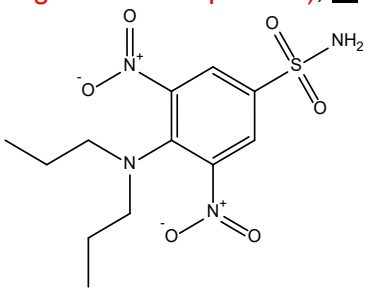
sodium 2,4-dimethylbenzenesulfonate

d) three or more **fused aromatic** and/or **heteroaromatic** rings that can extend conjugation through ring substituents (N or C=O) with the formation of a zwitterion *(*e.g., N^+ and O^-), or



C.I. Disperse Blue zwitterion

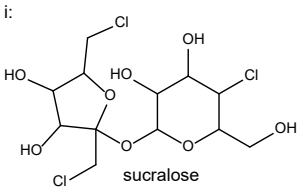
e) a mono-**aromatic** benzenesulfonamide ($-S(=O)_2NH_2$) (not methanesulfonamide ($H_3CS(=O)_2NH-$) and not a **connector** between rings) containing ≤15 total Cs (no other rings should be present), or



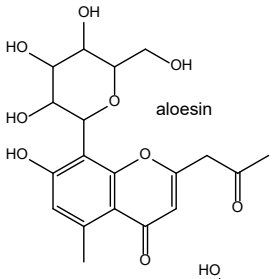
oryzalin

f) **i)** at least two or more alicyclic ring(s), nonaromatic heterocyclic ring(s) (only O is allowed as ring heteroatom), and/or aromatic ring(s) and at least one of the rings is a tetrahydropyran (oxane) ring and the tetrahydropyran ring is either **singly bonded** or connected to the rest of the molecule by an -O- in the position next to the tetrahydropyran O and all other (i.e., four) tetrahydropyran ring carbons are substituted by any combination of -OH (minimum 2 must be present), $-CH_2-OH$, -COOH, and/or Cl (a maximum of 1 Cl). Additionally, except for the fully substituted tetrahydropyran ring, every other ring should have at least two substitutions. The allowed substitutions are any combination of -OH, -COOH, =O, and alkyl (with or without sidechain substitutions), or **ii)** steviol and its glycosides, or

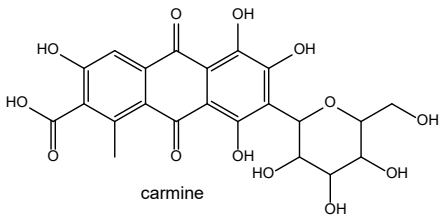
i:



sucralose

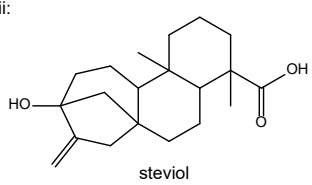


aloesin

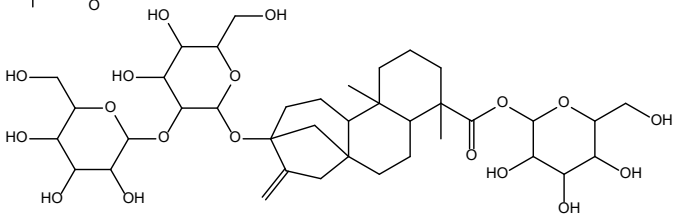


carmine

ii:

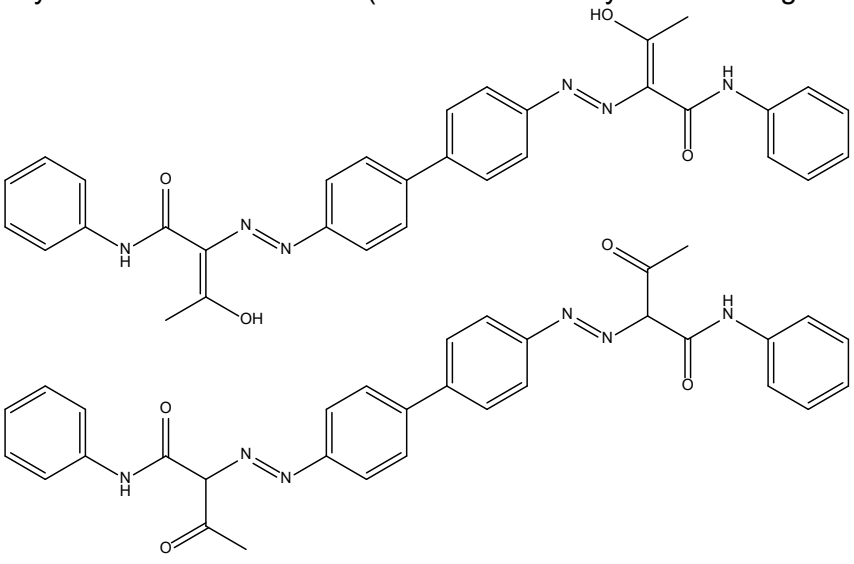


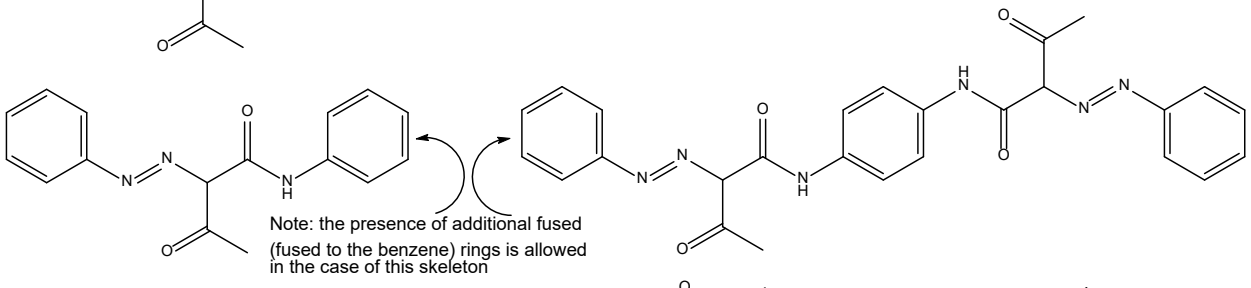
steviol



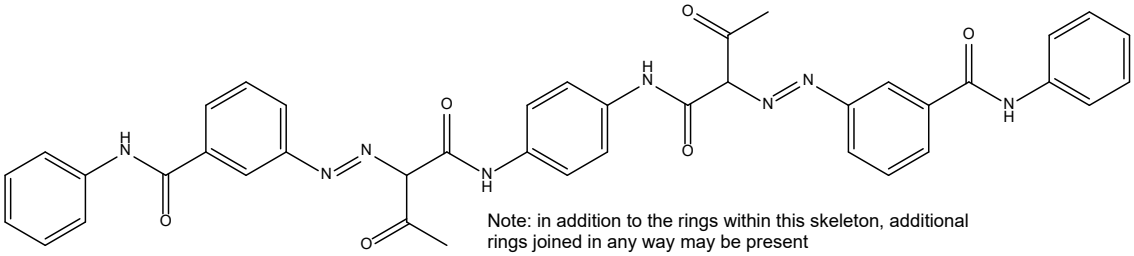
stevioside

g) any of the **skeletons** below (with or without any aromatic ring substitution)?





Note: the presence of additional fused (fused to the benzene) rings is allowed in the case of this skeleton

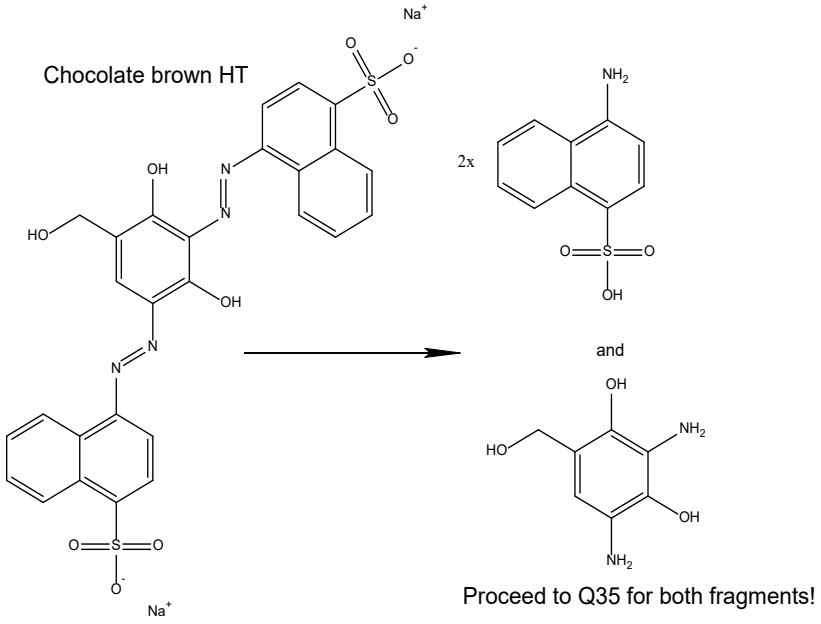


Note: in addition to the rings within this skeleton, additional rings joined in any way may be present

i) If yes to a(i)), b), f(ii)), or g), assign to Class I.

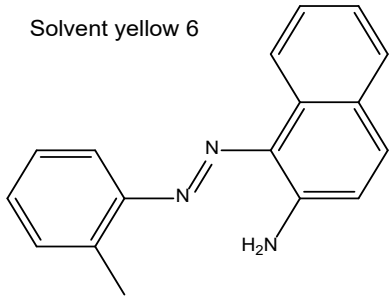
ii) If yes to a(ii)), assume the reductive cleavage of the azo function(s). For all **heteroaromatic** fragment(s) produced after **reduction**, proceed to Q19. For all

heterocyclic (nonaromatic) fragment(s), proceed to Q11. For all **aromatic** fragment(s), proceed to Q35. For all alicyclic fragments containing amine substituent, assign the fragment to Class IV. (Note: each azo function may undergo reduction in the intestinal lumen resulting in the formation of the corresponding amine fragment. Please note that N-N=C-C=O ↔ -N=N-C=C-O group can participate in azo type reduction.)
Example:



Proceed to Q35 for both fragments!

iii) If yes to c), d), or f(i)), assign to Class II.
iv) If yes to e), assign to Class III.
v) If no to a), b), c), d), and e), assign to Class IV.
Example for no:



4.6 Analysis of the Data in the Combined EDT DB

4.6.1 Description of the Combined EDT DB

Upon the completion of the validation, the original (i.e., pre-validation) EDT DB was combined with the external validation EDT DB to create the combined EDT DB. The combined EDT DB contains data on 3,141 substances. Regarding the species included in the combined EDT DB, studies were conducted using the following: rat (2,573), dog (313),

mouse (202), rabbit (22), monkey (19), hamster (6), pig (5), and unspecified (1), with the numbers in parentheses indicating the count of substances.

As the EDT was designed to sort chemicals based on/according to their chronic toxic potential through oral exposure only, we aimed at collecting oral studies for both the original (pre-validation) DB and the external validation DB. Unfortunately, for certain congeneric groups either no or inadequate number of oral studies are available; hence, to help us more confidently assign these compounds to an EDT class and to have as broad as possible applicability domain, we had to allow some non-oral studies to be present in our DBs. 96.4% of the studies in the combined EDT DB were performed via the oral route of administration.[4] No oral studies existed for 3.6% of the substances in the combined EDT DB. The breakdown of the type of studies in the combined EDT DB by the route of administration are presented in Table 6 below.

Table 6. Distribution of studies in the combined EDT DB based on the route of exposure

Exposure route	Number of studies	Percent of all studies (%)
Dermal	1	0.032
Osmotic minipump	4	0.13
Subcutaneous	8	0.25
Intraperitoneal	24	0.76
Intravenous/Infusion	29	0.92
Inhalational	48	1.53
Oral	3,027	96.37
Total	**3,141**	**99.99**

Substances in combined EDT DB are not equally distributed into the 6 classes: with Class VI having many fewer substances than the other five classes. This is unsurprising as Class VI aims at capturing the most toxic substances that exist. Only a limited number of these extremely toxic substances have been tested in subchronic and/or chronic toxicological studies due to their extreme toxicity. The distribution of compounds into the various EDT Classes in the combined EDT DB is displayed in Table 7 below.

Table 7. The distribution of substances into the various EDT Classes in the Combined EDT DB

EDT Class	I	II	III	IV	V	VI	Total
Total # of substances	341	637	572	1065	435	91	3141
% of total # of substances	11	20	18	34	14	3	100
# of substances used for TTC calculation	243	405	366	736	239	70	2059

Regarding the chemistries (i.e., chemical classes) associated with each EDT class, the FDA notes that the chemistries within each class are highly varied and encompass

[4] For the purposes of the EDT, oral studies are any studies where the test material was delivered into the stomach (i.e., feed, gavage, drinking water (or juice), capsule administration, and nasogastric intubation).

compounds with broad structural diversity. Compounds with markedly different structures, metabolic pathways, toxicity endpoints, and modes of toxic action may be assigned the same EDT class and, hence, to the predictive safe intake level (i.e., TTC level). For instance, Class I includes a wide range of predictive safe compounds and those with very low toxic potential, such as aliphatic linear and methyl-substituted primary alcohols, certain dicarboxylic acids, amino acids, various lactones, sugars, sugar alcohols and acids, bile acids, benzoic acid and related compounds, among numerous other chemical classes.

Moreover, even within the same chemical class, compounds can be categorized into different EDT classes. For example, linear or simply branched aliphatic acyclic hydrocarbons can be placed into different classes based on their specific structures. While hexane is classified under Class IV at Q28d(i)), substances with a terminal double bond conjugated with another double bond (i.e., terminal dienes) may be classified into either Class IV or III at Q28s(i)) or Q28s(ii)), respectively. Other compounds within this chemical class may be categorized into Class I at Q9.

Furthermore, assigning compounds to a single representative chemical class can be fraught with difficulties. While structurally simple compounds such as ethanol, acetaldehyde, or acetic acids can be easily assigned to primary alcohols, aldehydes, and carboxylic acids, respectively, assigning compounds with more than one functional groups and/or complex skeletons to a single representative chemical class, for the purposes of describing the compounds sorted into the various EDT classes, can be extremely difficult as their toxic potential and mode of toxic action are a result of their various structural features and are often not due to a single structural feature. For example, the chemical classifications of 1) disodium;4-[4-[[5-(2-bromoprop-2-enoylamino)-2-sulfonatophenyl]diazenyl]-3-methyl-5-oxo-4H-pyrazol-1-yl]-2,5-dichlorobenzenesulfonate (Lanasol Yellow 4G, CAS 70247-70-0), 2) ethyl 2-[[(1R)-1-cyclohexyl-2-[(2S)-2-[[4-[(Z)-N'-hydroxycarbamimidoyl]phenyl]methylcarbamoyl]azetidin-1-yl]-2-oxoethyl]amino]acetate (Exanta, CAS 192939-46-1), and 3) methyl 3-chloro-5-[(4,6-dimethoxypyrimidin-2-yl)carbamoylsulfamoyl]-1-methylpyrazole-4-carboxylate (Halosulfuron-methyl, CAS 100784-20-1) into a single chemical class is not possible due to their structural complexities (see Figures A, B, and C below where the potential major chemical classifications and the corresponding structural features are color coded).

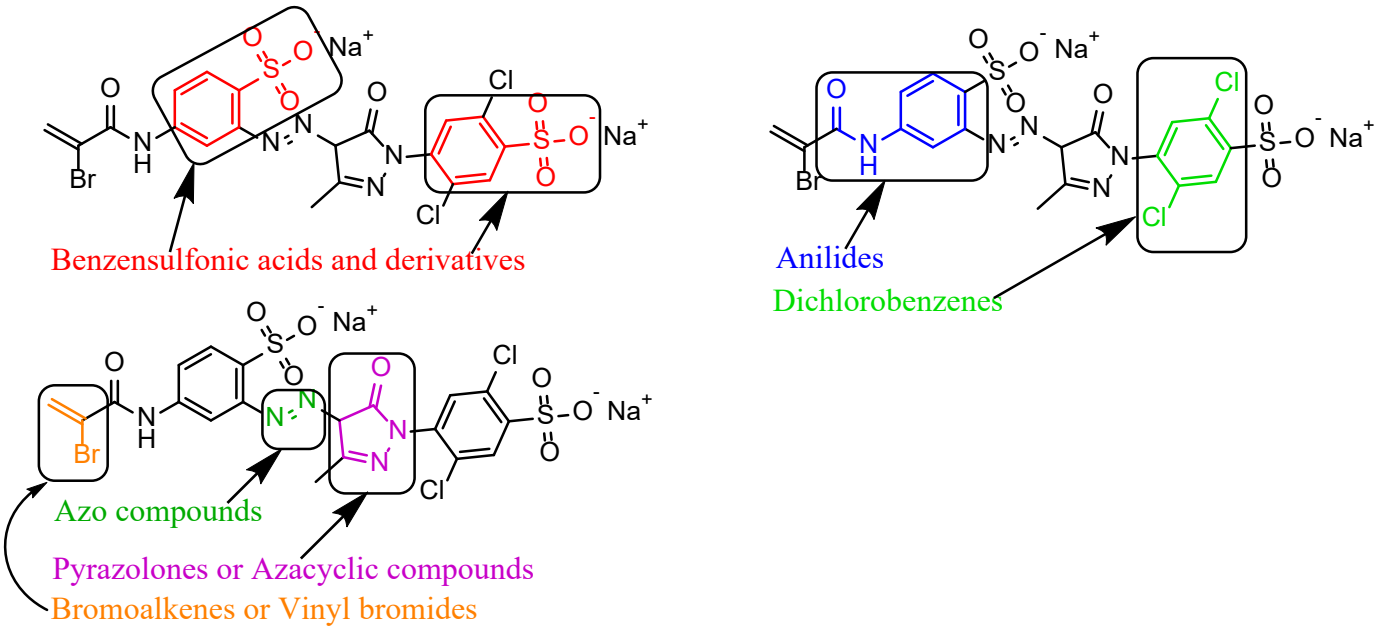


Figure A. Potential chemical classes of Lanasol Yellow 4G

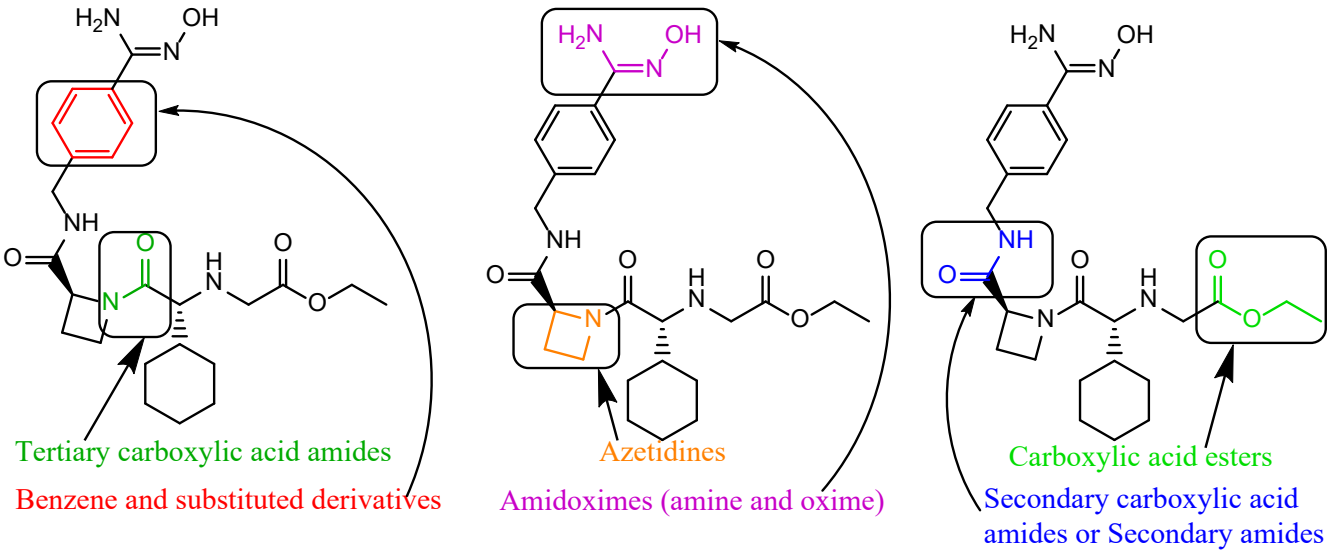


Figure B. Potential chemical classes of Exanta

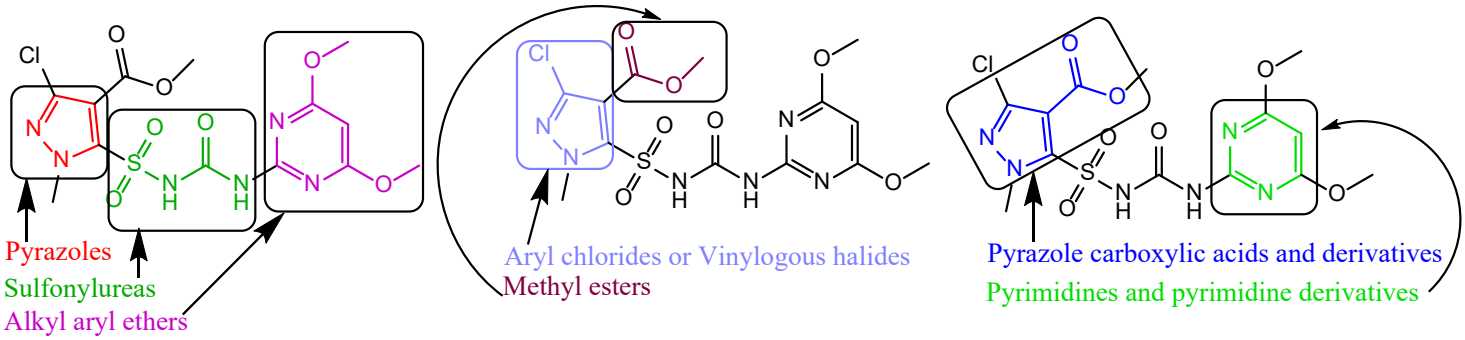


Figure C. Potential chemical classes of Halosulfuron-methyl

FDA notes that most compounds in the EDT DB are structurally complex, often containing multiple functional groups and/or intricate skeletons. As a result, assigning these compounds to a single chemical group is frequently not possible. To enable analysis of the chemical space covered by the EDT DB, we used the Classyfire software tool (http://classyfire.wishartlab.com/) to assign each compound to its respective chemical classes (Feunang et al., 2016). Given the structural complexity of many compounds, most can be categorized into multiple chemical classes. To address this, alternative chemical classifications are also provided in the EDT DB. For example, in the case of Lanasol Yellow 4G (see Figure A above), the EDT DB lists its chemical class as:

"Amino acids, peptides and analogues/Amino acids and derivatives/Alpha amino acids and derivatives **OR** Benzenesulfonic acids and derivatives **OR** 1-Sulfo, 2-unsubstituted aromatic compounds **OR** Anilides **OR** Benzenesulfonyl compounds **OR** Dichlorobenzenes **OR** N-arylamides **OR** Aryl chlorides **OR** Pyrazolones **OR** Sulfonyls **OR** Organosulfonic acids **OR** Azo compounds **OR** Secondary carboxylic acid amides **OR** Bromoalkenes **OR** Vinyl bromides **OR** Azacyclic compounds **OR** Organobromides **OR** Organochlorides **OR** Carbonyl compounds**."**

Here, **"OR"** denotes alternative chemical classifications, reflecting the compound's ability to fit into multiple categories based on its structure. FDA notes that while Classyfire places this compound into "Alpha amino acids and derivatives" likely because it contains functional groups or moieties that overlap with descriptors used for amino acids, Lanasol Yellow 4G does not contain amino acid(s) in its structure.

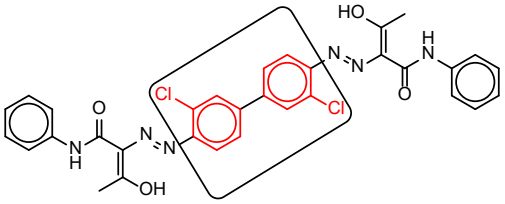
Below, to further demonstrate the difficulty of defining the EDT classes based on chemical classes, selected compounds from the EDT DB belonging to the chemical class of chlorobenzenes (compounds where one or more chlorine atoms are directly bonded to the carbon atoms of a benzene ring) and chlorobenzene derivatives will be utilized. 'Chlorobenzenes and chlorobenzene derivatives' are a subgroup within the chemical class of 'aryl chlorides' (compounds in which one or more chlorine atoms are directly bonded to an aromatic ring). In turn, 'aryl chlorides' is a subgroup within 'organochlorides', which, in turn, is a subgroup of 'organohalides'.

The compounds within the chemical group of chlorobenzenes and chlorobenzene derivatives display broad structural variation, hence broad range of toxicity, endpoints of

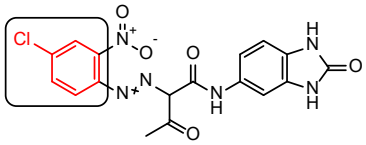
toxicity, and mode of toxic action. Therefore, unsurprisingly, chlorobenzenes and chlorobenzene derivatives may be placed into any of the six EDT classes (see Figure D below after the reference) and they exhibit broad structural variation (with multiple possible additional chemical classifications/grouping) even within the same EDT class.

In summary, placing structurally complex compounds into a single chemical group and describing EDT classes solely in terms of the chemical classes they encompass are fraught with difficulties and, therefore, will not be attempted at this time. As mentioned earlier, the possible chemical classes of all compounds are provided in the EDT DB and allow for the search of specific chemical groups by interested parties for their own analysis.

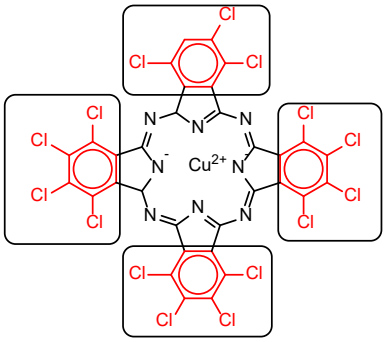
Examples of EDT Class I Chlorobenzenes and chlorobenzene derivatives:



Diarylanilide Yellow (CAS 6358-85-6)

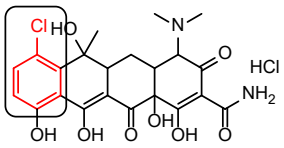


Copper phthalocyanine (CAS 147-14-8)

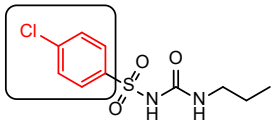


C.I. Pigment Orange 36 (CAS 12236-62-3)

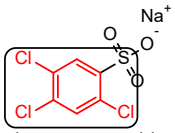
Examples of EDT Class II Chlorobenzenes and chlorobenzene derivatives:



Chlortetracycline hydrochloride (CAS 64-72-2)

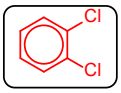


Chlorpropamide (CAS 94-20-2)

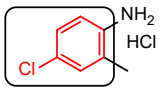


Sodium;2,4,5-trichlorobenzenesulfonate (CAS 53423-65-7)

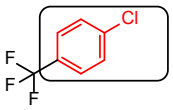
Examples of EDT Class III Chlorobenzenes and chlorobenzene derivatives:



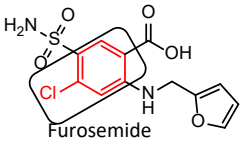
1,2-Dichlorobenzene (CAS 95-50-1)



4-Chloro-2-methylaniline HCl (CAS 3165-93-3)

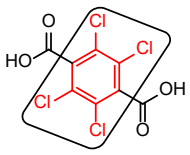


4-Chlorobenzotrifluoride (CAS 98-56-6)

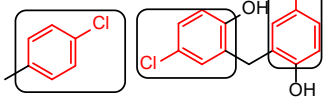


Furosemide (CAS 54-31-9)

Examples of EDT Class IV Chlorobenzenes and chlorobenzene derivatives:

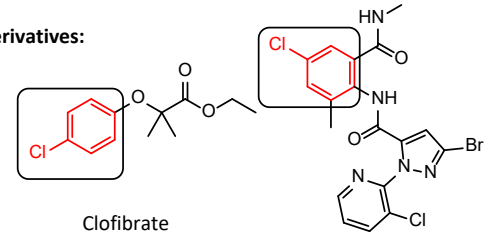


Chlorthal (CAS 2136-79-0)



4-Chlorotoluene (CAS 106-43-4)

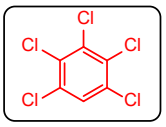
Dichlorophen (CAS 97-23-4)



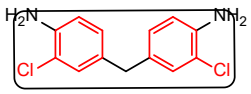
Clofibrate (CAS 637-07-0)

Chlorantraniliprole (CAS 500008-45-7)

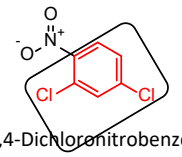
Examples of EDT Class V Chlorobenzenes and chlorobenzene derivatives:



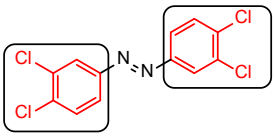
Pentachlorobenzene (CAS 608-93-5)



4,4'-Methylenebis(2-chloroaniline) (CAS 101-14-4)

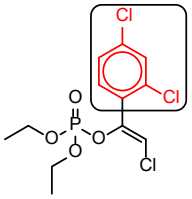


2,4-Dichloronitrobenzene (CAS 611-06-3)

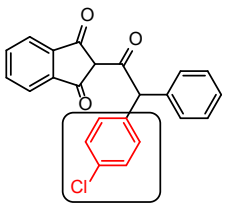


3,4,3',4'-Tetrachloroazobenzene (CAS 14047-09-7)

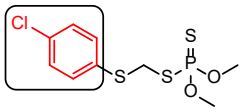
Examples of EDT Class VI Chlorobenzenes and chlorobenzene derivatives:



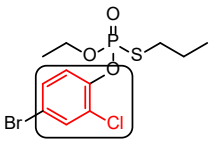
Chlorfenvinphos (CAS 470-90-6)



Chlorophacinone (CAS 3691-35-8)



Carbophenothion (CAS 786-19-6)



Profenofos (CAS 41198-08-7)

Figure D. Examples of chlorobenzenes and chlorobenzene derivatives from the EDT DB spanning the six EDT classes displaying broad structural variation

4.6.2 Cumulative Distribution of NELs in Various EDT Classes for the Combined EDT DB

We have prepared NEL distribution plots to help visualize the overlap amongst the toxic potentials of substances in the various EDT classes. We also compared the resolution of the toxic potentials of substances found in the original (pre-validation) EDT DB classified using the pre-validation EDT (red lines) and the toxic potentials of substances found in the combined EDT DB classified using the post-validation EDT (green lines) (see Figures 3 and 4).

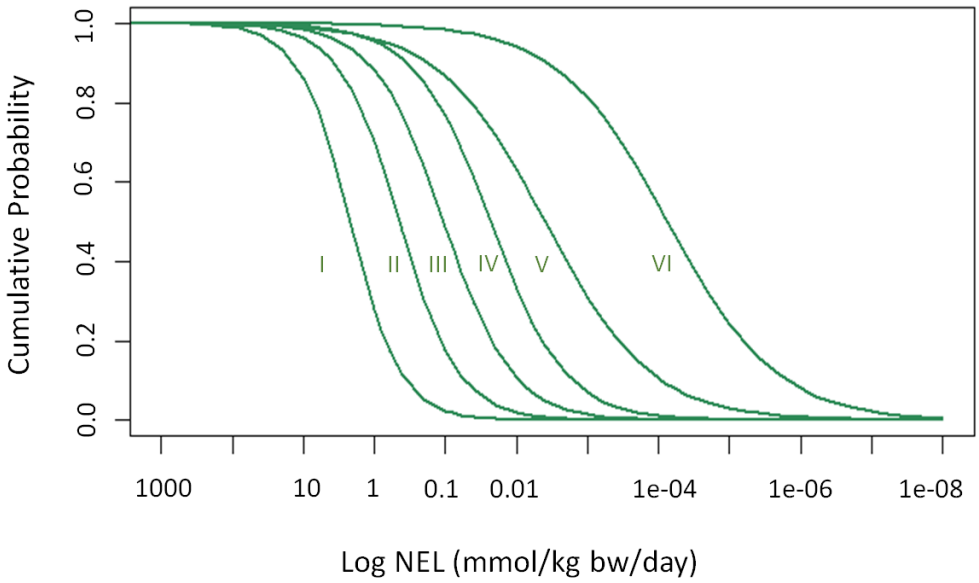


Figure 3. NEL distribution plot for the six EDT classes employing the post-validation EDT for the substances found in the combined EDT DB

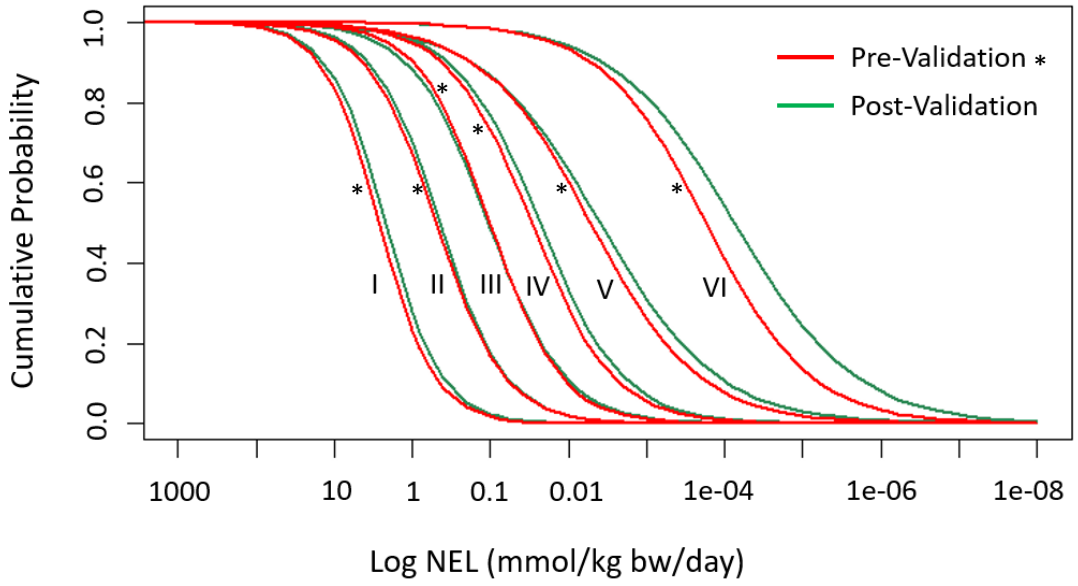


Figure 4. Comparison of the NEL distributions of substances found in the various EDT classes using the pre-validation EDT (red lines, as applied to the original (pre-validation) EDT DB) with the post-validation EDT (green lines, as applied to the combined EDT DB)

The pre-validation EDT questions were developed with the help of the original (pre-validation) EDT DB; hence, unsurprisingly, the red lines in Figure 4 show a good resolution of the toxic potentials of substances found in the six EDT classes in the original (pre-validation) EDT DB when these substances were classified using the pre-validation EDT. A large number of naïve substances became available through the external validation DB. Some of the naïve substances had structural features, such as functional groups, moieties, or skeletal structures, that either had no representative substances or only a very limited number of representative substances in the original (pre-validation) EDT DB (see section 4.4.3). Therefore, we found that the pre-validation EDT needed to be updated based on the results of the external validation (see section 4.4 for details) to either newly address or refine the sorting of functional groups, moieties, and skeletal features that were either not represented or were underrepresented in the original (pre-validation) EDT DB to ensure the broad applicability domain of the EDT and its maximal performance for substances with very wide structural variation. The validation suggested that certain groups of compounds were 'underclassified' (i.e., placed into a class lower than they should have been (e.g., organotins containing halogens covalently bound to tin, aflatoxins, brevetoxins, ochratoxins, and others)) while some other groups of compounds were 'overclassified' (i.e., placed into a class of higher concern than they should have been (e.g., certain antibiotics, epoxidized fatty acids, various colors and pigments, steviol and its glycosides, and others)) (see section 4.4.3). All of these issues were rectified during the validation improving the accuracy of the EDT classifications and ensuring optimal resolution of the varying toxic potentials of substances with broad structural variation with many and varied applications. Based on Figure 4, considering that the post-validation EDT was applied to a much larger dataset with much broader structural variation than the pre-validation EDT with a smaller DB that was used for the development of the tool, and the extensive validation of the EDT, it is clear that the post-validation EDT can resolve the toxic potentials of compounds even better than the pre-validation EDT.

4.6.3 Derivation of the Finalized TTCs Based on the Combined EDT DB

Table 8 provides the finalized (post-validation) EDT TTCs calculated based on the data contained in the combined EDT DB according to the method described in section 3.3. The EDT TTCs have not changed much as a result of the validation even though the number of substances based on which they were calculated was expanded. Moreover, the TTCs remained relatively stable in spite of the updates and refinements to the pre-validation EDT questions indicating the reliability of the tool. Going forward, the finalized EDT TTCs will be used.

Table 8. The finalized (post-validation) EDT TTCs

EDT Class	I	II	III	IV	V	VI
Pre-validation EDT TTC (µg/kg bw/d)	403	51	14	2.9	0.031	0.00030
Finalized EDT TTC (µg/kg bw/d)	**385**	**45**	**12**	**2.9**	**0.052**	**0.00053**
Finalized EDT TTC (µmol/kg bw/d)	2.14	0.241	0.0624	0.0104	0.000170	1.63E-06
5th percentile NEL (µmol/kg bw/day)	214	24.1	6.24	1.04	0.0170	1.63E-04
95th percentile NEL (µmol/kg bw/day)	19629	4457	1615	548.0	291.9	7.491
Median MW	180.16	187.22	187.12	277.27	302.38	323.44
MW range* (5th to 95th percentile range)	42.08-1844.62 (75.17-890.89)	30.03-1701.21 (89.04-566.88)	34.08-1755.63 (90.16-589.73)	40.05-2285.61 (94.84-598.66)	56.06-1921.69 (121.75-855.26)	140.09-1111.30 (192.83-593.53)
Total No. of substances	341	637	572	1065	435	91
No. of substances used for TTC calculation**	243	405	366	736	239	70

*Adjusted based on the number of subunits (in case of substances with two or more identical (non-covalently bonded) subunits.[5]
**The discrepancy between the total number of substances in each class and those used for TTC calculations arises because some studies provided only LOAELs and no NOAELs, which prevented their inclusion in the calculations. Additionally, NO(A)ELs from studies shorter than 84 days were excluded from TTC calculations for Classes I-V.

As stated before, there are 3,141 substances in the combined EDT DB. For 2,757 of these substances, the best representative study yielded a NEL. (As we did not use studies with a duration of less than 84 days to calculate chronic class TTCs except for Class VI (as this class had the fewest number of representative substances in the combined EDT DB), NELs for 2,057 substances were used for TTC calculations.) When comparing all 2,757 NELs (directly taken from the toxicology studies using units of mg/kg bw/day but adjusted for duration) with their corresponding class TTCs, no Class I, II, III, IV, V, or VI substance has a duration adjusted NEL below its pre- or post-validation class TTC (in units of mg/kg bw/day); hence, both the pre-validation and post-validation TTCs are protective when applied to a dataset of 2,757 unique substances with very broad structural variation.

We note that while the original (pre-validation) EDT DB contained only 52 Class VI substances, of which 46 could be used for the Class VI TTC calculation, the combined EDT DB—upon which the finalized TTCs are based—now includes 91 Class VI substances, three times the number used in the original Munro Class II TTC. For the Class VI TTC

[5] For example, zinc diamyldithiocarbamate (CAS 15337-18-5) has two identical diamyldithiocarbamate subunits. Therefore, its MW was divided by two.

calculations, NELs for 70 of these 91 substances were utilized, which is more than double the number used for the original Munro Class II TTC calculation.

Class VI aims to capture the most toxic substances available. Due to their extreme toxicity and often limited commercial applicability, Class VI substances are not typically tested in oral studies other than acute. Consequently, despite a thorough screening of a wide range of toxicological databases, we were unable to identify additional Class VI substances with subacute, subchronic, chronic, or reproductive and developmental toxicity studies for inclusion in the combined EDT DB.

Nevertheless, given the extremely low EDT Class VI TTC value of 0.00053 µg/kg bw/day, we believe this threshold is protective for any potential substances that may be assigned to Class VI. Additionally, this Class VI TTC is lower than any other published TTC values, being only one-fifth of the genotoxicity carcinogenicity threshold of 0.15 µg/person/day (0.0025 µg/kg bw/day) (Kroes et al., 2005). Thus, the FDA anticipates that the Class VI TTC level will effectively protect all substances classified within EDT Class VI.

5. Conclusions

The EDT questions were created based on one of the most important toxicological principles stating that chemicals that are similar structurally and are, or expected to be, similar metabolically, are also expected to be toxicologically similar. Structurally and metabolically similar compounds often belong to the same congeneric group/category and, as they are expected to behave in a toxicologically similar manner, within the same congeneric group toxicological data for data rich compounds within that congeneric group can be used to aid the safety evaluation of data poor substances (read-across) in the same group. Based on the publicly available toxicological and ADME data for a wide range of chemicals with broad structural variation, we tried to group chemicals, identify trends, and make reliable predictions based on the now current state of science to predict the relative chronic oral toxic potentials of chemicals. Most EDT questions were designed to enable the user to perform read-across by capturing toxicologically related substances at the same question, sub-question, or sub-sub-question. Performing read-across will be automated in the EDT software currently under development.

While for certain groups of chemicals a large amount of safety data exists to make reliable and robust predictions, for some groups, only limited or no data are available at this time. The external validation of the EDT showed that when additional information and data become available, the EDT can be further refined to make it more precise. In the future, FDA may review and update the EDT questions and classifications to ensure that new information is incorporated into the EDT and the EDT reflects the current state of science. In addition to ensuring that the EDT questions are continually improved, the periodic review will also help reaffirm and strengthen the scientific basis of the current questions of the EDT.

FDA considers the EDT to be a tool for toxicologists that can help sort chemicals based on/according to their relative chronic oral toxic potential and provide predictive safe intake levels (TTC) for a wide variety of data poor substances. We do not believe that the EDT should be used as the sole source of toxicological evaluation of any new or otherwise data poor compounds. The EDT should be seen as one more tool to help toxicologists evaluate the safety of compounds but must not be used to replace professional judgement in the safety evaluation of chemicals. Over time and with experience and refining, we hope that the

EDT, in conjunction with other new approach methodologies, may help refine, reduce, and replace animal testing.

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Appendix 2: The Combined (Post-validation) EDT Database

The combined EDT DB is available as one of the documents posted on FDA's Peer Review website.

Appendix 3: Organization of the Post-validation EDT

Figure 2 visually represents the decision-making flow within the EDT, outlining how responses to each of the 47 main questions guide the classification of a compound. For users of assistive technology or readers requiring a text-based version, the following description outlines the logic sequence step-by-step.

Detailed Description:

1. **Question 1**
   - Yes: Assign to Class I
   - No: Go to Q2
2. **Question 2**
   - Before assigning the substance to a class at Q2, the user is directed to crosscheck against Qs 5 and 6; that is, if due to the presence of a toxic element addressed at Q5 or a structural moiety of high toxic potential at Q6 the substance would go to a higher class at either Q5 or Q6 than it would at Q2, the substance will be assigned to the highest class it would get at either Q2, Q5, or Q6.
   - Yes: Assign to Class II, III, V, or VI.
   - No: Go to Q3
3. **Question 3**

- Before assigning the substance to a class at Q3, the user is directed to crosscheck against Qs 5, 6, 43, and 44; that is, if due to the presence of a toxic element addressed at Q5 or a structural moiety of high toxic potential at Q6 the substance would go to a higher class at either Q5 or Q6 than it would at Q3, the substance will be assigned to the highest class it would get at either Q3, Q5, Q6, Q43, or Q44.
- Yes: Assign to Class II, III, IV, or V.
- No: Go to Q4

4. **Question 4**
   - Yes: Go to Q5
   - No: Go to Q6
5. **Question 5**
   - Yes: Go to Q6 or assign to Class II, III, IV, V, or VI
   - No: Assign to Class IV, V, or VI
6. **Question 6**
   - Yes: Assign to Class I, II, IV, V, or VI
   - No: Go to Q7
7. **Question 7**
   - Yes: Go to Q8 or assign to Class II, III, IV, or V
   - No: Go to Q9
8. **Question 8**
   - Yes: Assign to Class III, V, or VI
   - No: Go to Q11 or Q33 or assign to Class IV
9. **Question 9**
   - Yes: Assign to Class I
   - No: Go to Q10
10. **Question 10**
    - Yes: Go to Q11
    - No: Go to Q23
11. **Question 11**
    - Yes: Go to Q1, Q12, Q13, Q14, or Q33
    - No: Go to Q12 or Q33
12. **Question 12**
    - Yes: Go to Q1, Q10, Q30, or Q33 or assign to Class IV
    - No: Go to Q13
13. **Question 13**
    - Yes: Go to Q14
    - No: Go to Q15
14. **Question 14**
    - Yes: Go to Q33 or assign to Class II, III, or V
    - No: Assign to Class IV
15. **Question 15**
    - Yes: Go to Q28
    - No: Go to Q16
16. **Question 16**
    - Yes: Assign to Class III
    - No: Go to Q17
17. **Question 17**
    - Yes: Go to Q19
    - No: Go to Q18
18. **Question 18**
    - Yes: Assign to Class III or V or go to Q28. If no at Q28, go to Q47 for final class

assignment.
    - No: Go to Q28
19. **Question 19**
    - Yes: Assign to Class III, IV, or V
    - No: Go to Q20
20. **Question 20**
    - Yes: Go to Q21 or Q47 or assign to Class III, IV, or V. Before assigning the compound to a class at Q47, crosscheck against Q43. Assign the substance to the highest class it would get at either Q47 or Q43.
    - No: Go to Q21
21. **Question 21**
    - Yes: Go to Q28
    - No: Go to Q22
22. **Question 22**
    - Yes: Assign to Class III
    - No: Go to Q47, but crosscheck against Q43. Assign the substance to the highest class it would get at either Q43 or Q47.
23. **Question 23**
    - Yes: Go to Q24
    - No: Go to Q29
24. **Question 24**
    - Yes: Go to Q1 or Q25 or assign to Class I or III
    - No: Go to Q25
25. **Question 25**
    - Yes: Assign to Class III
    - No: Go to Q26 or Q47
26. **Question 26**
    - Yes: Go to Q27
    - No: Go to Q47. Before assigning the substance to a class at Q47, crosscheck against Q28. Assign the substance to the highest class it would receive at either Q47 or Q28.
27. **Question 27**
    - Yes: Assign to Class IV or V
    - No: Go to Q28
28. **Question 28**
    - Yes: Go to Q1 or assign to Class III, IV, or V
    - No: Assign to Class II or III
29. **Question 29**
    - Yes: Go to Q33
    - No: Go to Q30
30. **Question 30**
    - Yes: Go to Q31 or assign to Class I, III, or IV
    - No: Go to Q47. Before assigning the substance to a class at Q47, crosscheck against Q28. Assign the substance to the highest class it would receive either at Q28 or Q47.
31. **Question 31**
    - Yes: Assign to Class III
    - No: Go to Q32
32. **Question 32**
    - Yes: Assign to Class III or IV
    - No: Go to Q28. Before assigning the substance to a class at Q28, crosscheck

against Q24. Assign the substance to the highest class it would receive either at Q28 or Q24.

33. **Question 33**
    - Yes: Assign to Class IV or V
    - No: Go to Q34
34. **Question 34**
    - Yes: Go to Q1 or assign to Class III or IV
    - No: Go to Q35
35. **Question 35**
    - Yes: Go to Q36 or Q38
    - No: Go to Q47
36. **Question 36**
    - Yes: Go to Q37 or Q41
    - No: Go to Q47
37. **Question 37**
    - Yes: Assign to Class III or V
    - No: Go to Q47
38. **Question 38**
    - Yes: Assign to Class IV
    - No: Go to Q39
39. **Question 39**
    - Yes: Assign to Class III
    - No: Go to Q40
40. **Question 40**
    - Yes: Assign to Class I or III
    - No: Go to Q41
41. **Question 41**
    - Yes: Assign to Class III
    - No: Go to Q42
42. **Question 42**
    - Yes: Assign to Class II or III
    - No: Go to Q43
43. **Question 43**
    - Yes: Assign to Class III, IV, or V
    - No: Go to Q44
44. **Question 44**
    - Yes: Assign to Class III, IV, or V
    - No: Go to Q45
45. **Question 45**
    - Yes: Go to Q46
    - No: Go to Q28
46. **Question 46**
    - Yes: Assign to Class II or III
    - No: Go to Q47
47. **Question 47**
    - Yes: Go to Q11, Q19, or Q35 or assign to Class I, II, III, or IV
    - No: Assign to Class IV