



Istituto Superiore di Sanità

ToxMic User Manual

ToxMic

**(Structure Alerts for the *in vivo*
micronucleus assay in rodents)**

Version 1.0 of 23 April 2008

User Manual

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Introduction

The *in vivo* mutagenicity studies, shortly followed by carcinogenicity, are posing high demand for test-related recourses. Among those, the micronucleus test in rodents is the most widely used, as follow up to positive *in vitro* mutagenicity results. A recent survey of the (Q)SAR models for mutagenicity and carcinogenicity has indicated that no (Q)SAR models for *in vivo* micronucleus are available in the public domain: therefore, the development and extensive use of estimation techniques such as (Q)SARs, read-across and grouping of chemicals, might have a huge saving potential for this endpoint.

The ToxMic rulebase provides a list of structural alerts (SAs, see section “Structural Alerts”) for a preliminary screening of potentially *in vivo* mutagens. These SAs are molecular functional groups or substructures that are known to be linked to the positive *in vivo* micronucleus assay.

The ToxMic rulebase is encoded as a plug-in to Toxtree application (<http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE>). This ensures that users being familiar with the Toxtree software can easily apply the features of the ToxMic plug-in to their problems.

Background

Mutagenicity testing is an important part of the regulatory hazard assessment of chemicals. It is undertaken for two main reasons: a) to detect chemicals that might cause genetic damage in germ cells, and thus increase the burden of heritable (genetic) disease in the human population; and b) to detect chemicals that might be carcinogenic (based on the assumption that mutagenesis, for example in somatic cells, is a key event in the process of carcinogenesis). Since no method is able alone to detect all possible genotoxic events, a wide array of test systems has been developed and accepted internationally in regulatory schemes.

Most often, these methods are used within a 2-tiered integrated testing approach: Tier 1 includes *in vitro* assays, and Tier 2 includes *in vivo* assays. As a matter of fact, mutagenicity testing was the first toxicity endpoint for which *in vitro* assays were accepted for regulatory testing, some 25 years ago. The latter usually comprise bacterial mutagenicity and cytogenetics tests, although gene mutation testing in cultured mammalian cells is sometimes also undertaken.

Tier 2 of the testing strategy involves the use of short-term *in vivo* studies (usually a bone-marrow cytogenetics assay) to assess whether any potential for genotoxicity detected at the Tier 1 *in vitro* stage is actually expressed in the whole animal. Thus, negative results *in vitro* are usually considered sufficient to indicate lack of mutagenicity, whereas a positive result is not considered sufficient to indicate that the chemical represents a mutagenic hazard (i.e. it could be a false positive). The above approach to genotoxicity testing has been adopted throughout the EU and has been recommended internationally as part of the strategy for predicting and quantifying mutagenic and carcinogenic hazard (see the Technical Guidance Documents at the European Chemicals Agency (ECHA): http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r7a_en.pdf?vers=20_08_08).

According to an assessment carried out by the former European Chemicals Bureau (ECB), the *in vivo* mutagenicity studies, shortly followed by carcinogenicity, are posing high demand for test-related recourses (Pedersen et al. 2003; Van der Jagt et al. 2004). Among those, the micronucleus test in rodents is the most widely used, as follow up to positive *in vitro* mutagenicity results. A recent survey of the (Q)SAR models for mutagenicity and carcinogenicity (performed jointly by ISS and ECB) has indicated that no (Q)SAR models for *in vivo* micronucleus are available in the public domain (Benigni et al. 2007): therefore, the development and extensive use of estimation techniques such as (Q)SARs, read-across and grouping of chemicals, might have a huge saving potential for this endpoint.

Structure Alerts for the *in vivo* micronucleus assay

A Structural Alert (SA) is a molecular functional group or substructure that is known to be linked to a certain chemical property or reactivity (Benigni and Bossa 2006; Benigni and Bossa 2008).

The compilation of SAs for the *in vivo* micronucleus assay in rodents provided here, is based on both the existing knowledge on the mechanisms of toxic action and a structural analysis of the chemicals tested in the assay. The development and scope of these SAs are described in detail in the accompanying scientific document (Benigni et al. 2009). The document can be freely downloaded at http://ecb.jrc.ec.europa.eu/DOCUMENTS/QSAR/EUR_23844_EN.pdf

The result is the optimized list of alerts in Appendix 1. The SAs included in ToxMic are 35; it includes a number of the Benigni / Bossa alerts for mutagenicity and carcinogenicity (Benigni et al. 2008) together with five additional substructures identified in the course of the research on *in vivo* micronucleus. For the sake of clarity, the codes of alerts of the Benigni / Bossa rulebase in Toxtree are maintained, whereas the five additional alerts have new codes.

Classification Scheme

The processing of a query chemical by the ToxMic plug-in for Toxtree can result in two different outcomes. According to the SAs that are utilized in the ToxMic rulebase, chemicals are classified into one of the following two categories:

- Class 1 (At least one positive structural alert for the micronucleus assay)
- Class 2 (No positive alert for the micronucleus assay)

If one or more SAs for the *in vivo* micronucleus assay are found the substance is classified as positive (Class 1). If no SAs for the *in vivo* micronucleus assay are found the substance is classified as Class 2.

The Toxtree ToxMic plug-in

The ToxMic plug-in for Toxtree is Java based and utilizes the Toxtree plug-in interface to make its functionality available to the user. The following two sections describe the installation and the usage of the ToxMic plug-in for Toxtree. For a better use of this plug-in, the user should previously become familiar with Toxtree and its functionalities, as described in detail in the Toxtree installation and user manuals (<http://ecb.jrc.ec.europa.eu/qsar/qsar-tools/index.php?c=TOXTREE>).

Installation

The implementation of the ToxMic plug-in follows the standard plug-in mechanism of Toxtree as used in the existing decision tree methods (Verhaar scheme, Cramer rules, etc.). The functionality of the ToxMic plug-in is included in the "ToxMic-ISS.jar" library file which has to be copied into the plug-in directory of the Toxtree installation as shown in Figure 1.

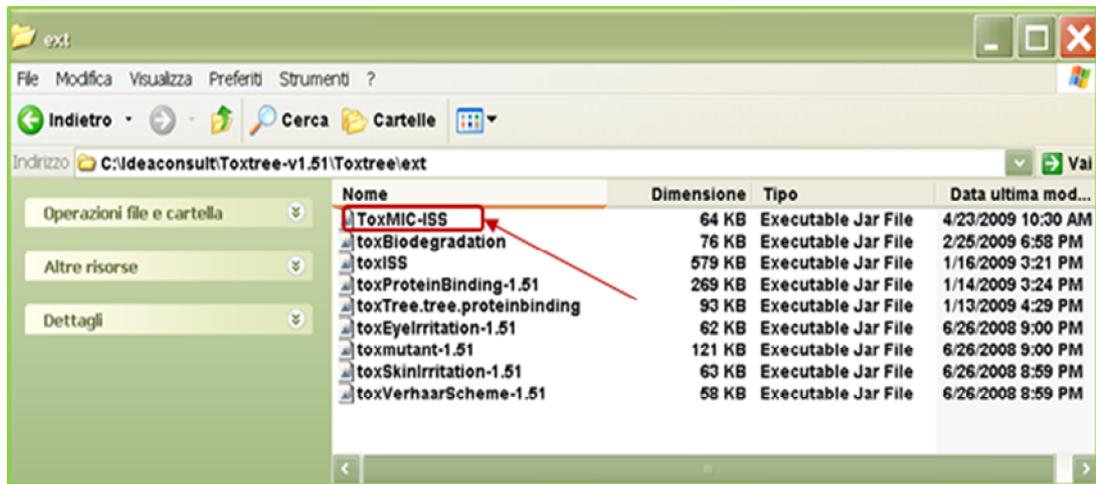


Figure 1 Installation of the ToxMic plug-in for Toxtree. The library file "ToxMic-ISS.jar" has to be copied into the "ext" plug-in directory of the Toxtree installation.

ToxMic usage

Once the ToxMic plug-in has been installed into Toxtree, it has to be activated. Within the “Select a tree” dialog window, the ToxMic plug-in can be selected from the list of available decision trees like any other Toxtree plug-in as illustrated in Figures 2 and 3.



Figure 2 The “Select a decision tree” option in the ToxMic menu bar.

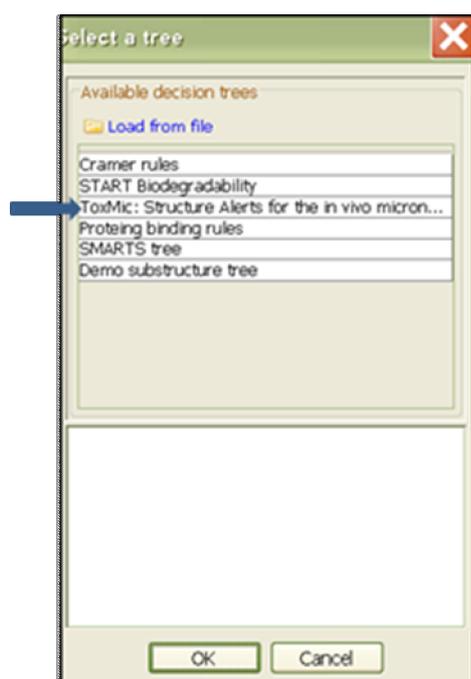


Figure 3 Decision tree selection in the “Select a tree” dialog window of Toxtree.

The Toxtree plug-in is now ready to use.

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As shown in Figure 4, the SMILES of the chemical under investigation can now be entered into the Toxtree main window, and then evaluated by pressing the "Estimate" button.

The screenshot shows the Toxtree (Estimation of Toxic Hazard - A Decision Tree Approach) v1.51 interface. The main window displays the SMILES string ClC=CCCC=O in the input field. Below the input field, the "Available structure attributes" table is visible, showing various attributes and their values. The "Structure diagram" section shows a ball-and-stick model of the chemical structure. The "Toxic Hazard" section displays the result: "At least one positive structural alerts for the micronucleus assay" (Class I) and "No alerts for the micronucleus assay (Class II)". The "Verbose explanation" section lists various structural alerts (QSA1-QSA35) and their status (Yes/No) for the in vivo micronucleus assay in rodents. The SMILES string ClC=CCCC=O is repeated next to each alert.

Alert	Status	SMILES
QSA1. Acyl halides	No	ClC=CCCC=O
QSA2. Alkyl (C₆) or benzyl ester of sulphonic or phosphonic acid	No	ClC=CCCC=O
QSA3. N-methylol derivatives	No	ClC=CCCC=O
QSA4. Monohaloalkene	Yes	ClC=CCCC=O
QSA5. 1 or 2 mustard	No	ClC=CCCC=O
QSA6. Propiolactone and propiolactone	No	ClC=CCCC=O
QSA7. Epoxides and aziridines	No	ClC=CCCC=O
QSA8. Aliphatic halogeno	No	ClC=CCCC=O
QSA9. Alkyl nitrite	No	ClC=CCCC=O
QSA10. α,β unsaturated carbonyls	No	ClC=CCCC=O
QSA11. Simple aldehyde	Yes	ClC=CCCC=O
QSA12. Quinone	No	ClC=CCCC=O
QSA13. Hydrazine	No	ClC=CCCC=O
QSA14. Aliphatic amine and amine	No	ClC=CCCC=O
QSA15. Isocyanate and isothiocyanate groups	No	ClC=CCCC=O
QSA16. Alkyl carbamate and thiocarbamate	No	ClC=CCCC=O
QSA18. Polycyclic Aromatic Hydrocarbons	No	ClC=CCCC=O
QSA19. Heterocyclic Polycyclic Aromatic Hydrocarbons	No	ClC=CCCC=O
QSA21. Alkyl and aryl N-nitroso groups	No	ClC=CCCC=O
QSA22. Amide and urethane groups	No	ClC=CCCC=O
QSA23. Aliphatic N-nitro	No	ClC=CCCC=O
QSA24. α,β unsaturated alkoxy	No	ClC=CCCC=O
QSA25. Aromatic nitroso group	No	ClC=CCCC=O
QSA26. Aromatic ring N-oxide	No	ClC=CCCC=O
QSA27. Nitro aromatic	No	ClC=CCCC=O
QSA28. Primary aromatic amine, hydroxyl amine and its derived esters (with rest)	No	ClC=CCCC=O
QSA28a. Aromatic mono- and dialkylamine	No	ClC=CCCC=O
QSA29. Aromatic diams	No	ClC=CCCC=O
QSA30. Coumarins and Furcoumarins	No	ClC=CCCC=O
QSA31. 1,2-dialkoxy-benzene	No	ClC=CCCC=O
QSA32. 1-phenoxy-benzene	No	ClC=CCCC=O
QSA34. N-acceptor-pyridyl-N-acceptor	No	ClC=CCCC=O
QSA35. Oxolane	No	ClC=CCCC=O
QSA36. Carbodiimides	No	ClC=CCCC=O
Query alert? At least one alert fired?	Yes	Class At least one positive

Figure 4 Example of the evaluation of sample chemical structure by ToxMic plug-in, with detailed description of the evaluation process in the main Toxtree window.

The compound under investigation is searched for the presence of all structural alerts for the *in vivo* micronucleus assay and the number of found alerts is kept.

If at least one alert -notifying a chemical potentially positive for the micronucleus assay- is found, the compound under investigation is declared as Class 1 ("At least one positive structural alert for the micronucleus assay").

If no alerts are found at all, the compound is declared as Class 2 ("No positive alert for the micronucleus assay")

The detailed summary of all alerts found in the compound under investigation is shown in the main Toxtree window (Figure 4).

Decision tree editing

The “Method”-Edit decision tree” menu of Toxtree can be used in order to edit an existing decision tree, for example ToxMic (Figure 5). The “Load from file” submenu can be used for loading any tree in the “Decision tree editor”.

The screenshot displays the Toxtree Decision Tree Editor. The main window shows a decision tree with nodes labeled SA19 through SA36. A sidebar on the left contains menu options for Decision tree, Decision node, Decision rules, and Categories. A 'Rule' dialog box is open, showing details for rule SA29 'Aromatic diazo'. The dialog includes a table with columns for ID, Title, SMARTS, and Explanation. Below the table, there is a 'Rule explanation' field containing text about sulfonic acid groups and a 'Rule explanation' field with a chemical structure of an aromatic diazo compound. At the bottom, there are radio buttons for 'Yes branch' and 'No branch', and an 'Edit example' button.

Figure 5. Decision tree editor on a copy of ToxMic rules

Before exiting from the Decision tree editor, users should save the edited decision tree by using the “File”-“Save” menu on the upper left corner of the main Decision tree editor window. A reminder is displayed if the user tries to exit the Decision tree editor without having saved his work.

References

Benigni R, Bossa C. 2006. Structural alerts of mutagens and carcinogens. *Curr Comput -Aid Drug Des* 2:169-176.

Benigni R, Bossa C. 2008. Structure Alerts for carcinogenicity, and the Salmonella assay system: a novel insight through the chemical relational databases technology. *Mutat Res Revs* 659:248-261.

Benigni, R., Bossa, C., Jeliaskova, N. G., Netzeva, T. I., and Worth, A. P. The Benigni / Bossa rulebase for mutagenicity and carcinogenicity - a module of Toxtree. EUR 23241 EN. 2008. Luxembourg, Office for the Official Publications of the European Communities. EUR - Scientific and Technical Report Series.

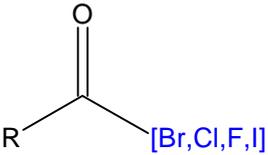
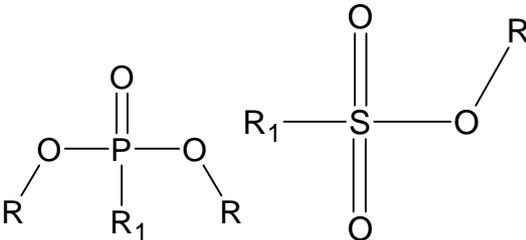
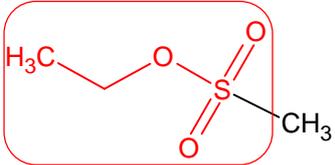
Benigni, R., Bossa, C., Netzeva, T. I., and Worth, A. P. Collection and evaluation of (Q)SAR models for mutagenicity and carcinogenicity. EUR 22772 EN. 2007. Luxembourg, Office for the Official Publications of the European Communities. EUR - Scientific and Technical Research Series.

Benigni, R., Bossa, C., Tcheremenskaia, O., and Worth, A. P. Development of Structure Alerts for the *in vivo* micronucleus assay in rodents. EUR 23844 EN. 2009. Luxembourg, Office for the Official Publications of the European Communities. EUR - Scientific and Technical Research Series.

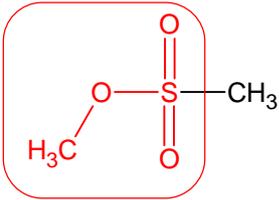
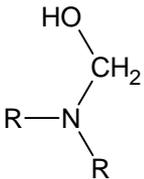
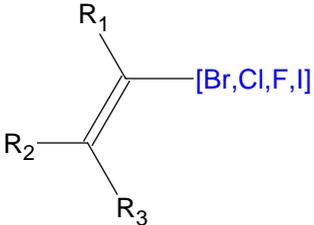
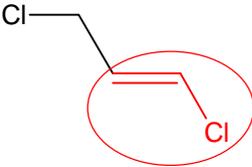
Pedersen, F., de Bruijn, J., Munn, S. J., and Van Leeuwen, K. Assessment of additional testing needs under REACH. Effects of (Q)SARs, risk based testing and voluntary industry initiatives. JRC report EUR 20863 EN. 2003. Ispra, EUR.

Van der Jagt, K., Munn, S. J., Torslov, J., and de Bruijn, J. Alternative approaches can reduce the use of test animals under REACH. Addendum to the Report "Assessment of additional testing needs under REACH. Effects of (Q)SARs, risk based testing and voluntary industry initiatives". JRC Report EUR 21405 EN. 2004. Ispra, European Commission Joint Research Centre.

APPENDIX 1. Structural Alerts included in the ToxMic plug-in.

STRUCTURAL ALERT	DETAILS AND EXAMPLES
<p>SA_1: Acyl halides</p> 	<p>R = any atom/group, except OH, SH</p> <hr/> <p>No representatives</p>
<p>SA_2: alkyl (C<5) or benzyl ester of sulphonic or phosphonic acid</p> 	<p>R= Alkyl with C<5 (potentially substituted by halogens), or benzyl</p> <p>R1= any atom/group except OH, SH, O-, S⁻</p> <hr/>  <p>Name: Ethyl Methanesulfonate</p> <p>CAS: 62-50-0</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP¹</p>

¹ National Toxicology Program, <http://ntp.niehs.nih.gov/>

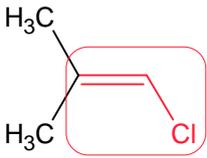
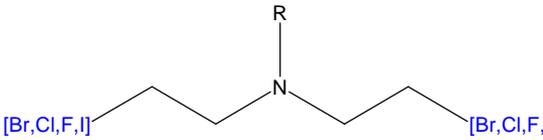
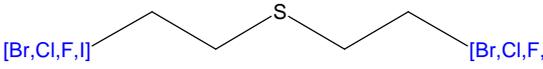
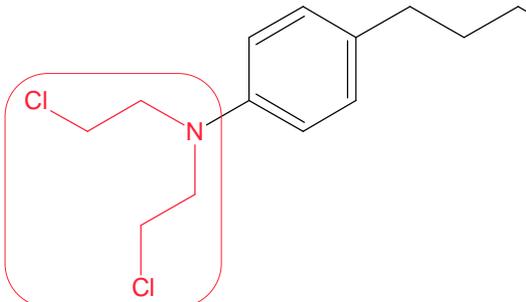
	 <p>Name: Methyl Methanesulfonate CAS: 66-27-3 In vivo Micronucleus (Rodent): Positive Reference: CCRIS²</p>
<p>SA_3: N-methylol derivatives</p> 	<p>R = any atom/group</p> <p>No positive representative</p>
<p>SA_4: Monohaloalkene</p> 	<p>R₁, R₂ (or R₃) = H or Alkyl R₃ (or R₂) = any atom/group except halogens</p>  <p>Name: 1,3-dichloropropene</p>

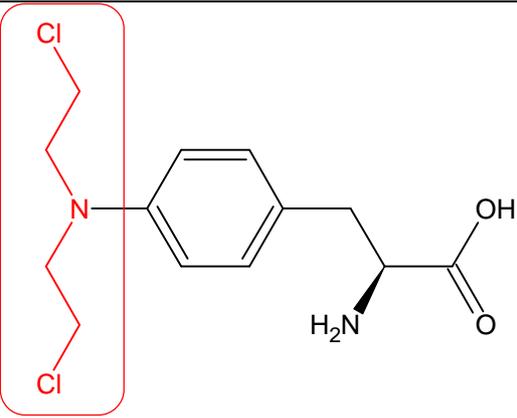
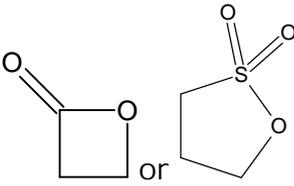
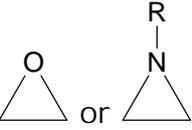
CAS: 542-75-6

² Chemical Carcinogenesis Research Information System, <http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS>

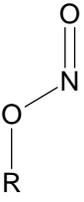
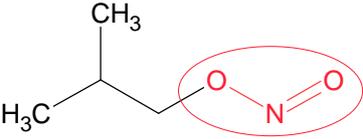
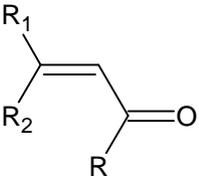
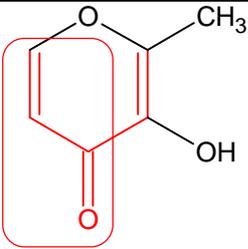
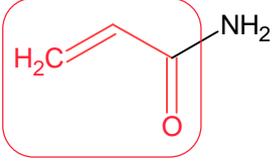
In vivo Micronucleus (Rodent): Positive

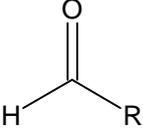
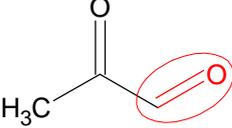
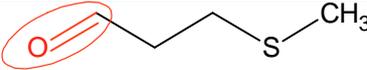
Reference: NTP

	 <p>Name: Dimethylvinyl Chloride</p> <p>CAS: 513-37-1</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
<p>SA_5: S or N mustard</p>  <p>or</p> 	<p>R = any atom/group</p>
	

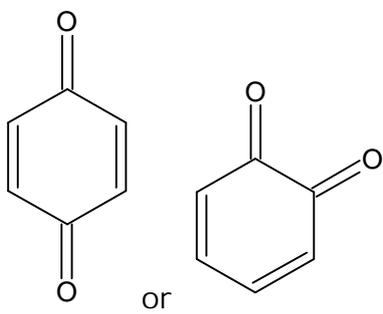
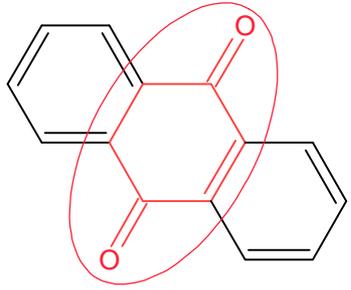
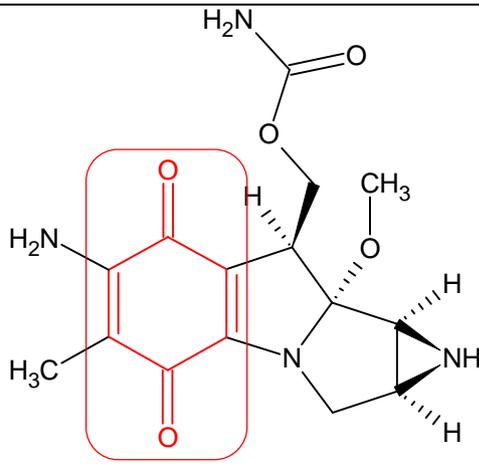
	<p>Reference: NTP</p>  <p>Name: Melphalan CAS: 148-82-3 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
<p>SA_6 Propiolactones or propiosultones</p> 	<p>Any substance with the displayed substructures</p> <p>No representatives</p>
<p>SA_7: Epoxides and aziridines</p> 	<p>R = any atom/group</p>
	 <p>Name: Ethylene Oxide CAS: 75-21-8</p>

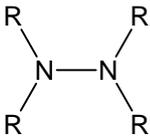
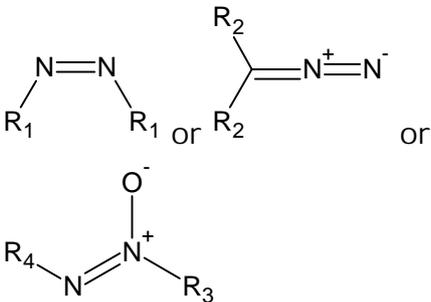
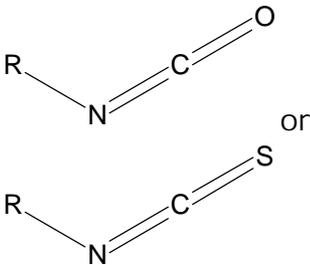
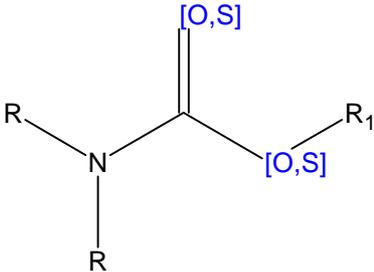
	<p>(Rodent): Positive Reference: CCRIS</p>
	<div data-bbox="885 343 1284 685" data-label="Chemical-Block"> </div> <p>Name: Triethylenemelamine CAS: 51-18-3 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
<p>SA_8: Aliphatic halogens</p> <div data-bbox="327 1162 614 1394" data-label="Chemical-Block"> </div>	<p>R = any atom/group</p> <div data-bbox="885 1231 1109 1336" data-label="Chemical-Block"> </div> <p>Name: 1,2-dibromoethane CAS: 106-93-4 In vivo Micronucleus (Rodent): Positive Reference: NTP</p> <div data-bbox="885 1754 1061 1917" data-label="Chemical-Block"> </div> <p>Name: 1,1-dichloroethane CAS: 75-34-3 In vivo Micronucleus</p>

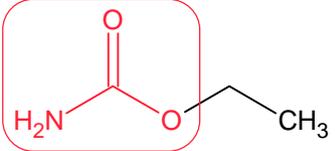
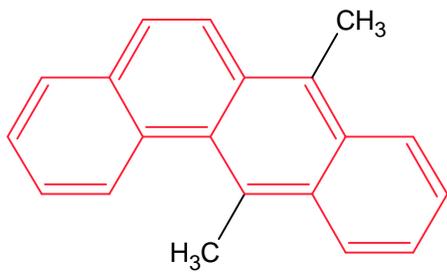
	<p>(Rodent): Positive Reference: CCRIS</p>
<p>SA_9: Alkyl nitrite</p> 	<p>R= any alkyl group</p>  <p>Name: Isobutyl Nitrite CAS: 542-56-3 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
<p>SA_10: Unsaturated carbonyls</p> 	<p>R1 and R2 = any atom/group, except alkyl chains with C>5 or aromatic rings. R= any atom/group, except OH, O⁻</p>  <p>Name: Maltol CAS: 118-71-8 In vivo Micronucleus (Rodent): Positive Reference: CCRIS</p> 

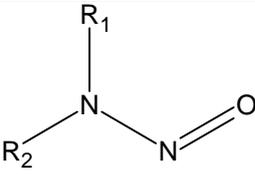
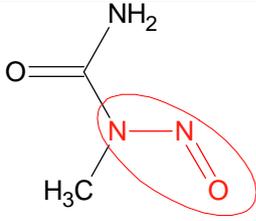
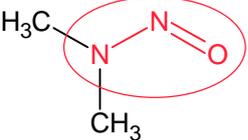
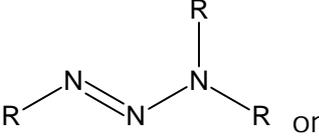
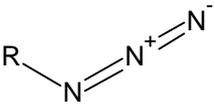
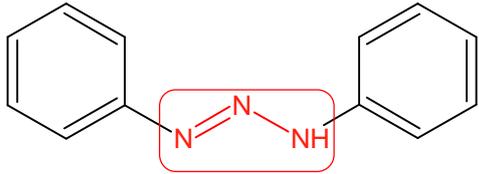
	<p>Name: Acrylamide</p> <p>CAS: 79-06-1</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>
<p>SA_11: Simple aldehyde</p> 	<p>R= aliphatic or aromatic carbon</p> <p>unsaturated aldehydes are excluded</p>  <p>Name: Pyruvaldehyde</p> <p>CAS: 78-98-8</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: Leadscope³</p>  <p>Name: 3-(methylthio)propionaldehyde</p> <p>CAS: 3268-49-3</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: Leadscope</p>

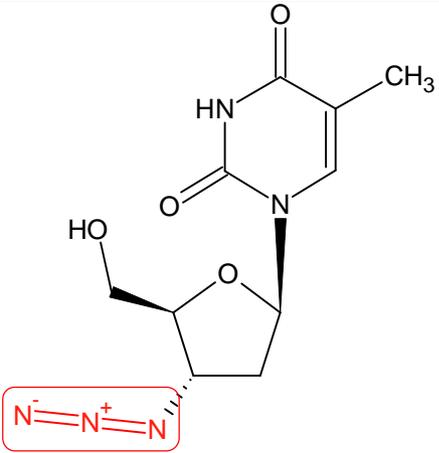
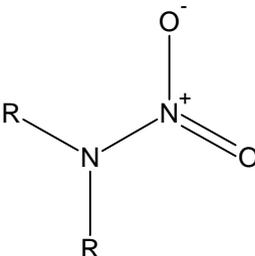
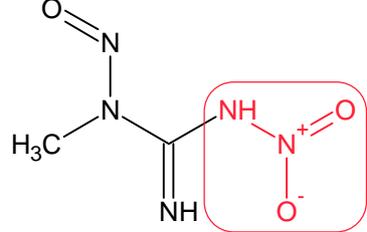
³ Leadscope Database, <http://www.leadscope.com/>

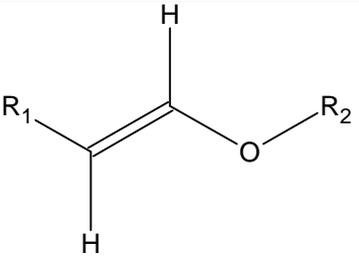
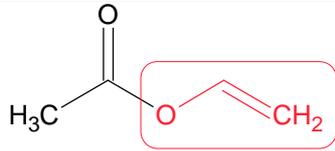
<p>SA_12: Quinones</p>  <p>or</p>	<p>Any substance with the displayed substructures</p>  <p>Name: 9,10-Anthraquinone CAS: 84-65-1 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
	 <p>Name: Mitomycin C CAS: 50-07-7 In vivo Micronucleus (Rodent): Positive Reference: CCRIS & NTP</p>

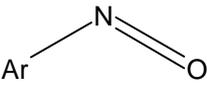
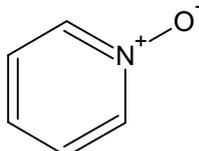
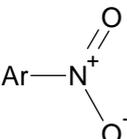
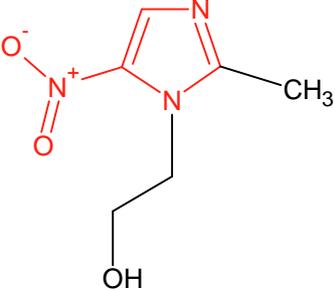
<p>SA_13: Hydrazine</p> 	<p>R= any atom/group</p> <p>No positive representative</p>
<p>SA_14: Aliphatic azo and azoxy</p> 	<p>R1 = Aliphatic carbon or hydrogen</p> <p>R2, R3 = Any atom/group</p> <p>R4 = Aliphatic carbon</p> <p>No representatives</p>
<p>SA_15: isocyanate and isothiocyanate groups</p> 	<p>R= any atom/group</p> <p>No positive representative</p>
<p>SA_16: alkyl carbamate and thiocarbamate</p> 	<p>R = Aliphatic carbon or hydrogen</p> <p>R1 = Aliphatic carbon</p>

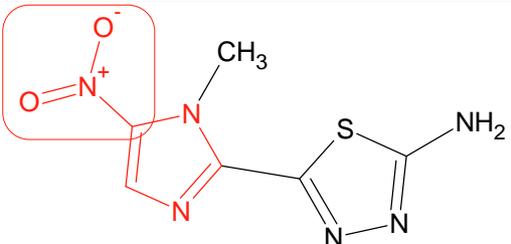
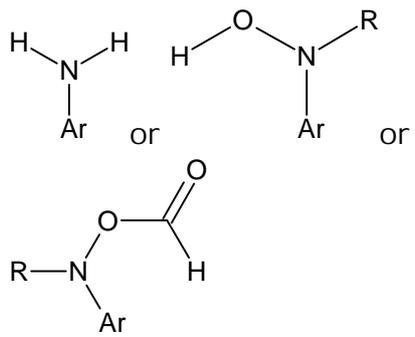
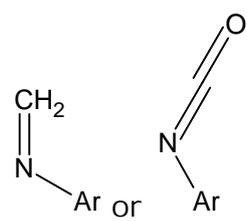
	 <p>Name: Urethane</p> <p>CAS: 51-79-6</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS & NTP</p>
<p>SA_18: Polycyclic Aromatic Hydrocarbons</p>	<p>Three or more fused rings, not heteroaromatic</p>  <p>Name: 7,12-Dimethylbenz(a)anthracene</p> <p>CAS: 57-97-6</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS & NTP</p>
<p>SA_19: Heterocyclic Polycyclic Aromatic Hydrocarbons</p>	<p>Three or more fused rings, heteroaromatic</p> <p>No positive representative</p>
<p>SA_21: alkyl and aryl N-nitroso groups</p>	<p>R1= Aliphatic or aromatic carbon,</p> <p>R2= Any atom/group</p>

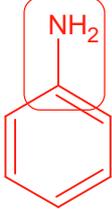
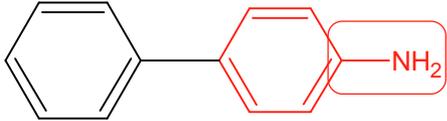
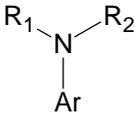
	 <p>Name: N-methyl-N-nitrosourea</p> <p>CAS: 684-93-5</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
	 <p>Name: N-nitrosodimethylamine</p> <p>CAS: 62-75-9</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>
<p>SA_22: azide and triazene groups</p>  <p>or</p> 	<p>R= Any atom/group</p>  <p>Name: Diazoaminobenzene</p> <p>CAS: 136-35-6</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS & NTP</p>

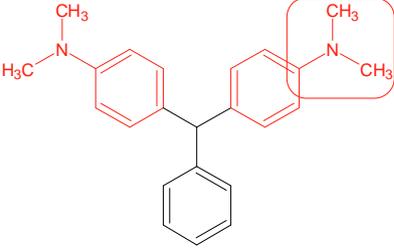
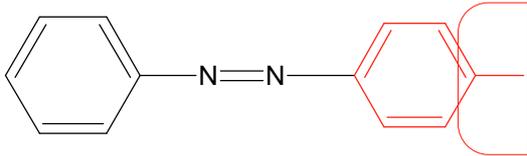
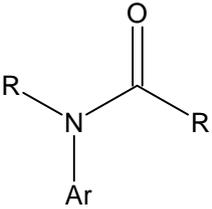
	 <p>The image shows the chemical structure of Zidovudine. It consists of a pyrimidine ring with a methyl group (CH₃) at the 5-position and a hydroxyl group (OH) at the 4-position. The nitrogen at the 1-position of the pyrimidine ring is linked to a ribose sugar ring. The ribose ring has a hydroxyl group (HO) at the 2' position. A red box highlights the triazene group (N=N=N) attached to the 3' position of the ribose ring.</p> <p>Name: Zidovudine CAS: 30516_87-1 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
<p>SA_23: aliphatic N-nitro group</p>  <p>The image shows the chemical structure of an aliphatic N-nitro group. It consists of a central nitrogen atom (N⁺) bonded to two R groups and a nitro group (O⁻ and O).</p>	<p>R= Aliphatic carbon or hydrogen</p>  <p>The image shows the chemical structure of N-methyl-N'-nitro-N-nitrosoguanidine. It consists of a central carbon atom double-bonded to two nitrogen atoms (NH) and single-bonded to a methyl group (H₃C) and a nitro group (N⁺ and O⁻). The nitro group is highlighted with a red box.</p> <p>Name: N-methyl-N'-nitro-N-nitrosoguanidine CAS: 70-25-7 In vivo Micronucleus (Rodent): Positive Reference: CCRIS</p>
<p>SA_24: Unsaturated aliphatic alkoxy group</p>	<p>R1= Any aliphatic Carbon R2 = Aliphatic or aromatic carbon</p>

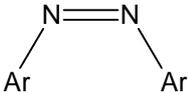
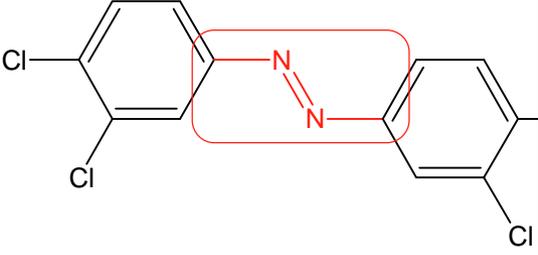
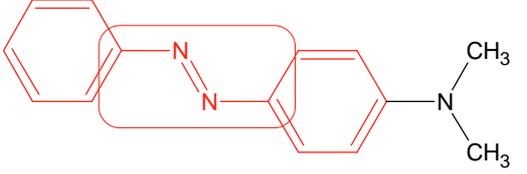
	 <p>Name: Vinyl Acetate</p> <p>CAS: 108-05-4</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
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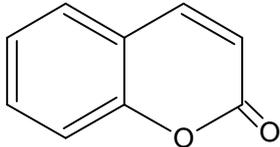
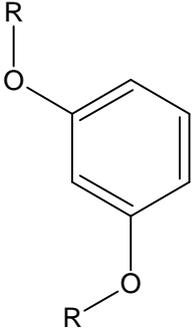
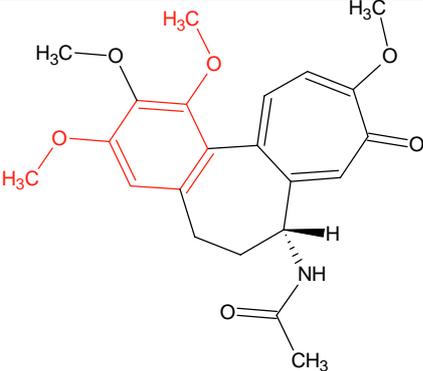
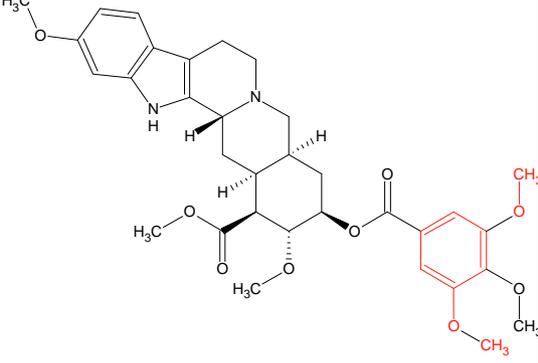
<p>SA_25: aromatic nitroso group</p> 	<p>Ar = Any aromatic/heteroaromatic ring</p> <p>No positive representative</p>
<p>SA_26: aromatic ring N-oxide</p> 	<p>Any aromatic or heteroaromatic ring</p> <p>No positive representative</p>
<p>SA_27: Nitro-aromatic</p> 	<p>Ar = Any aromatic/heteroaromatic ring:</p> <ul style="list-style-type: none"> -chemicals with ortho-disubstitution, or with an ortho carboxylic acid substituent are excluded; -chemicals with a sulfonic acid group (-SO₃H) on the same ring of the nitro group are excluded.
	 <p>Name: Metronidazole</p> <p>CAS: 443-48-1</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>

	 <p>Name: CL 64855</p> <p>CAS: 19622-55-0</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>
<p>SA_28: primary aromatic amine, hydroxyl amine and its derived esters</p>  <p>or amine generating group:</p> 	<p>Ar = Any aromatic/heteroaromatic ring</p> <p>R = Any atom/group</p> <p>-Chemicals with ortho-disubstitution, or with an ortho carboxylic acid substituent are excluded.</p> <p>-Chemicals with a sulfonic acid group (-SO₃H) on the same ring of the amino group are excluded.</p>

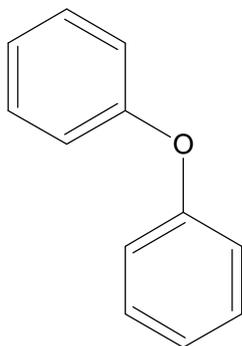
	 <p>Name: Aniline</p> <p>CAS: 62-53-3</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS & NTP</p>
	 <p>Name: 4-Biphenylamine</p> <p>CAS: 92-67-1</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
<p>SA_28bis: Aromatic mono- and dialkylamine</p> 	<p>Ar = Any aromatic/heteroaromatic ring</p> <p>R1 = Hydrogen, methyl, ethyl</p> <p>R2 = Methyl, ethyl</p> <p>-Chemicals with ortho-disubstitution, or with an ortho carboxylic acid substituent are excluded.</p> <p>-Chemicals with a sulfonic acid group (-SO₃H) on the same ring of the nitro group are excluded.</p>

	 <p>Name: Leucomalachite Green</p> <p>CAS: 129-73-7</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
	 <p>Name: 4-Dimethylaminoazobenzene</p> <p>CAS: 60-11-7</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>
<p>SA_28tris: aromatic N-acyl amine</p> 	<p>Ar = Any aromatic/heteroaromatic ring</p> <p>R = Hydrogen, methyl</p> <ul style="list-style-type: none"> • Chemicals with ortho-disubstitution, or with an ortho carboxylic acid substituent are excluded. • Chemicals with a sulfonic acid group (-SO₃H) on the same ring of the nitro group are excluded. <p>No positive representative</p>

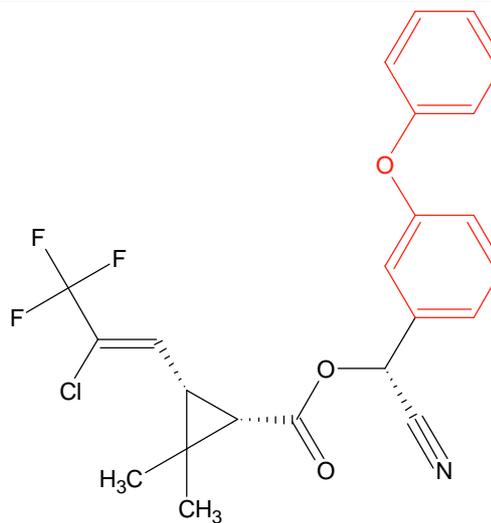
<p>SA_29: Aromatic diazo</p> 	<p>Ar = Any aromatic/heteroaromatic ring</p> <ul style="list-style-type: none"> Chemicals with a sulfonic acid group (-SO₃H) on both rings that contain linked to the diazo group are excluded.
	 <p>Name: 3,3',4,4'-Tetrachloroazobenzene</p> <p>CAS: 14047-09-7</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>
	 <p>Name: 4-Dimethylaminoazobenzene</p> <p>CAS: 60-11-7</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS</p>

<p>SA_30: Coumarins and Furocoumarins</p> 	<p>Any substance with the displayed substructure</p> <p>No positive representative</p>
<p>SA_32: 1,3-dialkoxy-benzene</p> 	<p>R= any alkyl group</p>  <p>Name: Colchicine CAS: 64-86-8 In vivo Micronucleus (Rodent): Positive Reference: CCRIS</p>
	 <p>Name: Reserpine CAS: 50-55-5 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>

SA_33: 1-phenoxy-benzene



Any substance with the displayed substructure.

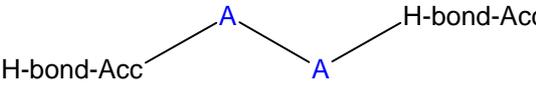
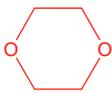
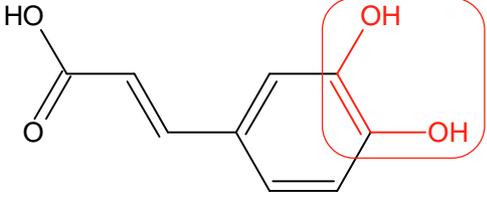


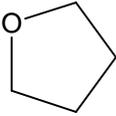
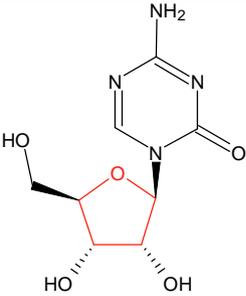
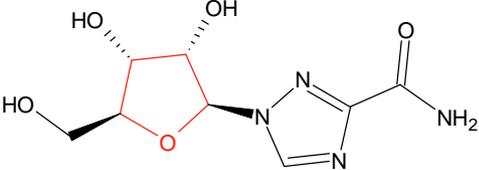
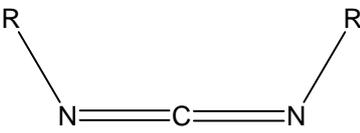
Name: Lambda-cyhalothrin

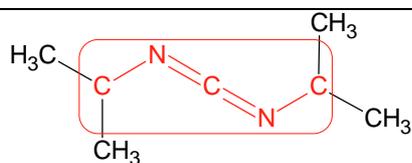
CAS: 91465-08-6

In vivo Micronucleus (Rodent):
Positive

Reference: CCRIS

<p>SA_34: hacceptor-path3-hacceptor</p> 	<p>A= Any atom, except Hydrogen</p> <p>H-bond-Acc= Any atom that is a potential Hydrogen bond acceptor:</p> <ul style="list-style-type: none"> a) any doubly bonded oxygen; b) any singly bonded oxygen, such as anion A-O- or hydroxyl A-OH; c) Uncharged imine, nitrile, or aromatic N. Examples of imines include C=NH, or C=N-Ak; aromatic N includes ARO -N-ARO, where both ARO-N bonds are cyclic, aromatic; d) an ether oxygen in the form C-O-C, where: neither C is substituted by a doubly-bonded N, S, or O; neither C is part of a carbonyl; at most one C is aromatic; the O is acyclic; e) C-O-C is cyclic, both C are sp³ hybridized; f) Any doubly bonded sulfur in thioxomethyl C=S, where S has no other attachments.
	 <p>Name: p-Dioxane</p> <p>CAS: 123-91-1</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: CCRIS & NTP</p>
	 <p>Name: 3,4-Dihydroxycinnamic acid</p> <p>CAS: 331-39-5</p> <p>In vivo Micronucleus (Rodent): Positive</p> <p>Reference: NTP</p>

<p>SA_35: Oxolane</p> 	<p>Any substance with the displayed substructure.</p>  <p>Name: 5-Azacytidine CAS: 320-67-2 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>  <p>Name: Ribavirin CAS: 36791-04-5 In vivo Micronucleus (Rodent): Positive Reference: NTP</p>
<p>SA_36: Carbodiimides</p> 	<p>R= any alkyl group</p>

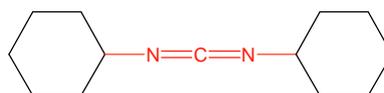


Name: Diisopropylcarbodiimide

CAS: 693-13-0

In vivo Micronucleus (Rodent):
Positive

Reference: NTP



Name: Dicyclohexylcarbodiimide

CAS: 538-75-0

In vivo Micronucleus (Rodent):
Positive

Reference: NTP