Training Manual on MetaPath Data Evaluation Record (DER) Composer

European Food Safety Authority (EFSA),

Acknowledgements: EFSA wishes to thank the following for the support provided to this scientific output: Richard Kolanczyk, Martina Panzarea, Juan Manuel Parra, Carla Dall'Agglio and Francesco Amoretti.

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1. Introduction

The Transparency Regulation, which amends the General Food Law (GFL), means that EFSA has new requirements for capturing, managing, handling and distributing data on plant protection products (PPP).

These changes require the specification of data formats for regulated product dossiers and allow documents to be submitted, searched, copied and printed, while ensuring compliance with legal requirements. It has been decided to use IUCLID formats and the IUCLID tool (managed by the European Chemicals Agency – ECHA) for data preparation, electronic submission and management of pesticide dossiers, by means of the ECHA Cloud platform. Furthermore, applicants should provide data on metabolism in the areas of residues and mammalian toxicology as attachments generated with the MetaPath software package called composers.

This Manual is focus on the MetaPath Data Evaluation Record (DER) composer. For MetaPath Metabolism Study Summary (MSS) Composer the applicant is referred to ANSES, 2020.

The training manual describes the different steps for data entry of mammalian (rat) metabolism studies submitted under the pesticide peer review process by using the MetaPath Data Evaluation Record (DER) composer.

The MetaPath DER composer is freely available in the software developer webpage together with MetaPath software and the MSS composer software: https://oasis-lmc.org/products/software/metapath.aspx

This Manual has been written in collaboration with US EPA.

Nomenclature and drawing instruction as described in the MetaPath MSS Composer Training Manual (ANSES, 2020) is also applicable to the DER composer. The applicant is advised first to read MetaPath MSS Composer Training Manual before this manual.

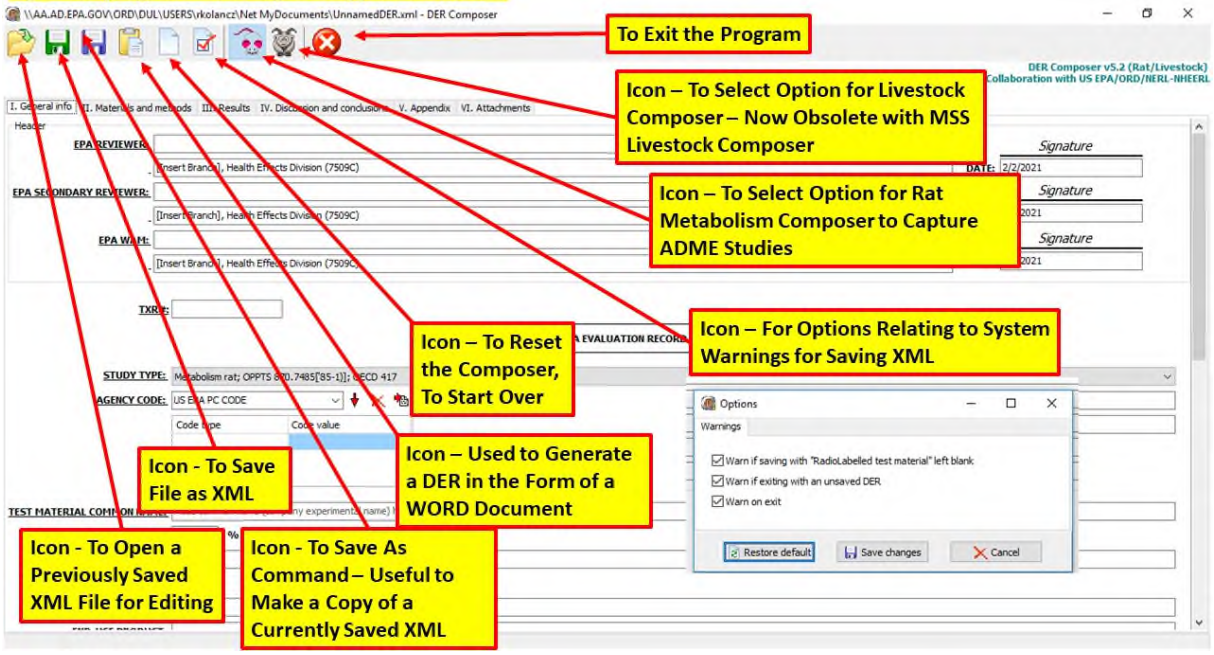
The xml file generated from the DER composer should be included as an attachment in IUCLID, under toxicokinetic study records as described in the EFSA MRL IUCLID Manual (EFSA, 2021).

2. Guideline for the use of DER/XML Composer – Rat Metabolism v.5.2

This guideline was prepared for use with DER Composer V5.2, a product resulting from the joint cooperation between the U.S. Environmental Protection Agency (USEPA) and the Laboratory of Mathematical Chemistry (LMC-Bourgas, Bulgaria).

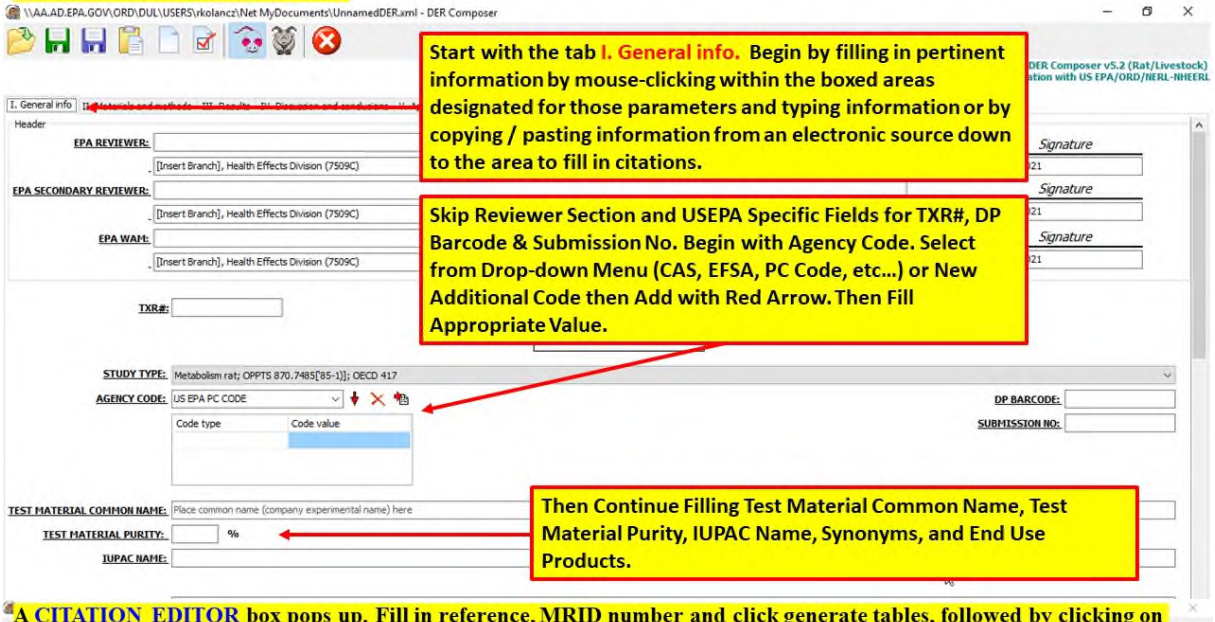
2.1. Opening DER Composer

Open the program – start up will appear as follows:

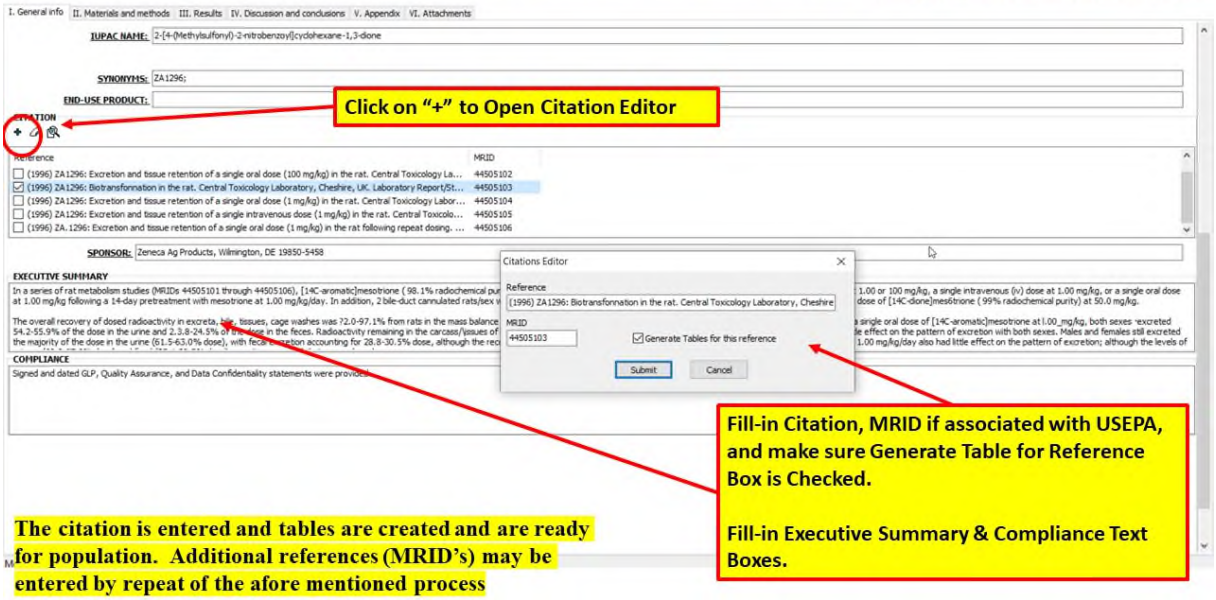


2.2. General information

Start with the tab I. General info. Begin by filling in pertinent information by mouse-clicking within the boxed areas designated for those parameters and typing information or by copying / pasting information from an electronic source down to the area to fill in citations.



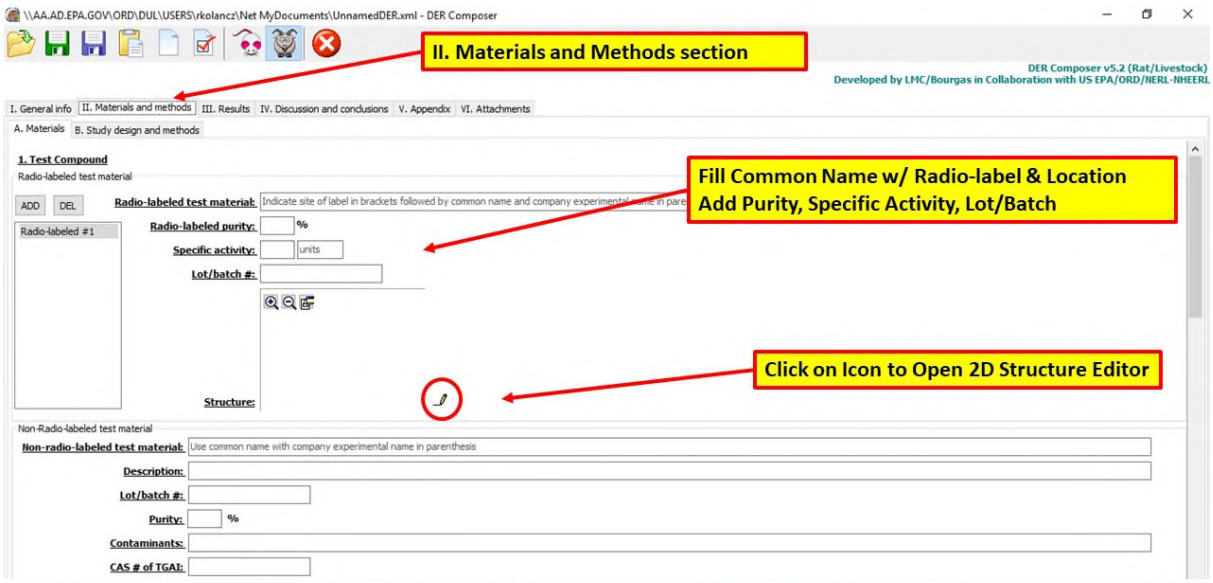
A CITATION EDITOR box pops up. Fill in reference, MRID number and click generate tables, followed by clicking on submit. If there are additional references repeat the process - click the + to add each, populate, and click submit.



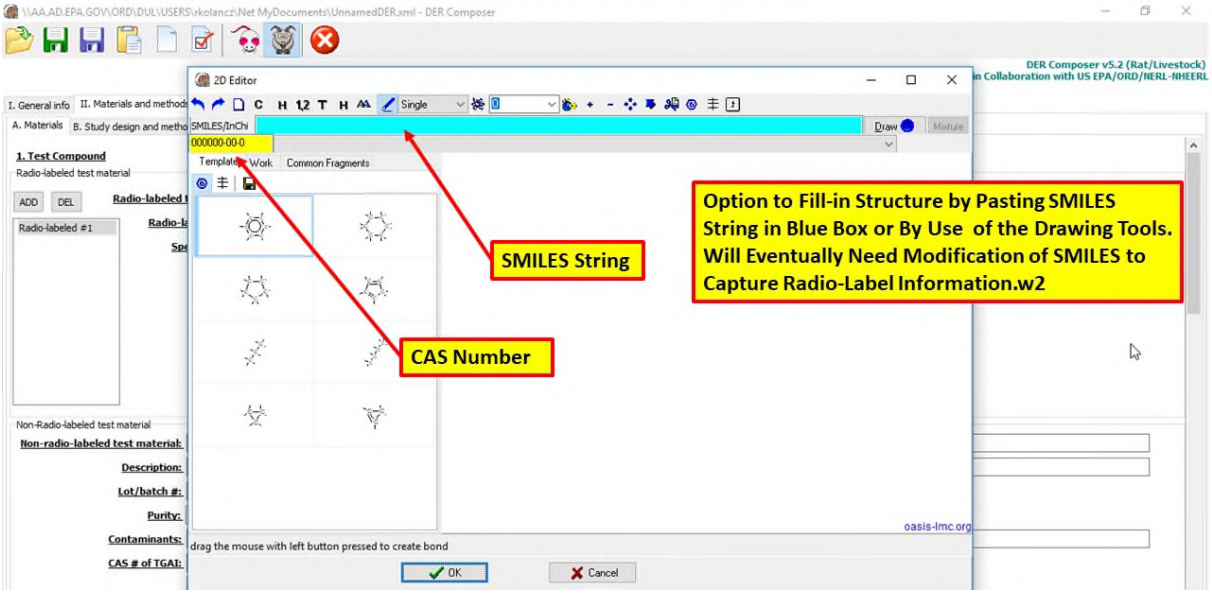
The citation is entered and tables are created and are ready for population. Additional references (MRID's) may be entered by repeat of the afore mentioned process

2.3. Material and methods

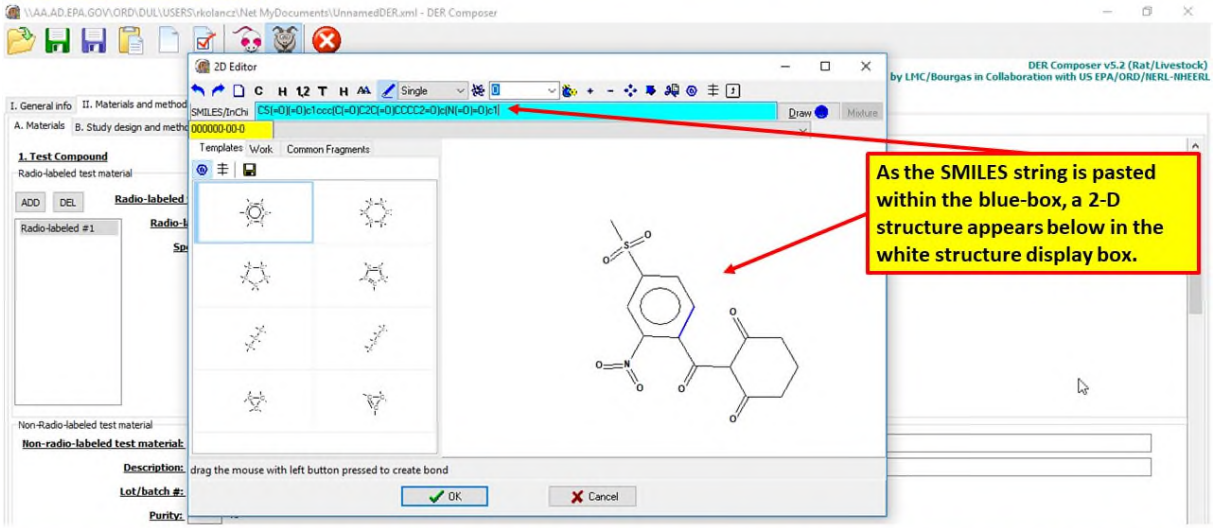
Next the tab II. Materials and methods and sub-tab A. Materials may be populated. Data is filled in via directly typing or copy/paste from electronic documents until reaching the structure entry. To enter the Radio-labeled Test Material and Non-radio-labeled Test Material structures:



Below is a graphic of the STRUCTURE DRAWING editor pop-up box. The large white area is the drawing workspace, the light-blue box is where a SMILES string may be entered or displayed, and the yellow box is where a CAS number can be entered. Scrolling over the top of the icons will give some indication of their utility.



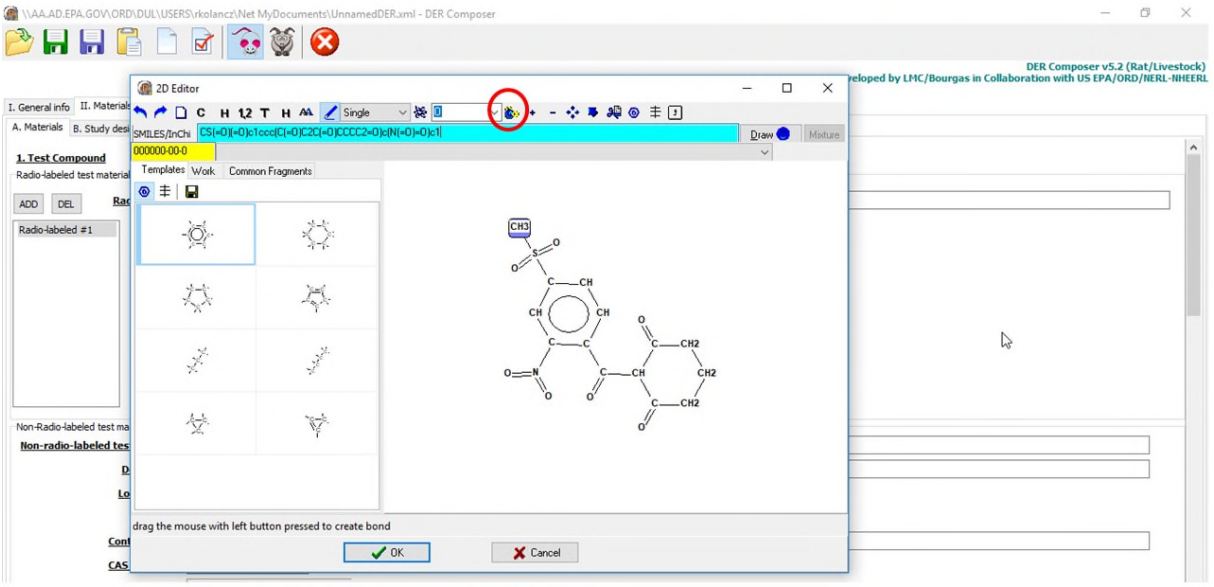
Perform a right-hand click of the mouse in the light-blue box of the STRUCTURE DRAWING package and select paste to enter the parent structure.



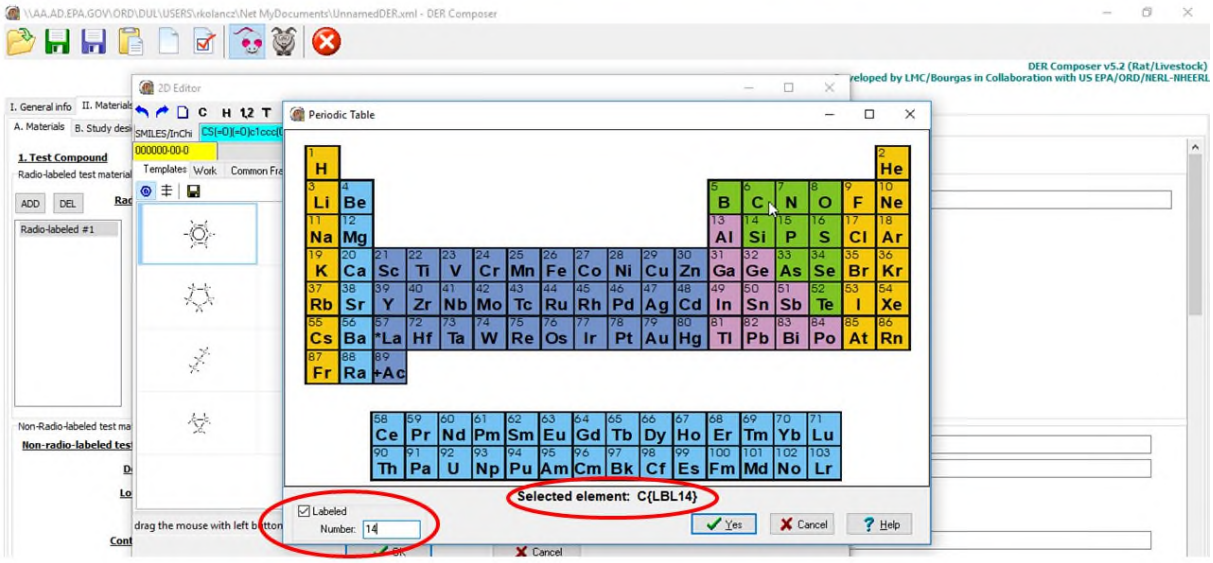
The parent structure (now present in the STRUCTURE DRAWING editor) may be modified utilizing tools within the editor. Specifically a label may be introduced in the structure of the radio-labeled parent.

Radio labeling of atoms

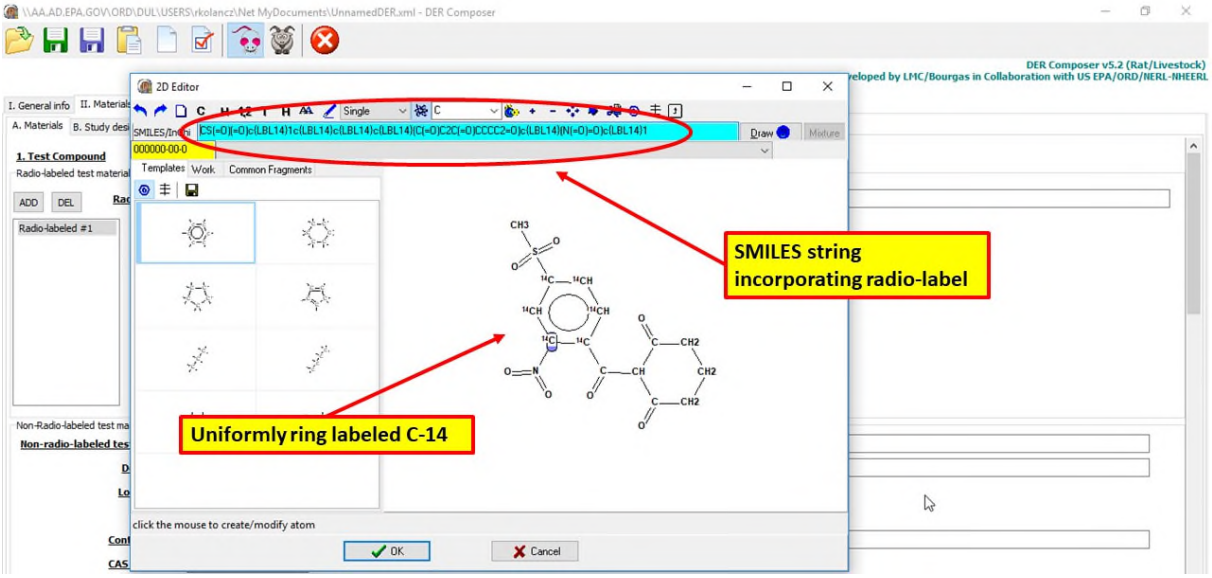
Within the STRUCTURE DRAWING window, open the periodic table by selecting the icon as circled in the figure below.

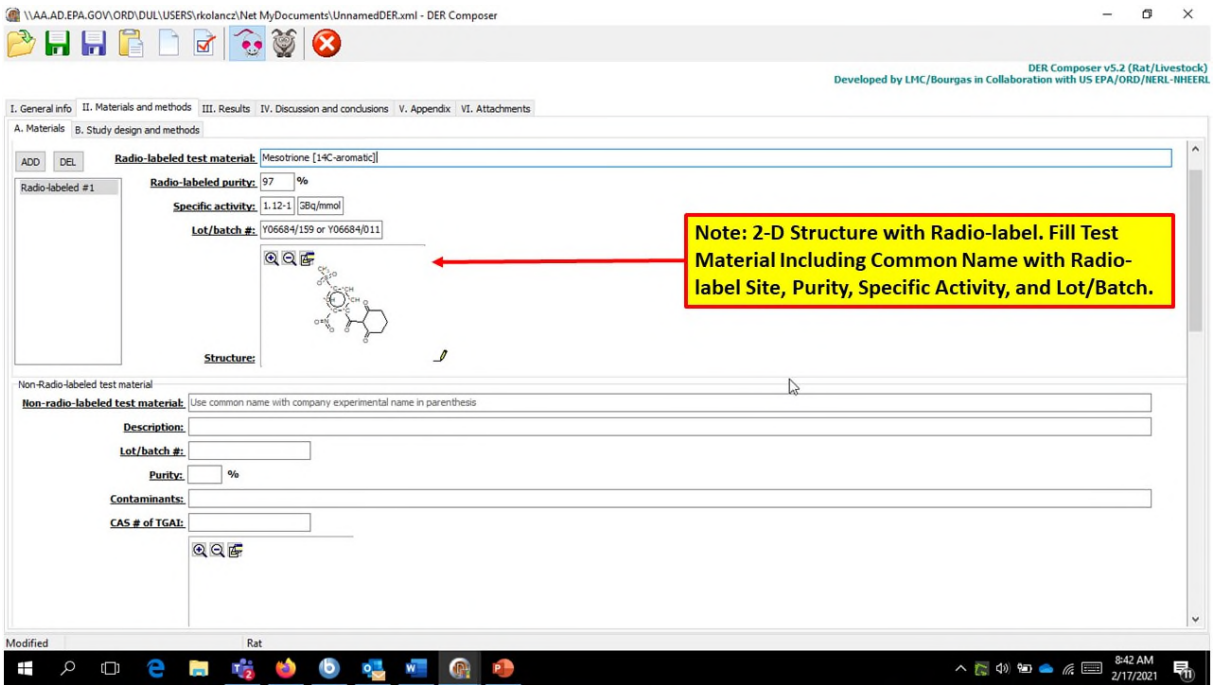


A periodic table screen comes up where you should check labeled, in this example add 14 in the number box and click on C for carbon. Then hit YES.

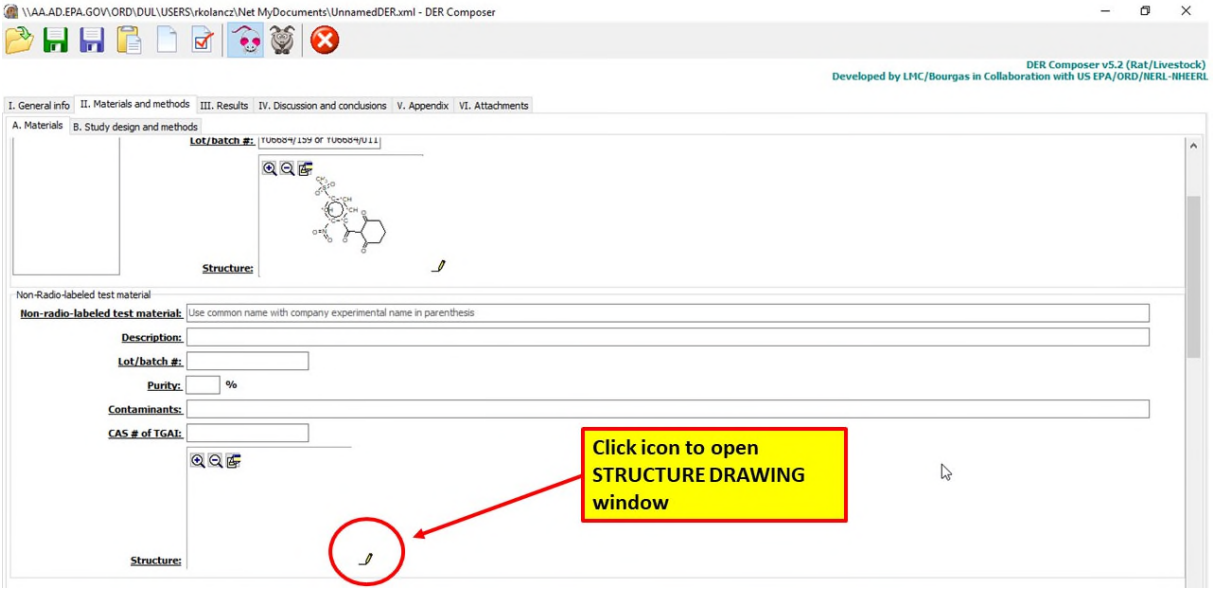


After clicking Yes on the previous screen, the periodic table closes, then you can add the C-14 label to each carbon in the example. The example below happens to be a uniformly labeled phenyl ring. Note that the information for the labeling is now contained in the SMILES.



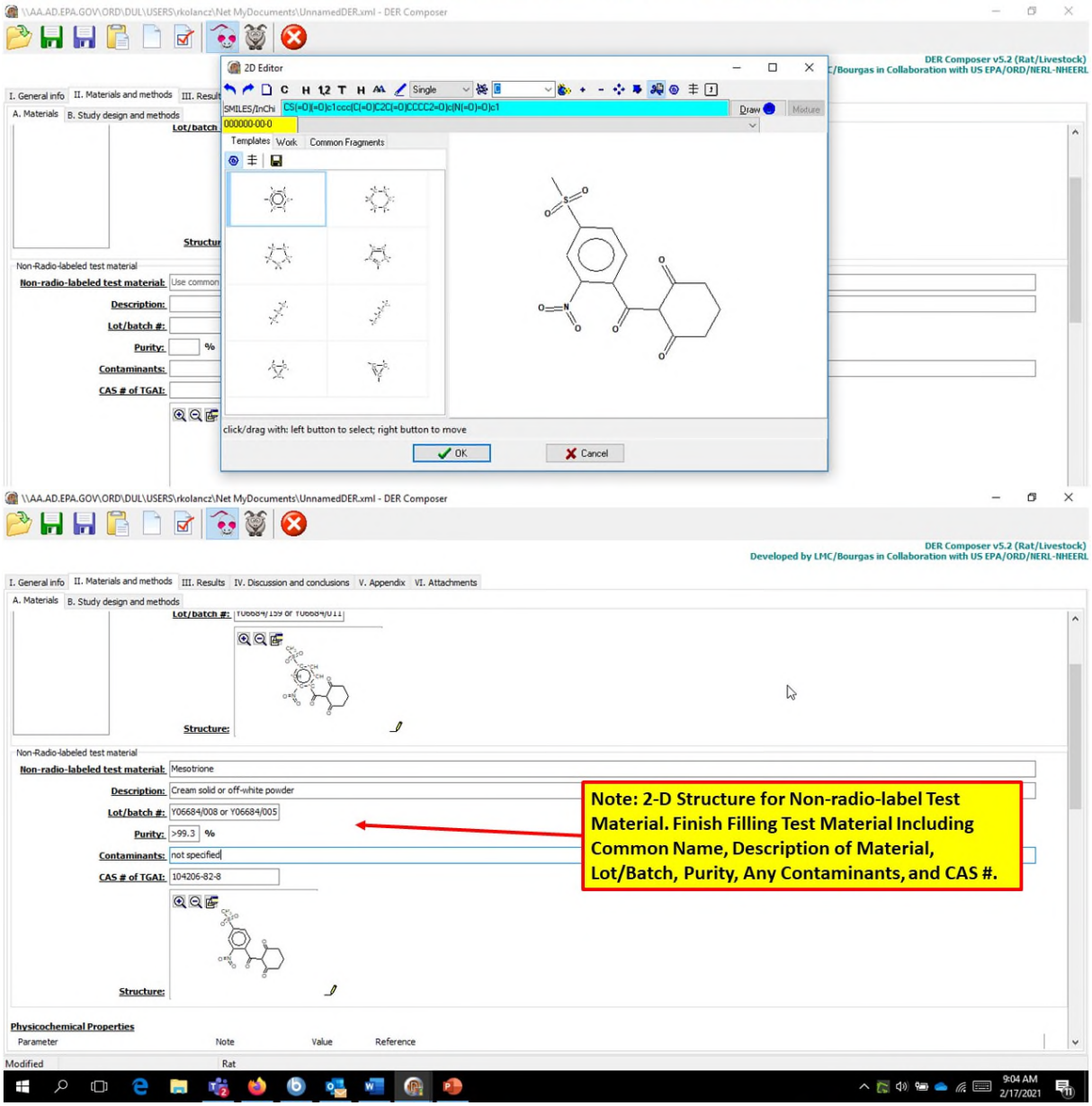


The Non-radiolabeled Test Material may be entered in the same fashion by opening the STRUCTURE DRAWING PACKAGE.

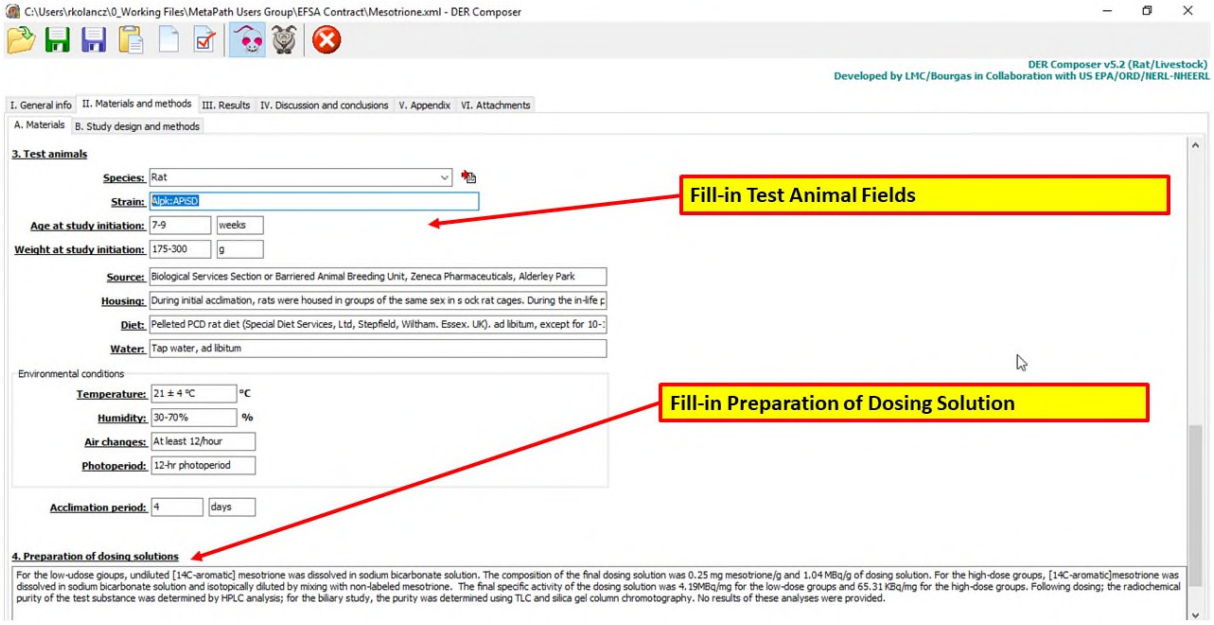


The SMILES string (from the excel list of parent structures) is entered in the light-blue box of the editor and the 2-D structure is immediately shown.

NOTE: The use of COPY/PASTE SMILES strings to generate the 2-D structures of parent chemicals serves to save time drawing structures, however the structures can be produced utilizing the tools of the drawing package.

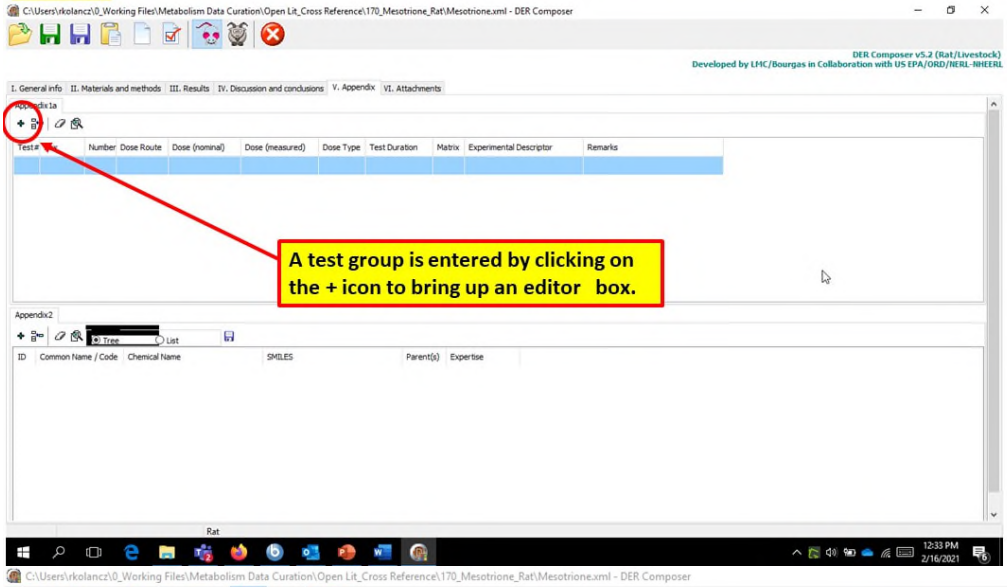


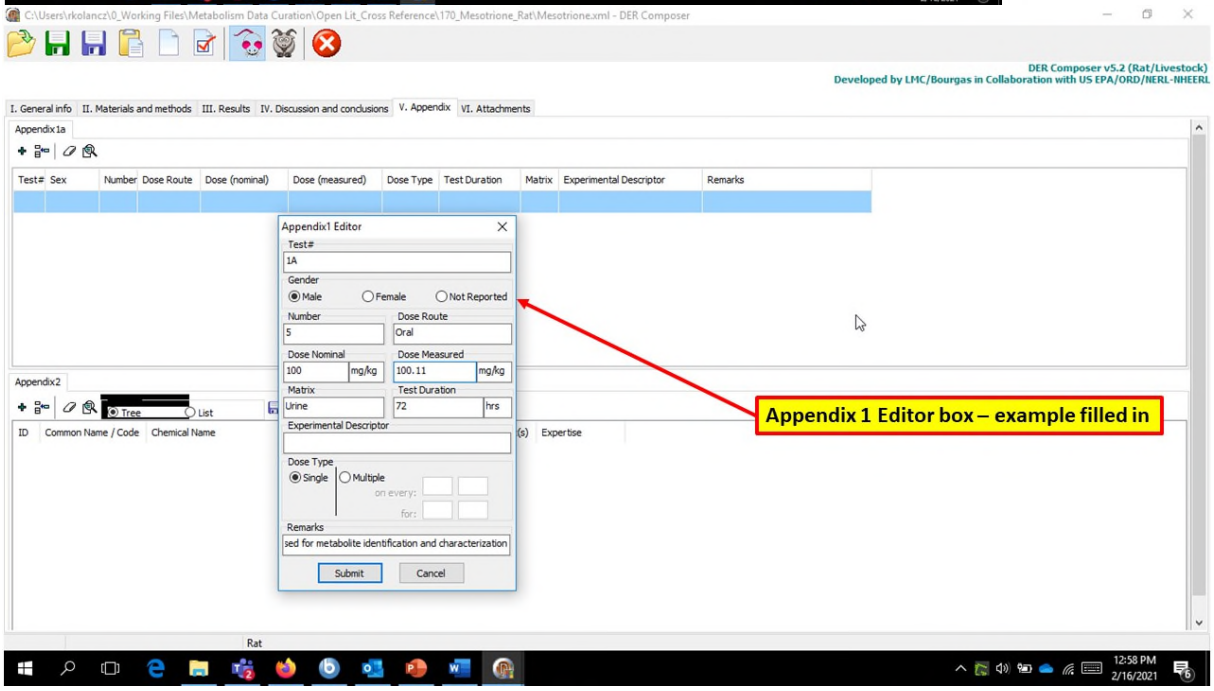
Continue filling out the rest of II. Material and methods A. Materials



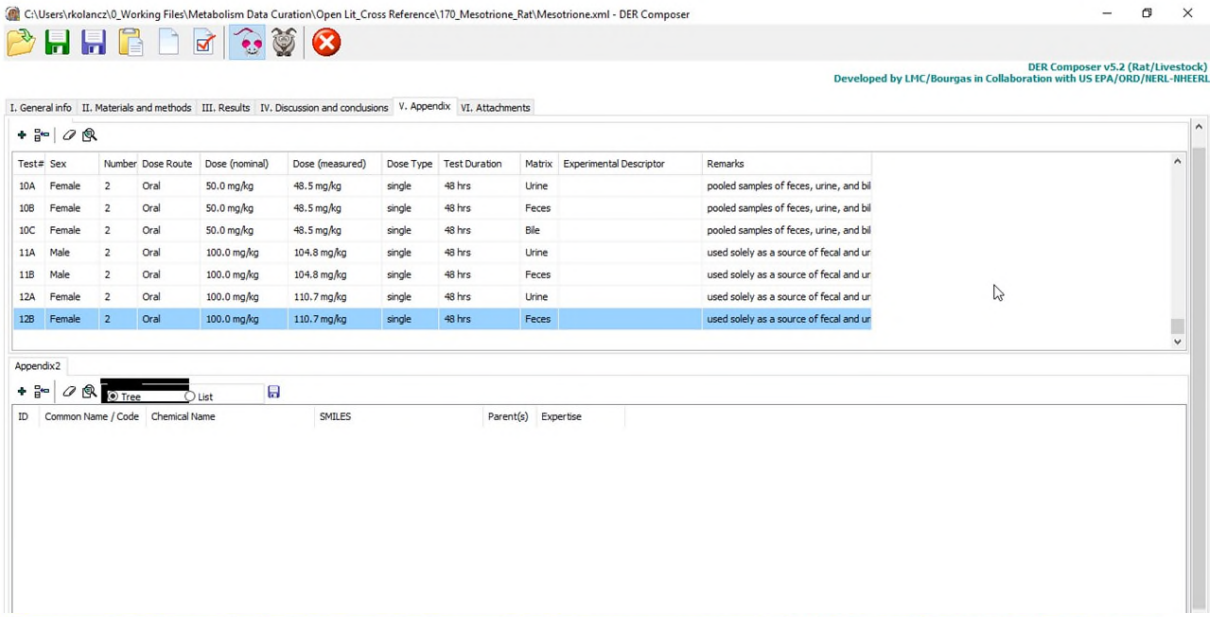
2.4. Appendix 1

Next go to tab V. Appendix – It is within this section that the various treatment groups are defined and listed as a TEST in the appendix 1 table below. A treatment group may be defined by gender, age, dose amount, dose route, sample matrix or other experimental descriptor (parameters that when varied may give rise to a different metabolic map for a particular chemical).

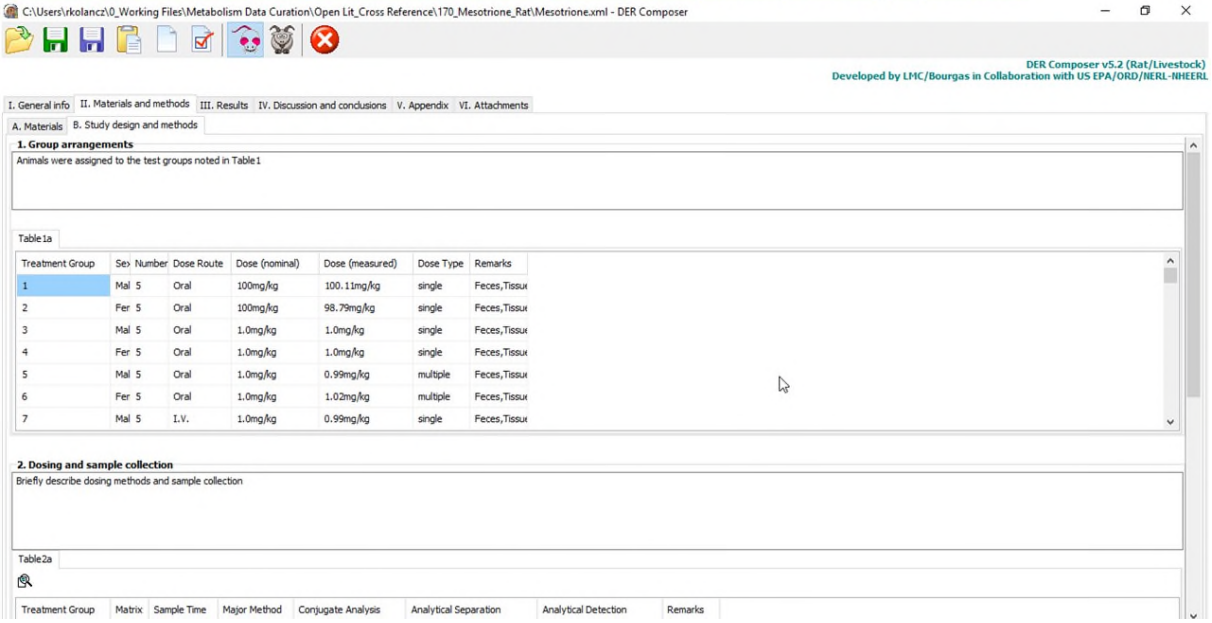




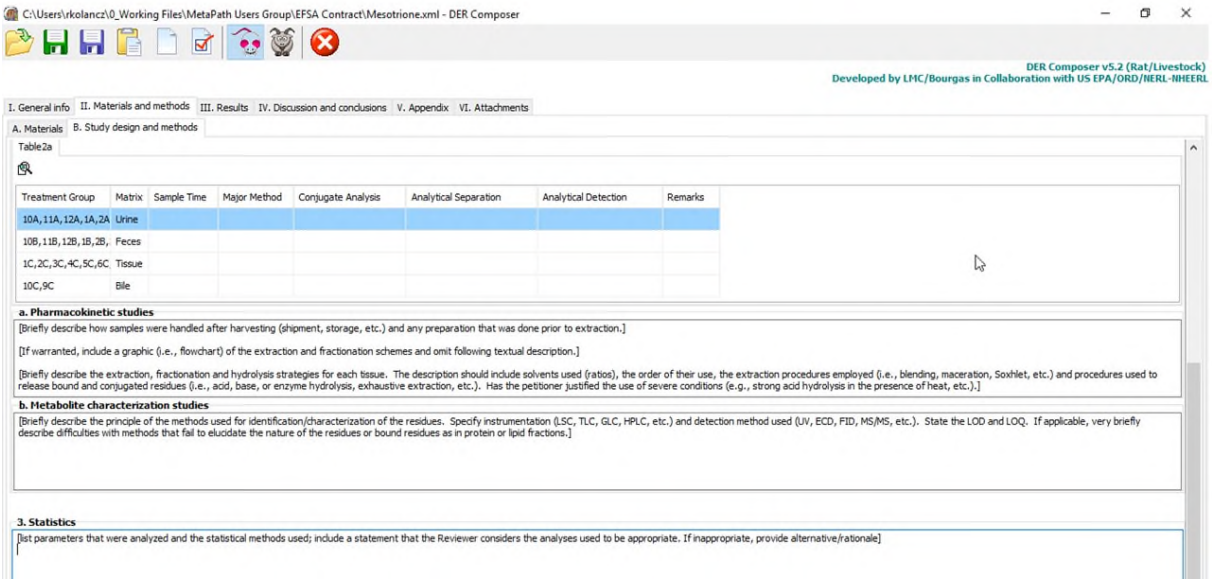
Click on submit to accept test – continue to add new tests via the same process until completed. Screen should appear as below:



The completed Appendix 1 will automatically populate the group arrangements Table 1 of section/tab II. Materials and methods sub-tab B. Study design and methods. This may be observed by clicking on the appropriate tabs.

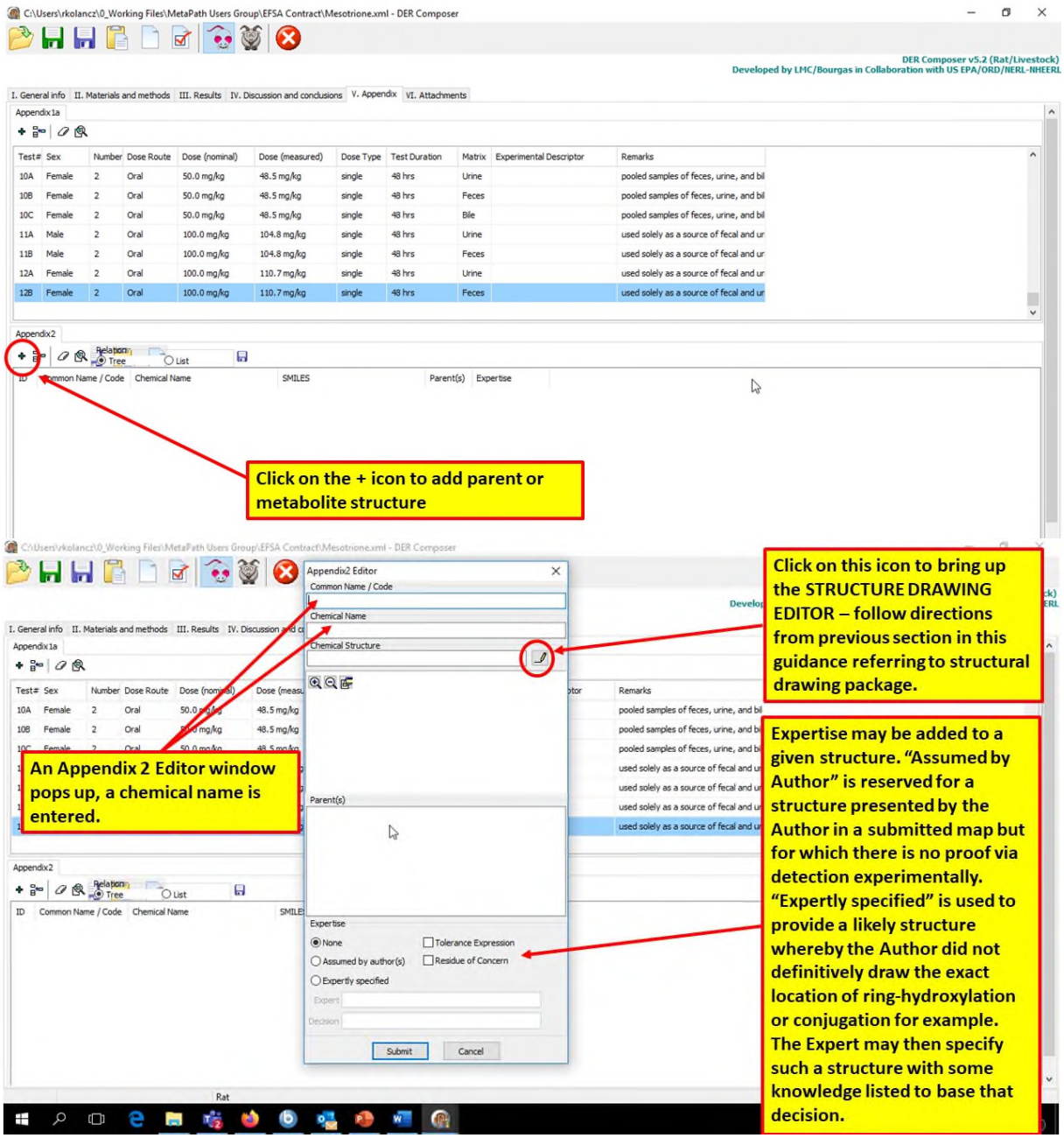


In addition, an automatic partial entry of the dosing and sample collection Table 2 of section/tab II. Materials and methods sub-tab B. Study design and methods takes place. We will return to complete this table after completion of Appendix 2.

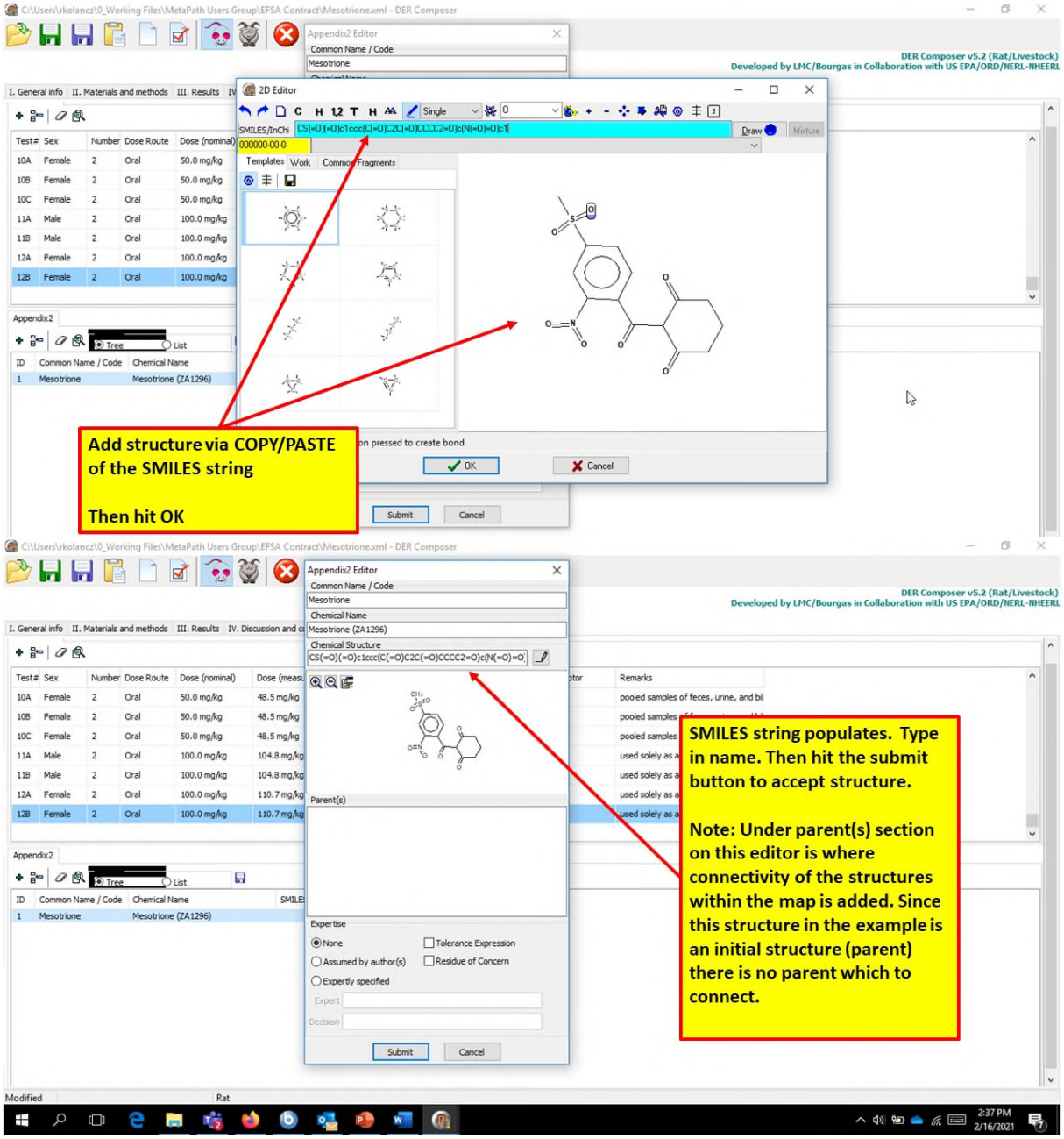


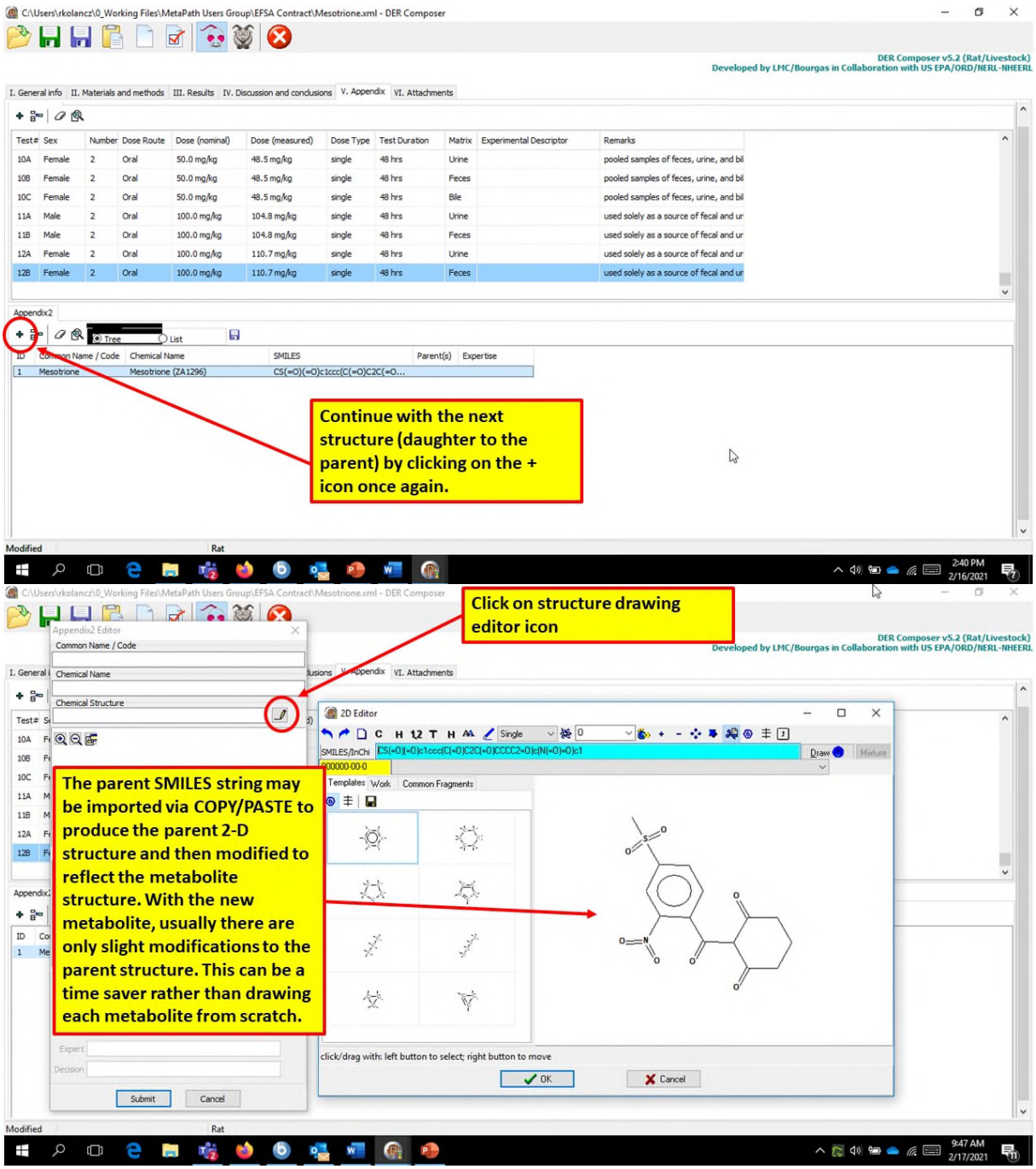
2.5. Appendix 2

Next a Metabolite Inventory table should be completed as Appendix 2 of tab V. Appendix.

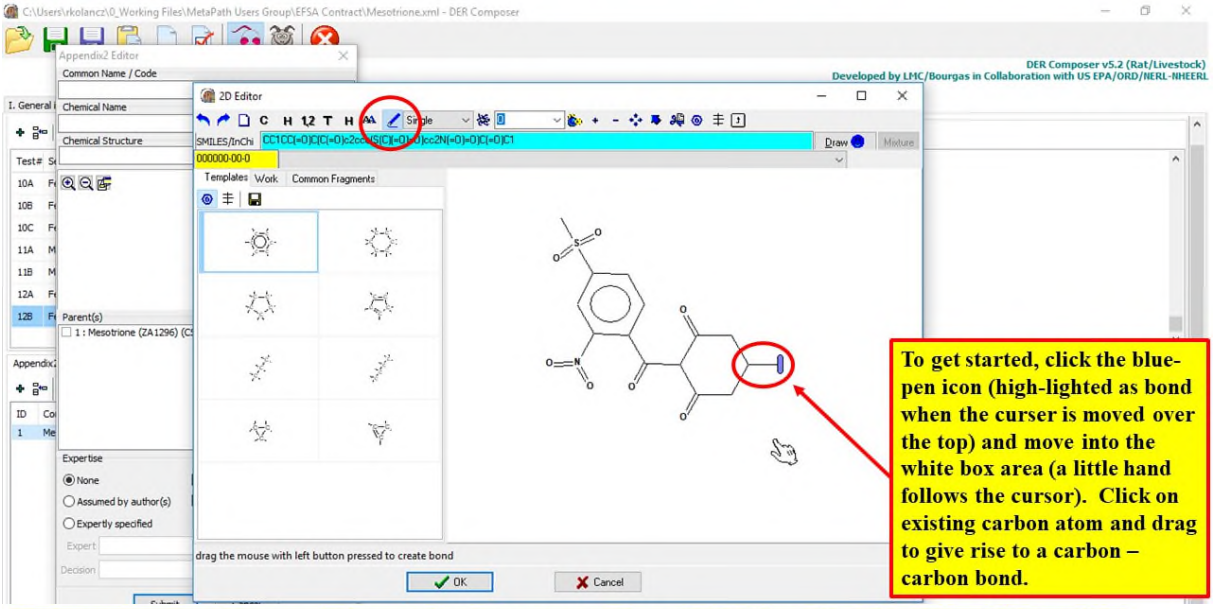


We will start by adding the parent structure – as was done in the materials & methods using COPY/PASTE of the SMILES string.

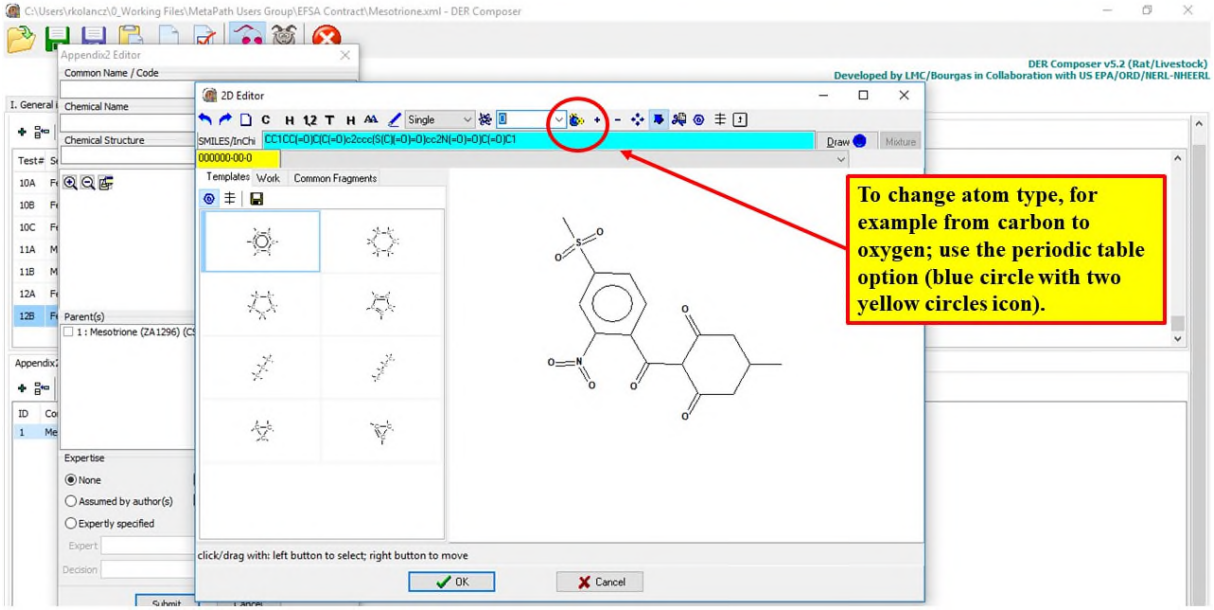



Continue with the next structure (daughter to the parent) by clicking on the + icon once again.
Click on structure drawing editor icon
The parent SMILES string may be imported via COPY/PASTE to produce the parent 2-D structure and then modified to reflect the metabolite structure. With the new metabolite, usually there are only slight modifications to the parent structure. This can be a time saver rather than drawing each metabolite from scratch.

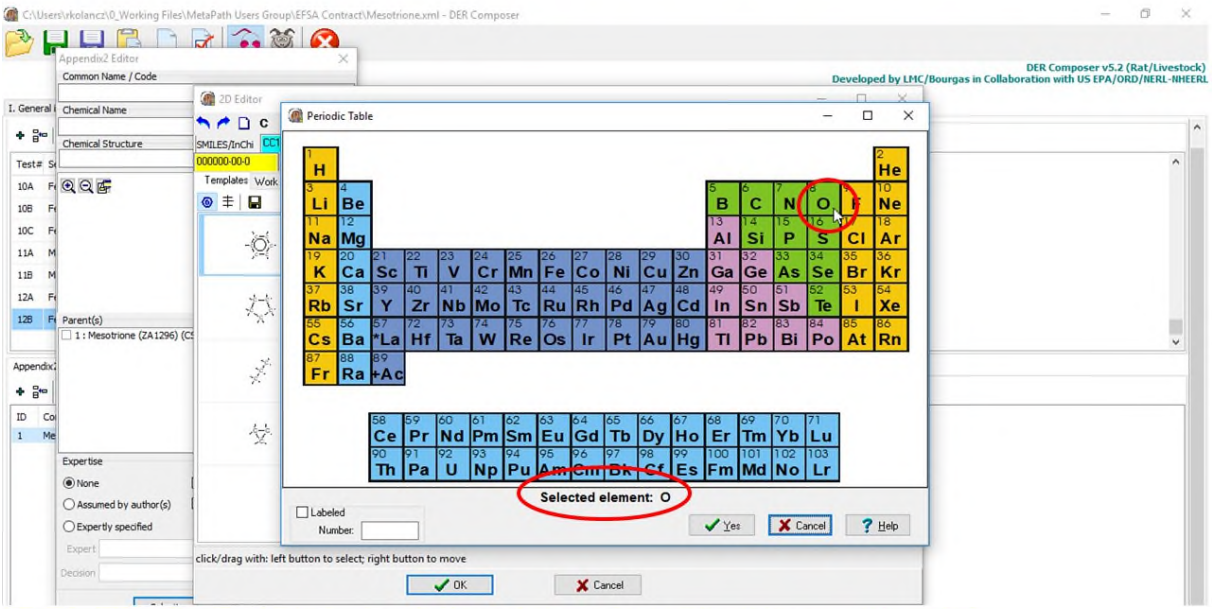
In this example the metabolite is 5-hydroxy-mesotrione. The following steps will introduce a hydroxy group in the 5-position of the dione ring.



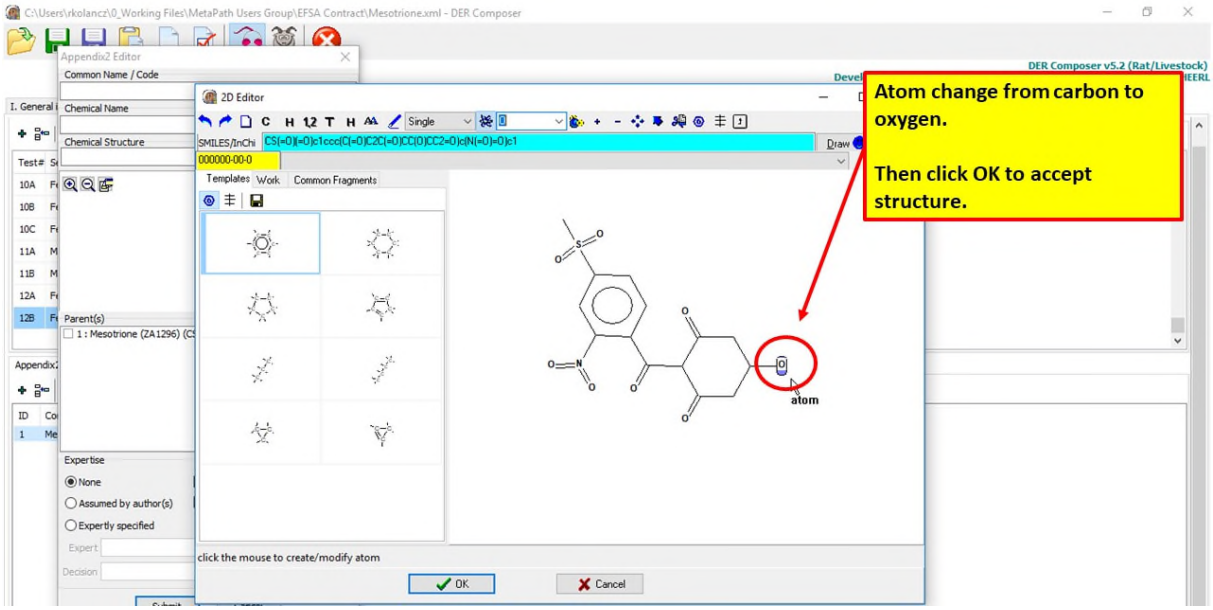
To change atom type, for example from carbon to oxygen; use the periodic table option (blue circle with two yellow circles icon).



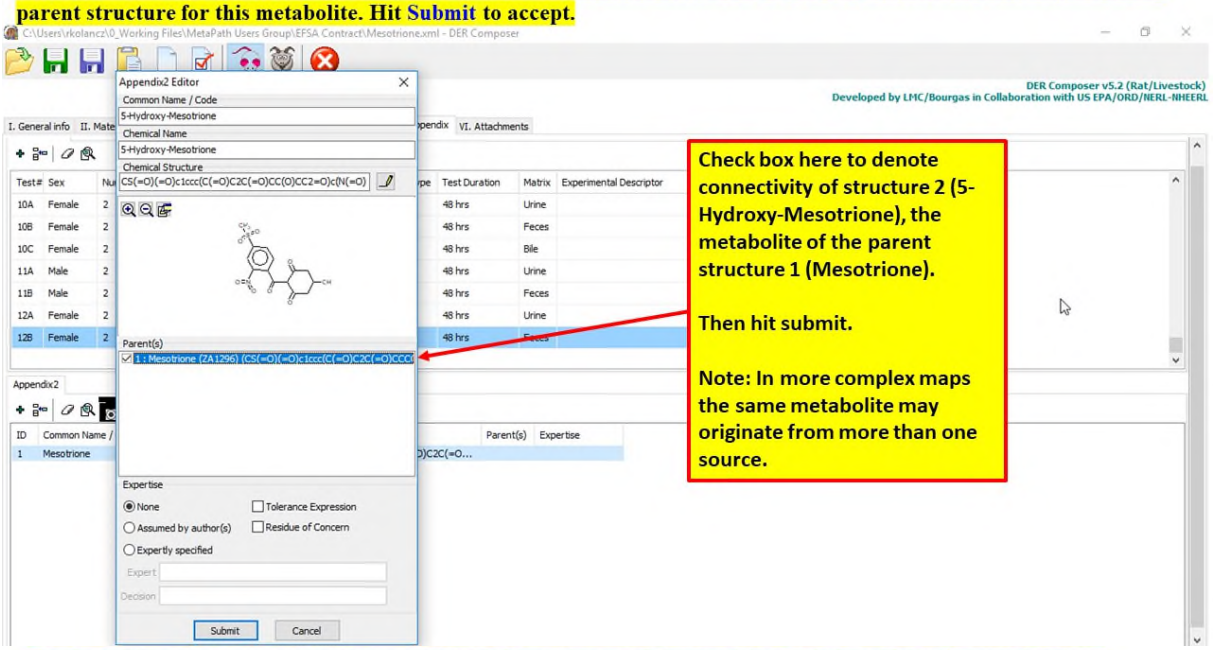
The periodic table opens, click on atom choice, click Yes to accept choice and the table goes away.



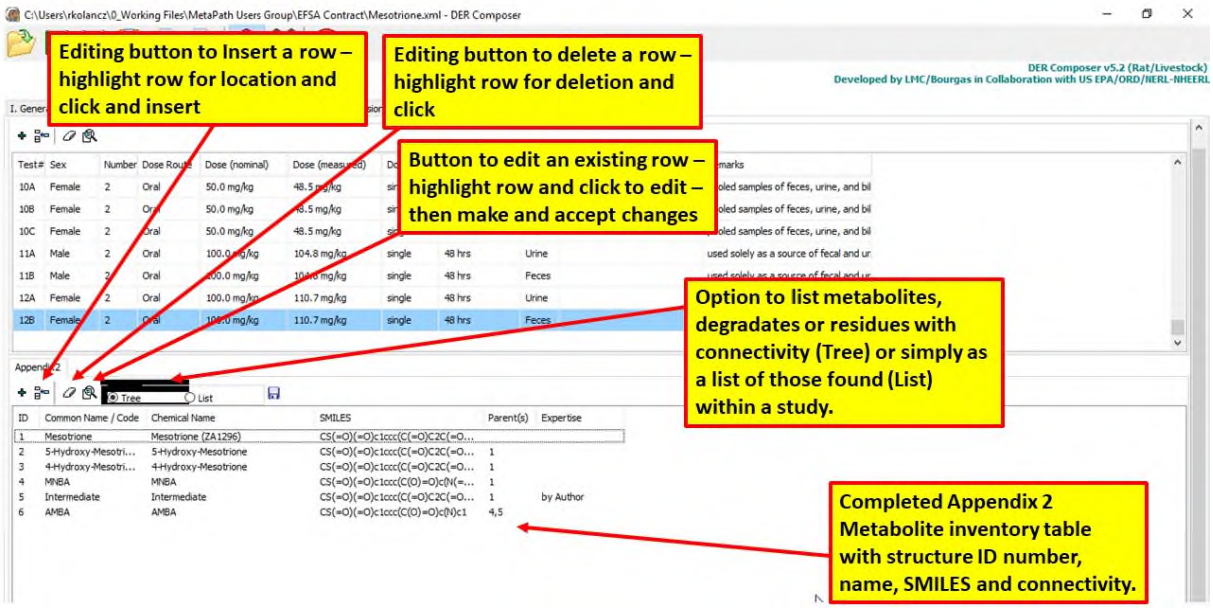
Simply click on the atom in the structure that you wish to replace and the substitution will be made.

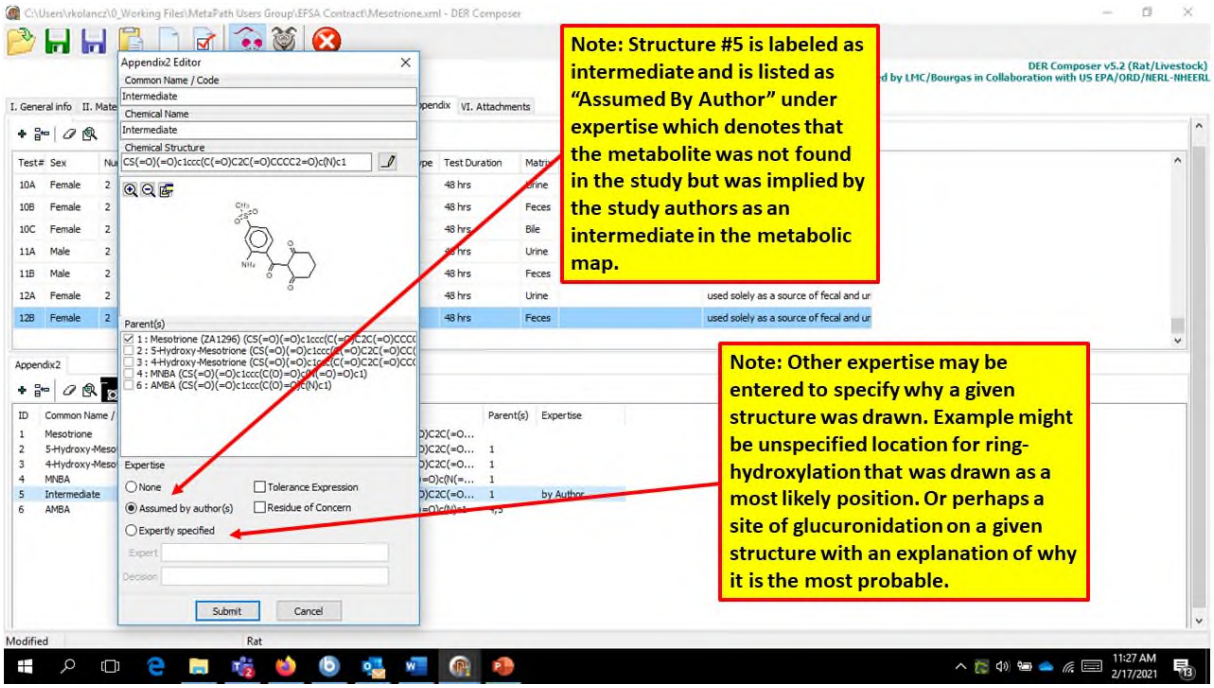


SMILES for metabolite is entered into EDITOR. Add metabolite name to Chemical Name and check affiliation box of parent structure for this metabolite. Hit Submit to accept.



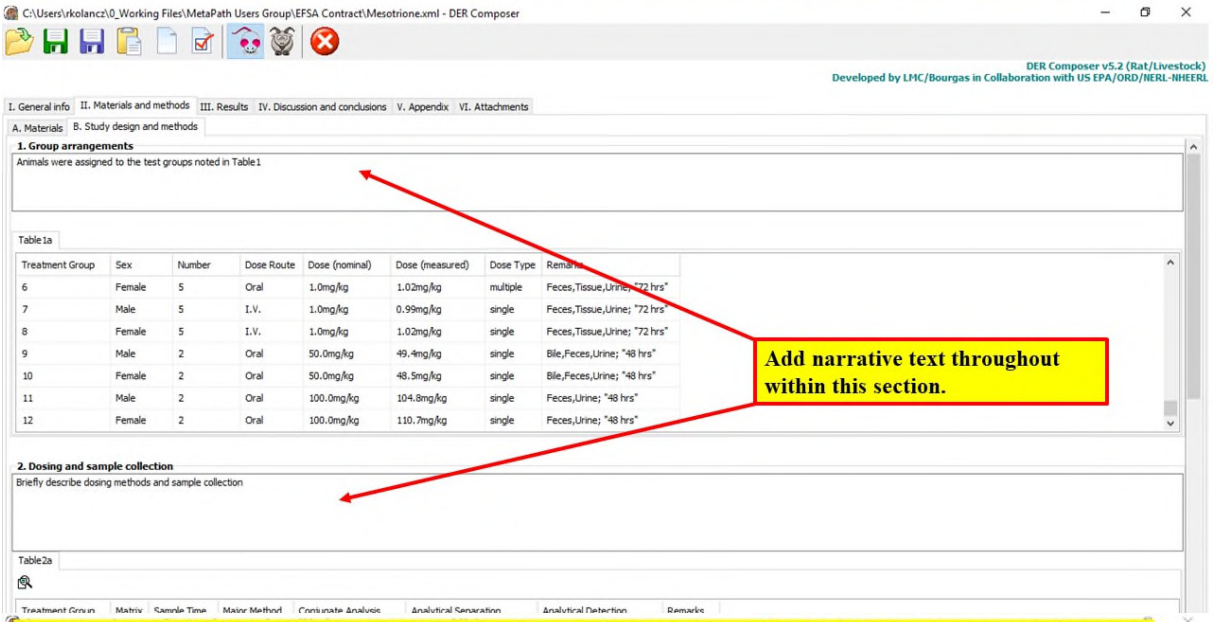
Continue filling in structures with connectivity information until the resulting table is sufficiently completed to represent the metabolic map.



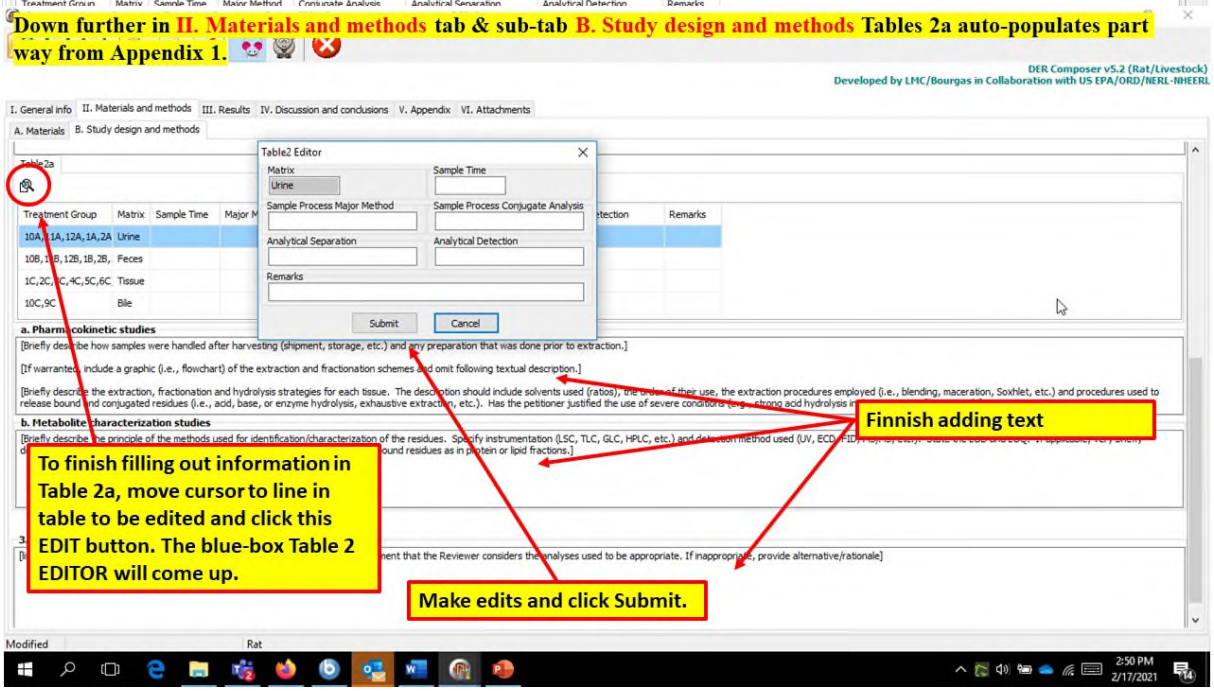

Note: Structure #5 is labeled as intermediate and is listed as "Assumed By Author" under expertise which denotes that the metabolite was not found in the study but was implied by the study authors as an intermediate in the metabolic map.
Note: Other expertise may be entered to specify why a given structure was drawn. Example might be unspecified location for ring-hydroxylation that was drawn as a most likely position. Or perhaps a site of glucuronidation on a given structure with an explanation of why it is the most probable.
Appendix2 Editor
Common Name / Code
Intermediate
Chemical Name
Intermediate
Chemical Structure
Parent(s)
Expertise
None
Tolerance Expression
Assumed by author(s)
Residue of Concern
Expertly specified
Expert
Decision
Submit
Cancel

2.6. Material and methods – Study design and methods

Next go back to II. Materials and methods tab & sub-tab B. Study design and methods and fill in narrative text sections under 1. Group arrangements and 2. Dosing and sample collection. Tables 1a auto-populates from Appendix 1.

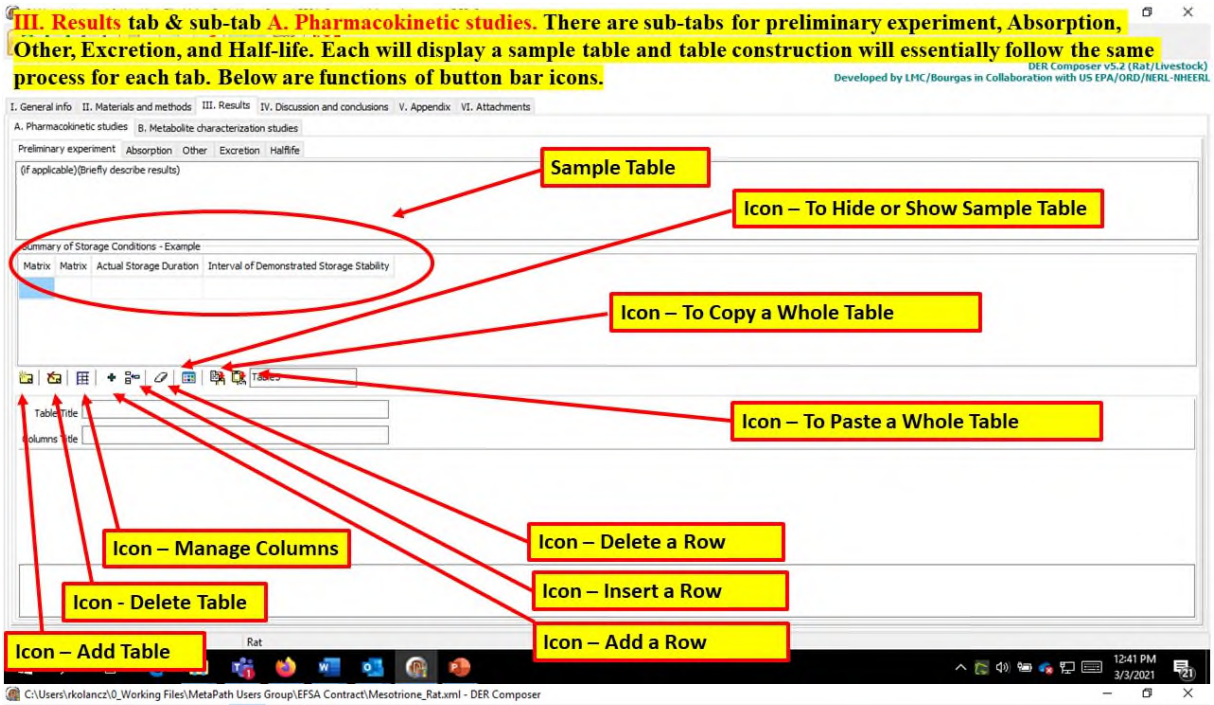


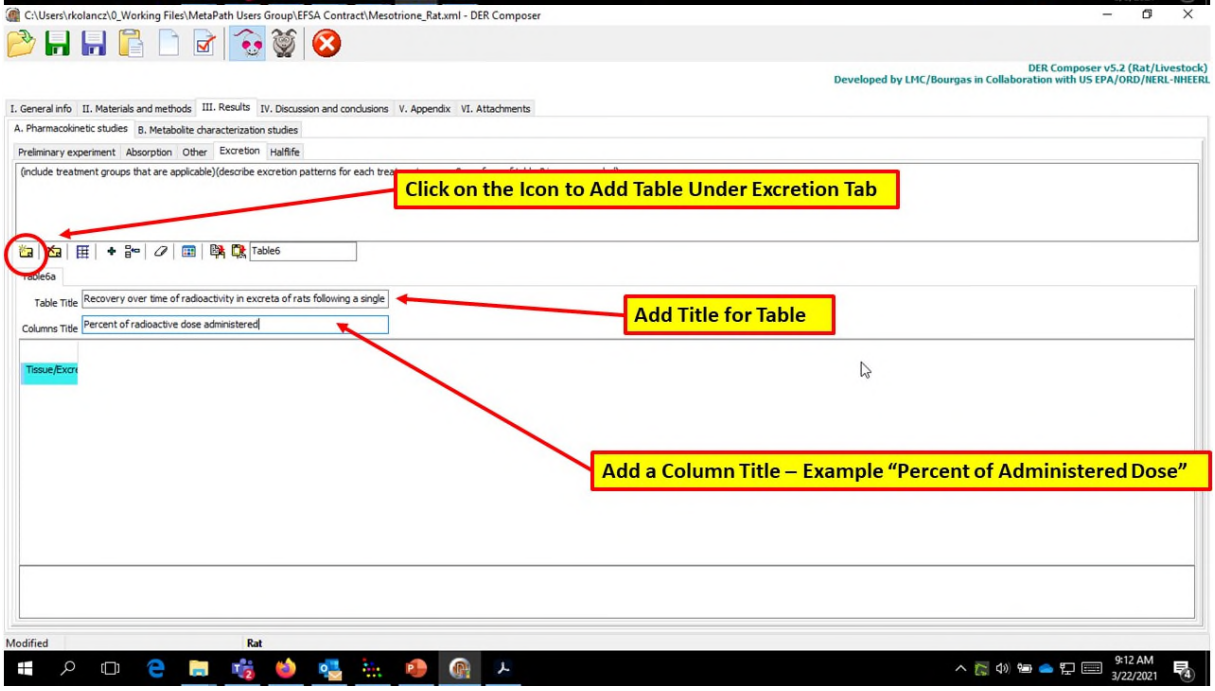
Down further in II. Materials and methods tab & sub-tab B. Study design and methods Tables 2a auto-populates part way from Appendix 1.

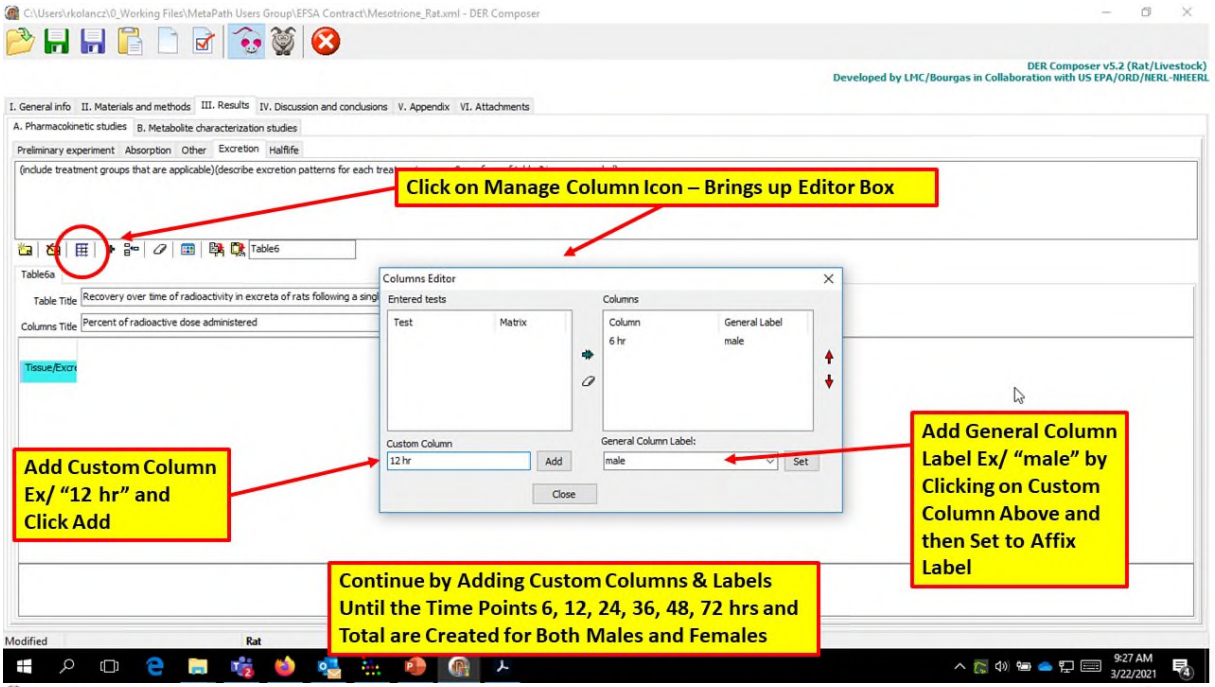


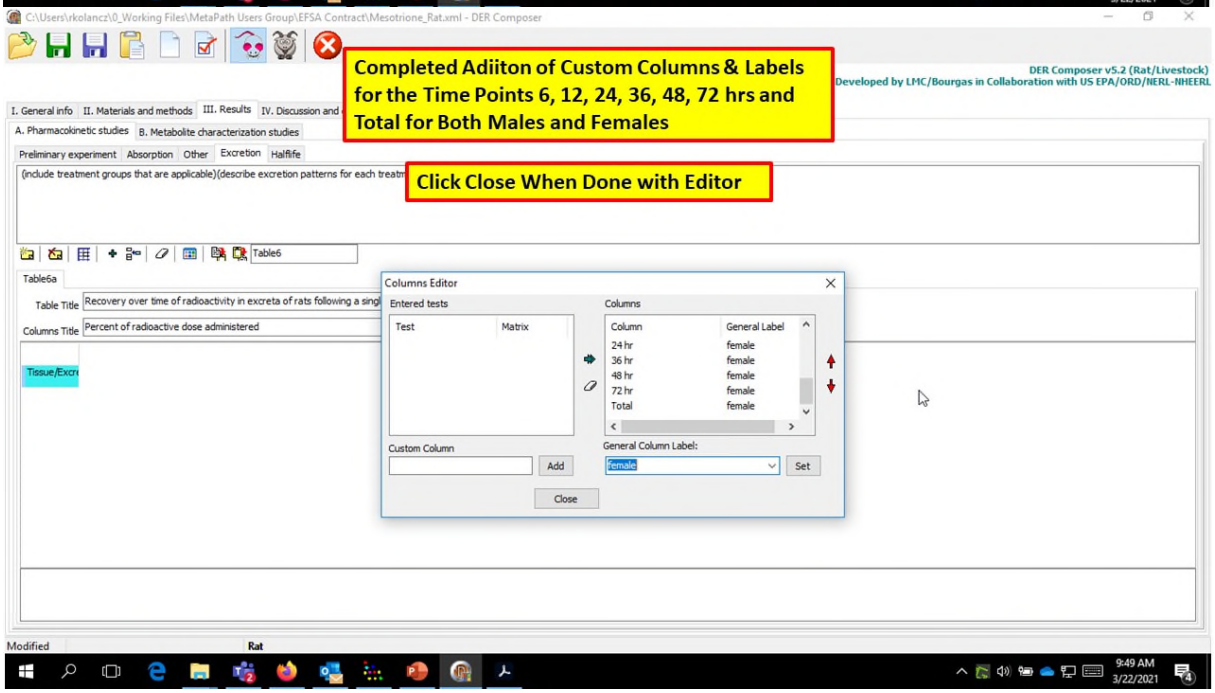
2.7. Results – Pharmacokinetic Studies

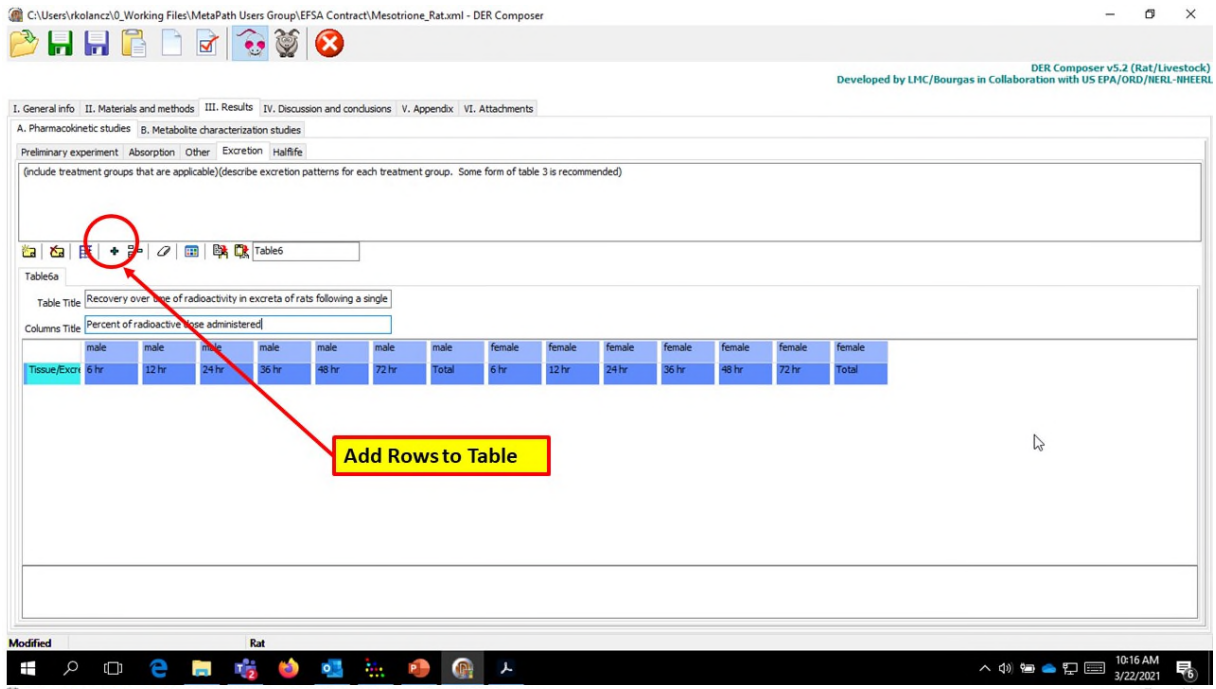
III. Results tab & sub-tab A. Pharmacokinetic studies. There are sub-tabs for preliminary experiment, Absorption, Other, Excretion, and Half-life. Each will display a sample table and table construction will essentially follow the same process for each tab. Below are functions of button bar icons.

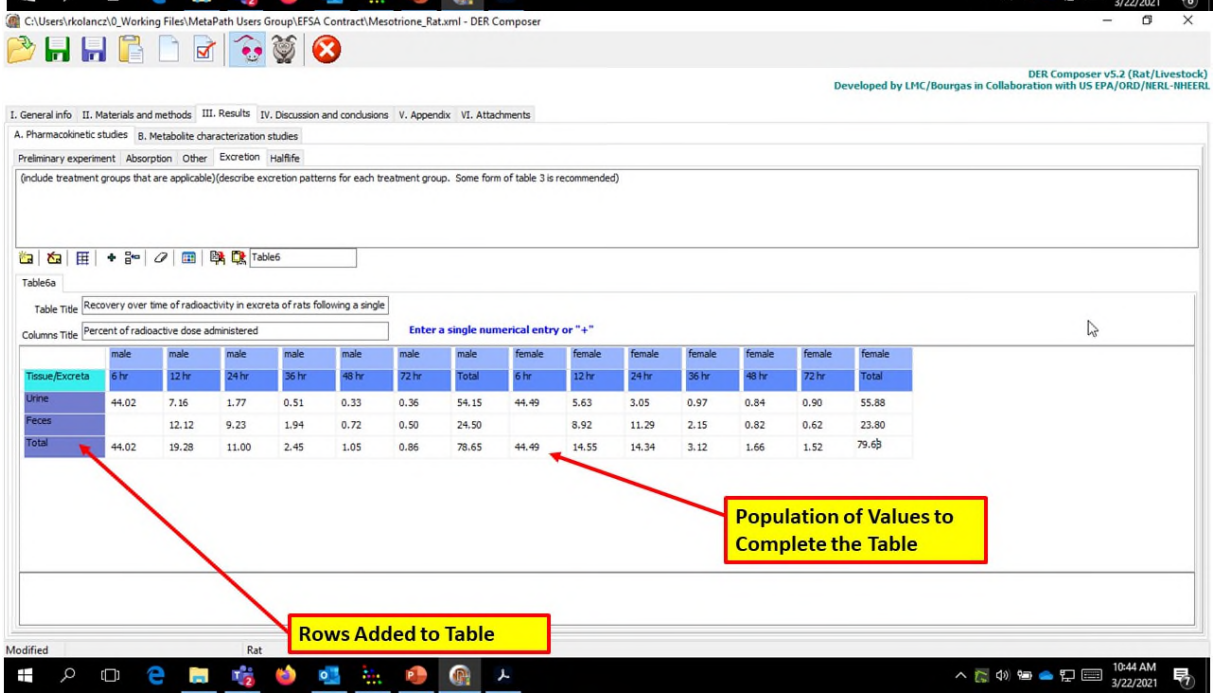


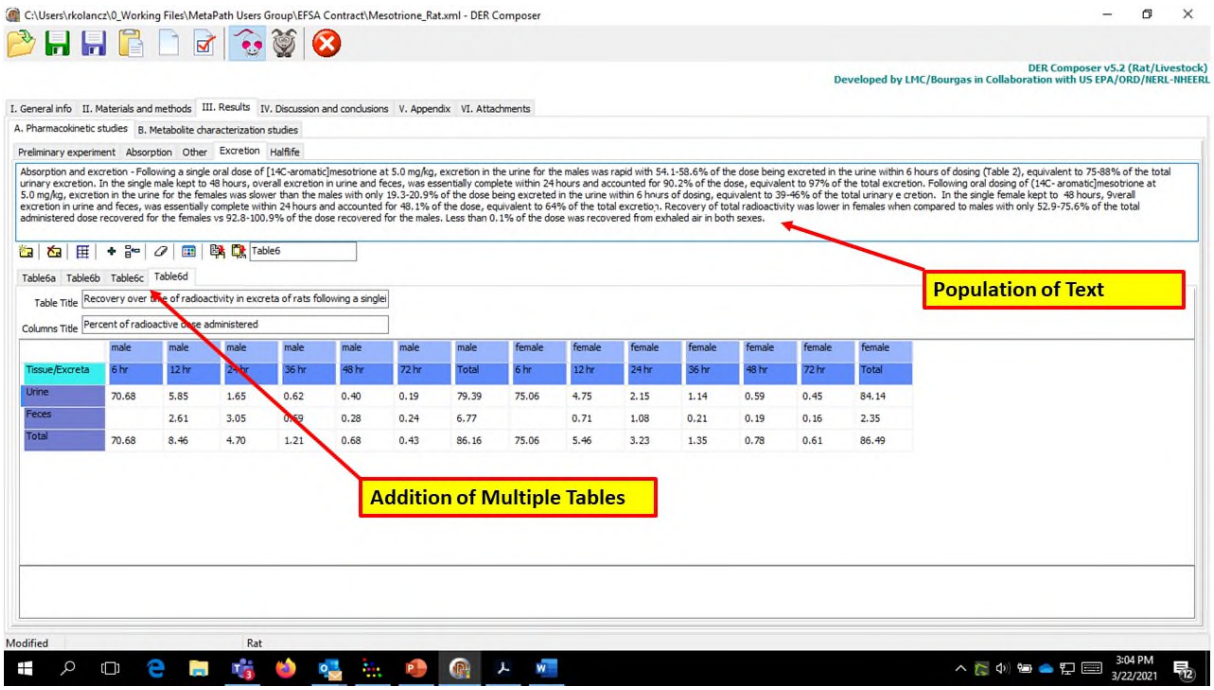



Click on Manage Column Icon – Brings up Editor Box
Columns Editor
Add Custom Column Ex/ "12 hr" and Click Add
Add General Column Label Ex/ "male" by Clicking on Custom Column Above and then Set to Affix Label
Continue by Adding Custom Columns & Labels Until the Time Points 6, 12, 24, 36, 48, 72 hrs and Total are Created for Both Males and Females


Completed Adiiton of Custom Columns & Labels for the Time Points 6, 12, 24, 36, 48, 72 hrs and Total for Both Males and Females
Click Close When Done with Editor
Columns Editor

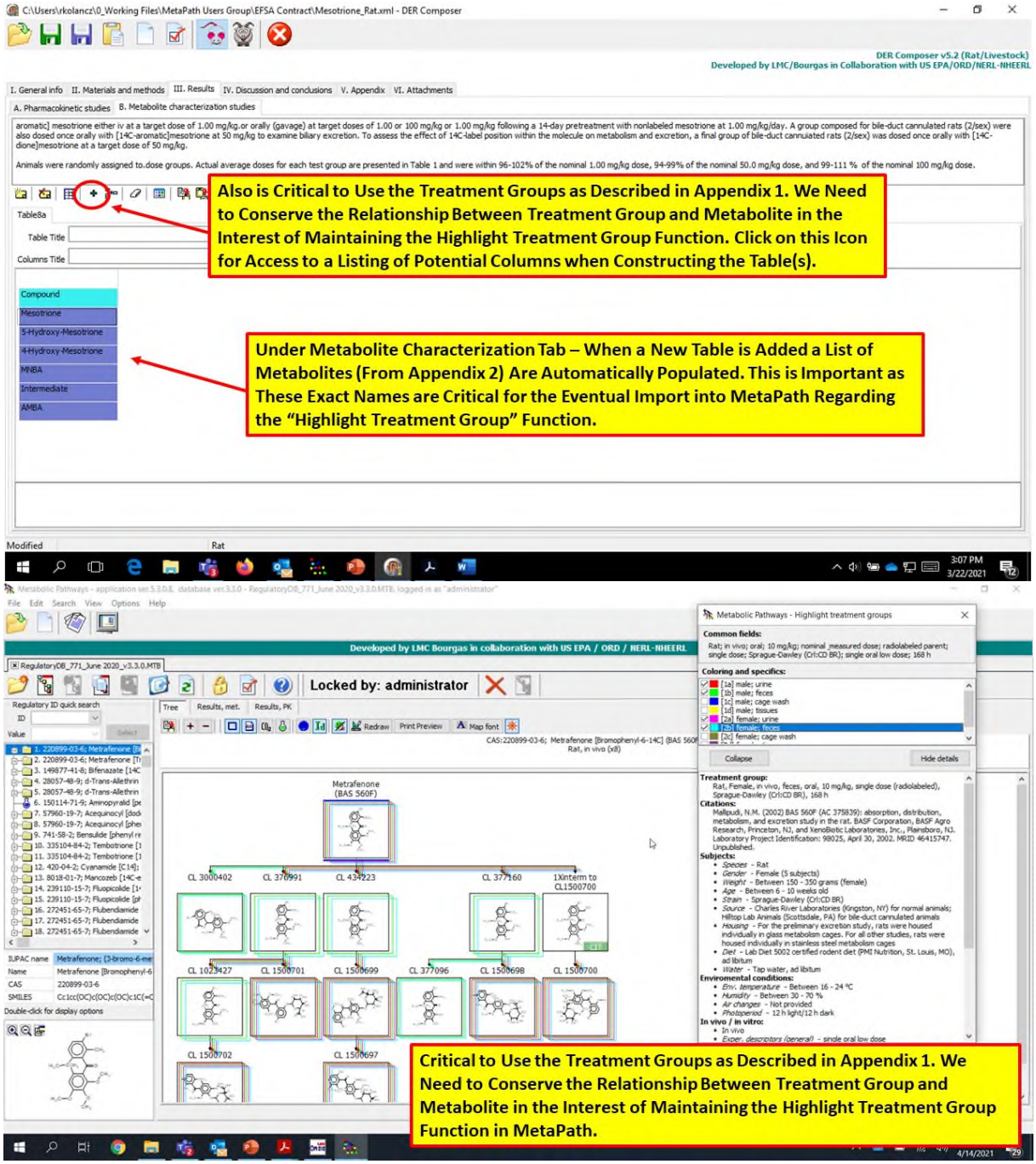

C:\Users\rkolancz\0_Working Files\MetaPath Users Group\EFSA Contract\Mesotrione_Rat.xml - DER Composer
DER Composer v5.2 (Rat/Livestock)
Developed by LMC/Bourgas in Collaboration with US EPA/ORD/NERL-NHEERL
I. General info
II. Materials and methods
III. Results
IV. Discussion and conclusions
V. Appendix
VI. Attachments
A. Pharmacokinetic studies
B. Metabolite characterization studies
Preliminary experiment
Absorption
Other
Excretion
Halflife
(include treatment groups that are applicable)(describe excretion patterns for each treatment group. Some form of table 3 is recommended)
Table6
Table6a
Table Title
Recovery over time of radioactivity in excreta of rats following a single
Columns Title
Percent of radioactive dose administered
Add Rows to Table
Modified
Rat

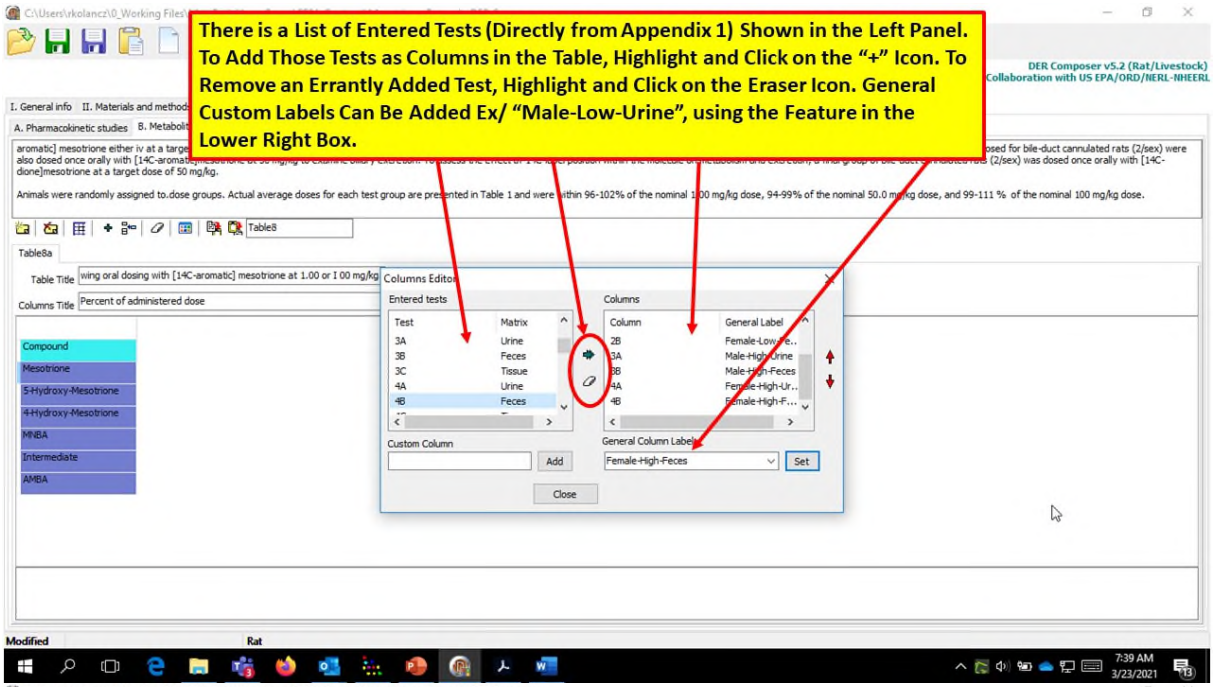

C:\Users\rkolancz\0_Working Files\MetaPath Users Group\EFSA Contract\Mesotrione_Rat.xml - DER Composer
DER Composer v5.2 (Rat/Livestock)
Developed by LMC/Bourgas in Collaboration with US EPA/ORD/NERL-NHEERL
Enter a single numerical entry or "+"
Population of Values to Complete the Table
Rows Added to Table
Modified
Rat

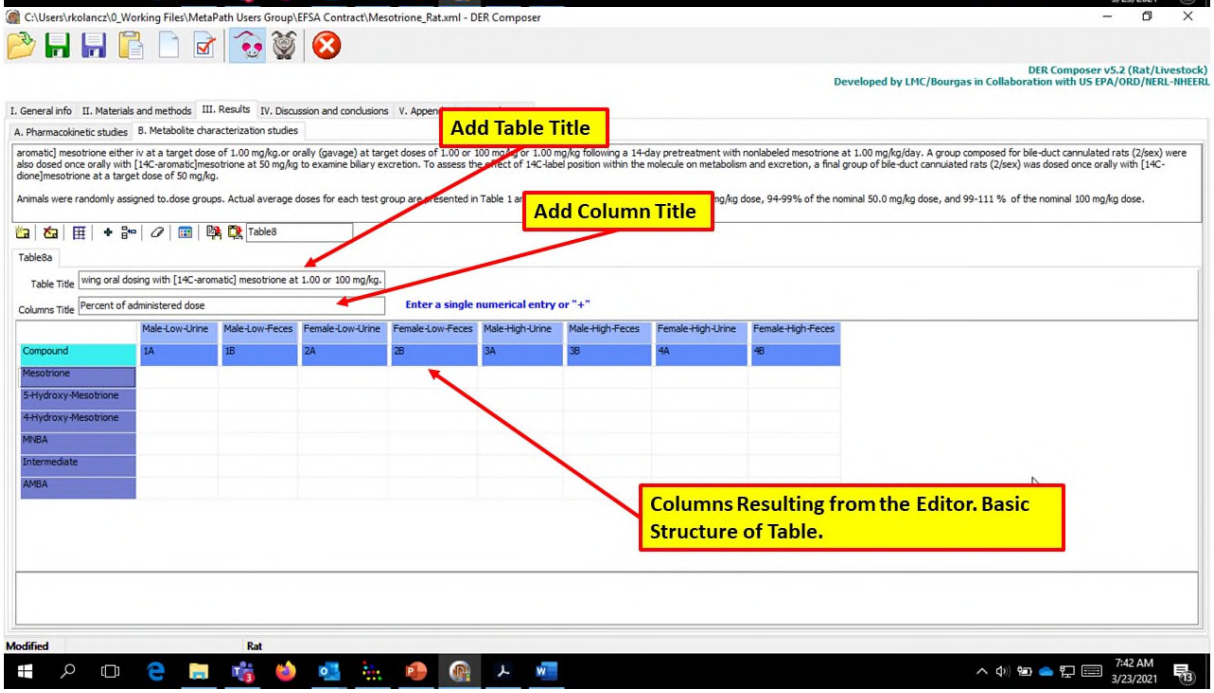


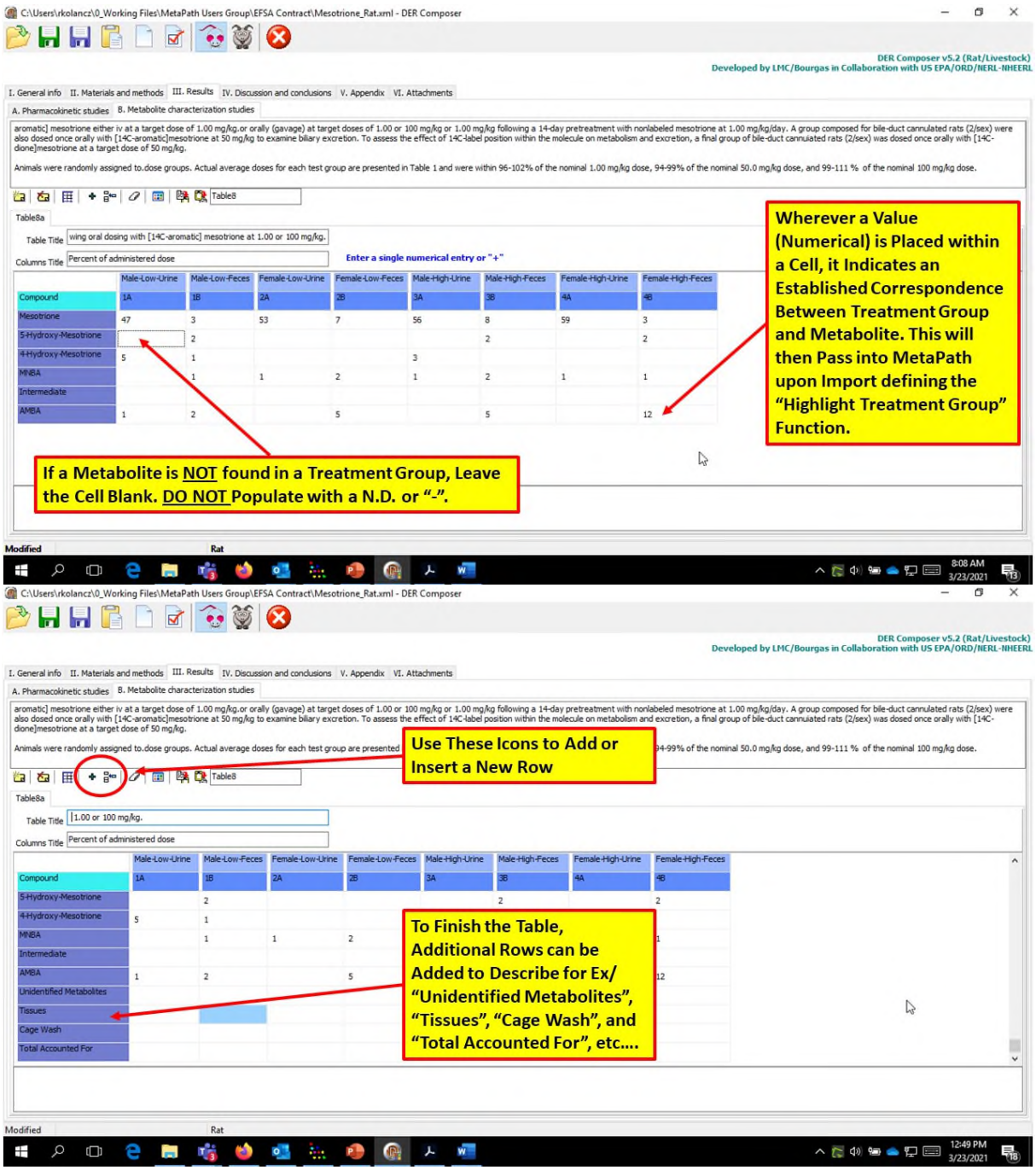
	male	male	male	male	male	male	male	female	female	female	female	female	female	female
Tissue/Excreta	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total	6 hr	12 hr	24 hr	36 hr	48 hr	72 hr	Total
Urine	70.68	5.85	1.65	0.62	0.40	0.19	79.39	75.06	4.75	2.15	1.14	0.59	0.45	84.14
Feces		2.61	3.05	0.59	0.28	0.24	6.77		0.71	1.08	0.21	0.19	0.16	2.35
Total	70.68	8.46	4.70	1.21	0.68	0.43	86.16	75.06	5.46	3.23	1.35	0.78	0.61	86.49

2.8. Results – Metabolite Characterization Studies




There is a List of Entered Tests (Directly from Appendix 1) Shown in the Left Panel. To Add Those Tests as Columns in the Table, Highlight and Click on the "+" Icon. To Remove an Errantly Added Test, Highlight and Click on the Eraser Icon. General Custom Labels Can Be Added Ex/ "Male-Low-Urine", using the Feature in the Lower Right Box.
DER Composer v5.2 (Rat/Livestock)
Collaboration with US EPA/ORD/NERL-NHEERL
Columns Editor
Entered tests
Columns
Custom Column
General Column Label
Add
Set
Close
Female-High-Feces
Table8a
Table Title
Columns Title
Percent of administered dose
Compound
Mesotrione
5-Hydroxy-Mesotrione
4-Hydroxy-Mesotrione
MNBA
Intermediate
AMBA
Modified
Rat


C:\Users\rkolancz\0_Working Files\MetaPath Users Group\EFSA Contract\Mesotrione_Rat.xml - DER Composer
DER Composer v5.2 (Rat/Livestock)
Developed by LMC/Bourgas in Collaboration with US EPA/ORD/NERL-NHEERL
Add Table Title
Add Column Title
Enter a single numerical entry or "+"
Table8a
Table Title
wing oral dosing with [14C-aromatic] mesotrione at 1.00 or 100 mg/kg.
Columns Title
Percent of administered dose
Male-Low-Urine
Male-Low-Feces
Female-Low-Urine
Female-Low-Feces
Male-High-Urine
Male-High-Feces
Female-High-Urine
Female-High-Feces
1A
1B
2A
2B
3A
3B
4A
4B
Compound
Mesotrione
5-Hydroxy-Mesotrione
4-Hydroxy-Mesotrione
MNBA
Intermediate
AMBA
Columns Resulting from the Editor. Basic Structure of Table.
Modified
Rat

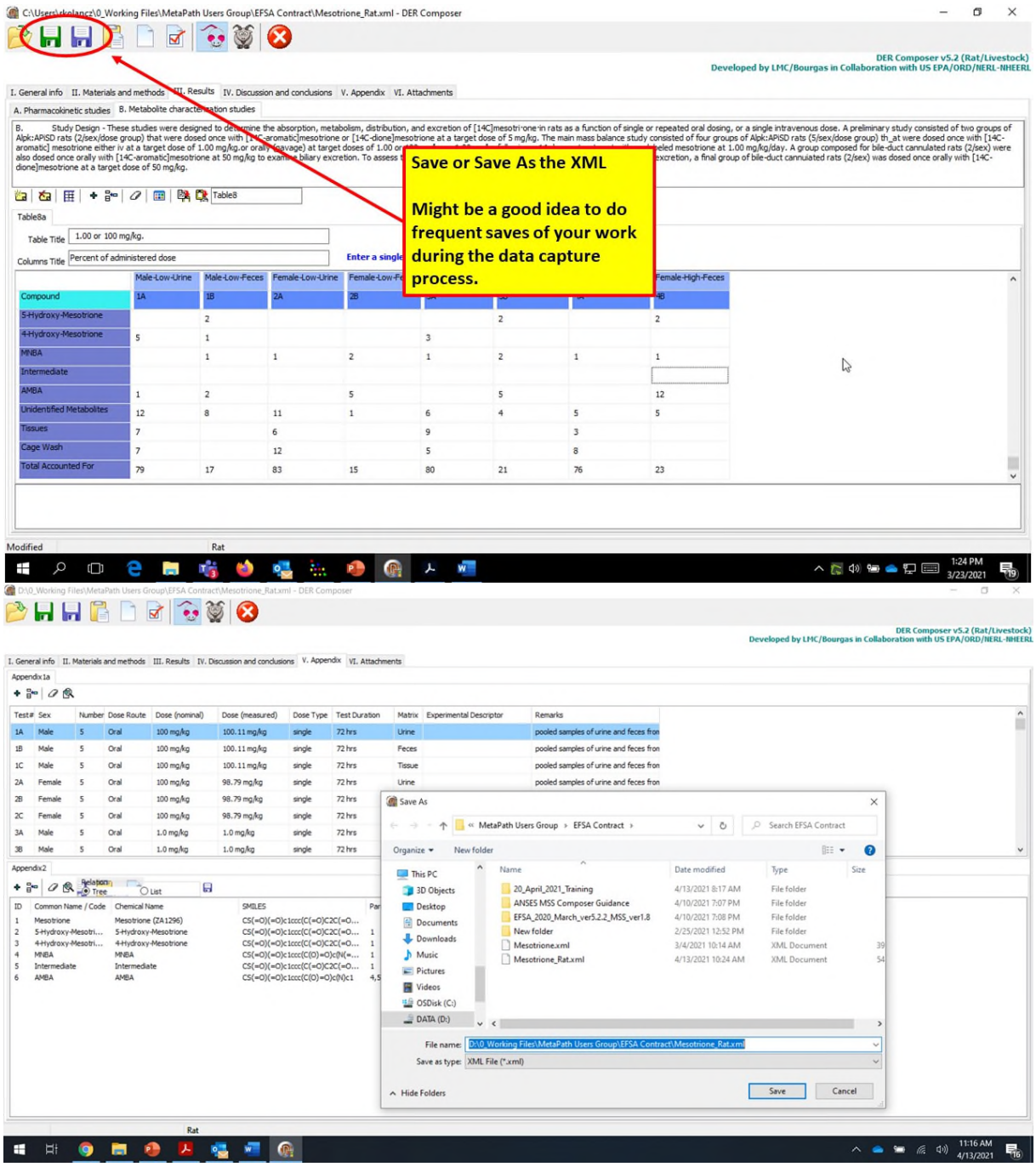


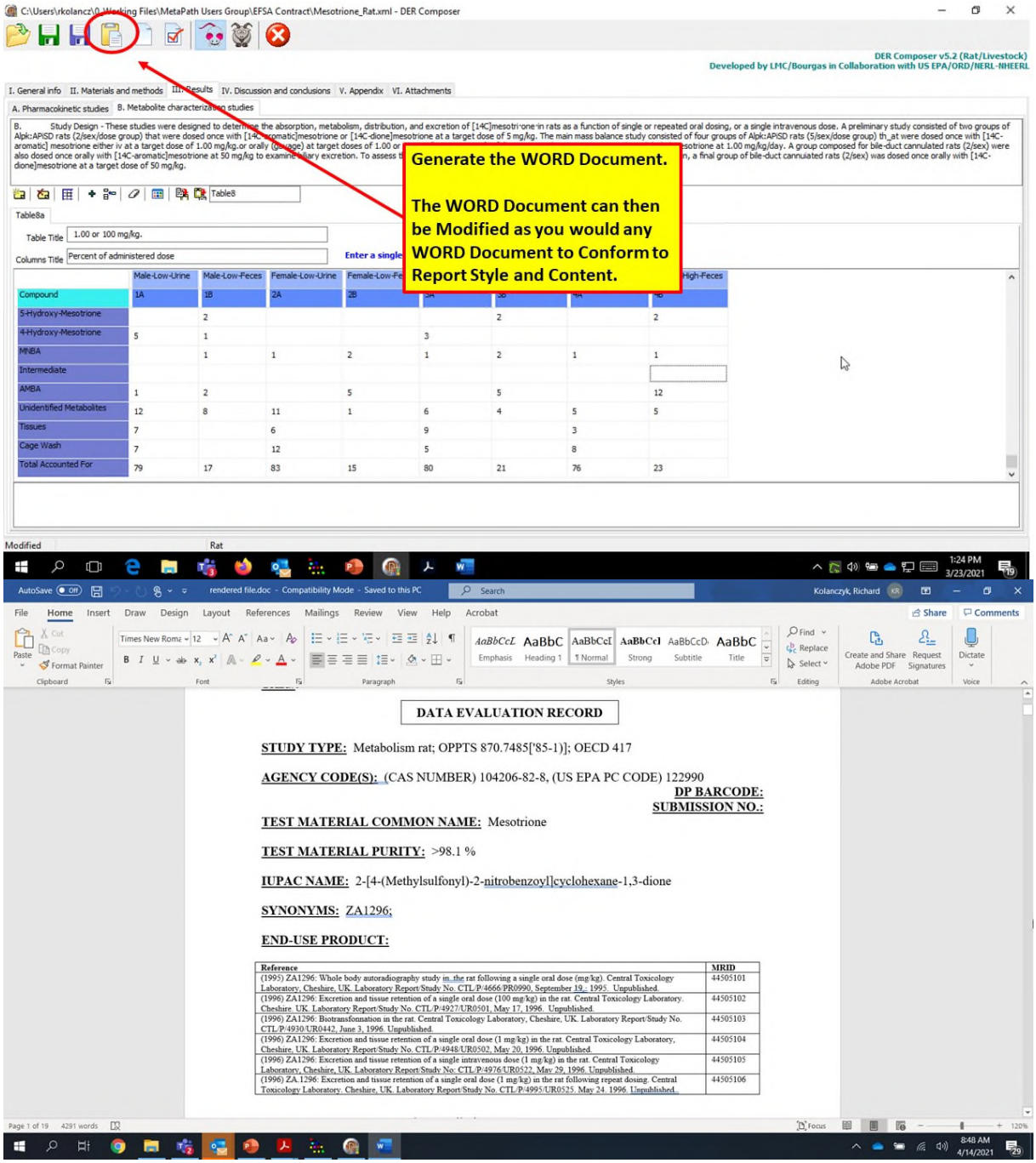
2.9. Conclusions, XML saving and Report generator

Please respect the following nomenclature when naming the DER XML file:

- Mammalian toxicology metabolism studies: id_activesubstance_mt_species_vX.xml (e.g. id_ quinmerac_mt_rat_v1).

Please noted that id means identification number. The applicant might decide to give an specific identification number to the file. EFSA will allocate an id called CardNo to the file once integrated into the regulatory MetaPath database.

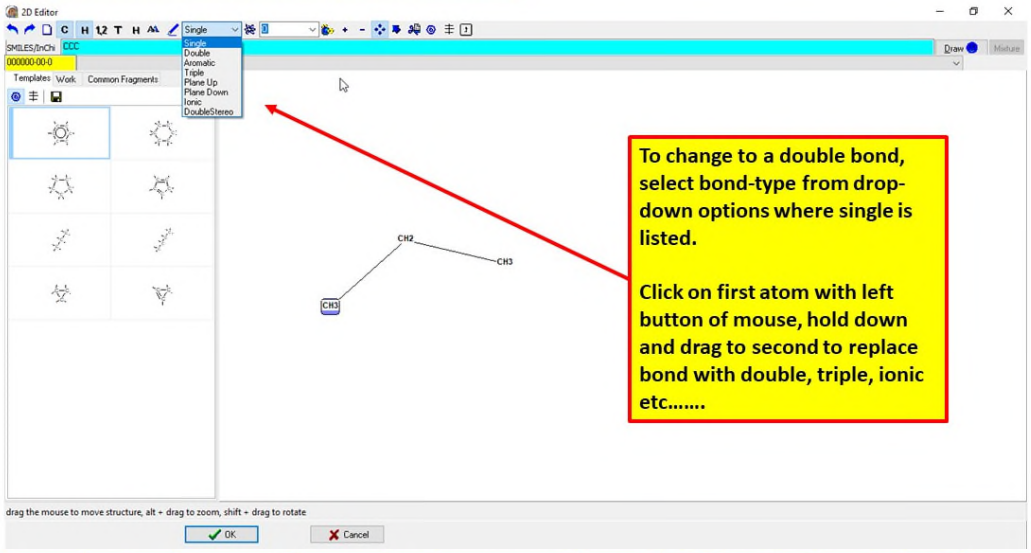

Save or Save As the XML
Might be a good idea to do frequent saves of your work during the data capture process.
Save As
Save
Cancel


Generate the WORD Document.
The WORD Document can then be Modified as you would any WORD Document to Conform to Report Style and Content.
DER Composer v5.2 (Rat/Livestock)
Developed by LMC/Bourgas in Collaboration with US EPA/ORD/NERL-NHEERL
DATA EVALUATION RECORD
STUDY TYPE: Metabolism rat; OPPTS 870.7485['85-1)]; OECD 417
AGENCY CODE(S): (CAS NUMBER) 104206-82-8, (US EPA PC CODE) 122990
DP BARCODE:
SUBMISSION NO.:
TEST MATERIAL COMMON NAME: Mesotrione
TEST MATERIAL PURITY: >98.1 %
IUPAC NAME: 2-[4-(Methylsulfonyl)-2-nitrobenzoyl]cyclohexane-1,3-dione
SYNONYMS: ZA1296;
END-USE PRODUCT:

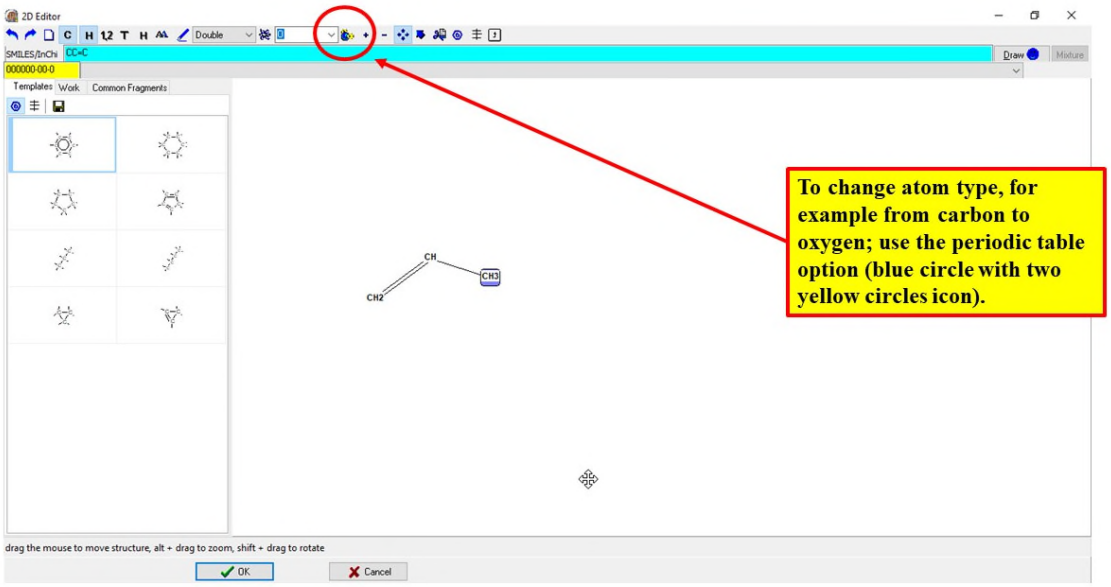
2.10. Drawing Tools, Structure Editor

STRUCTURE EDITING

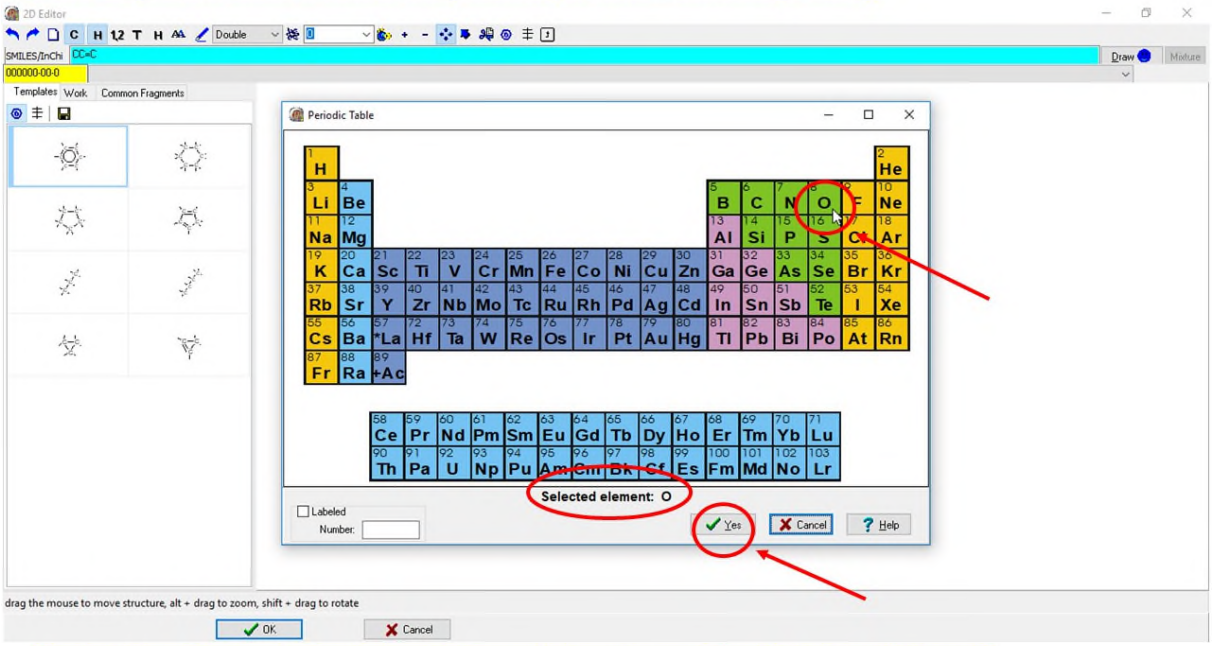
The following screen-shots illustrate some other functions of the STRUCTURE DRAWING package that may be used to modify/edit/draw 2-D structures of parent/metabolites.



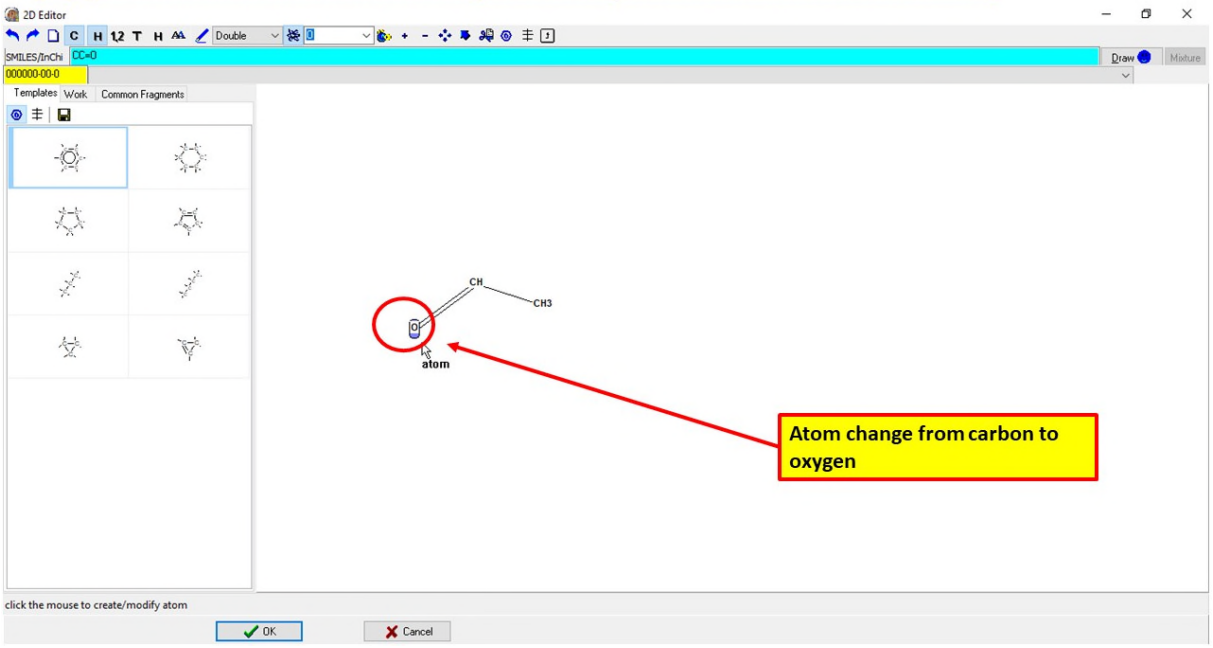
To change atom type, for example from carbon to oxygen; use the periodic table option (blue circle with two yellow circles icon).

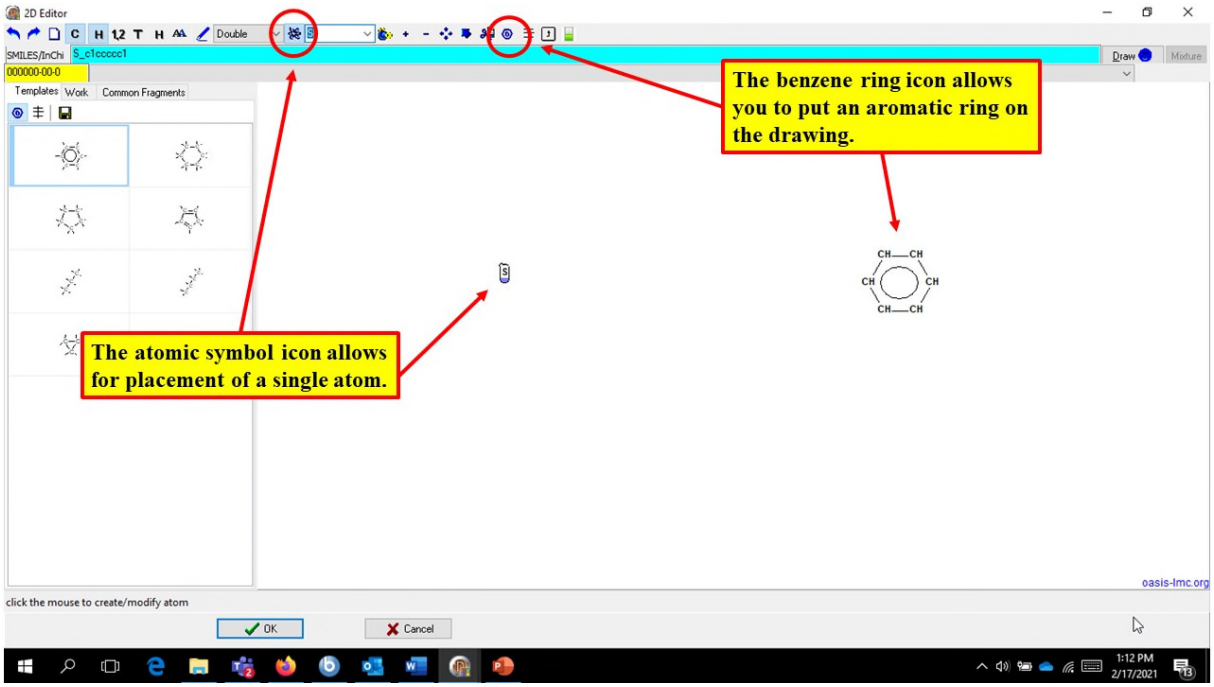


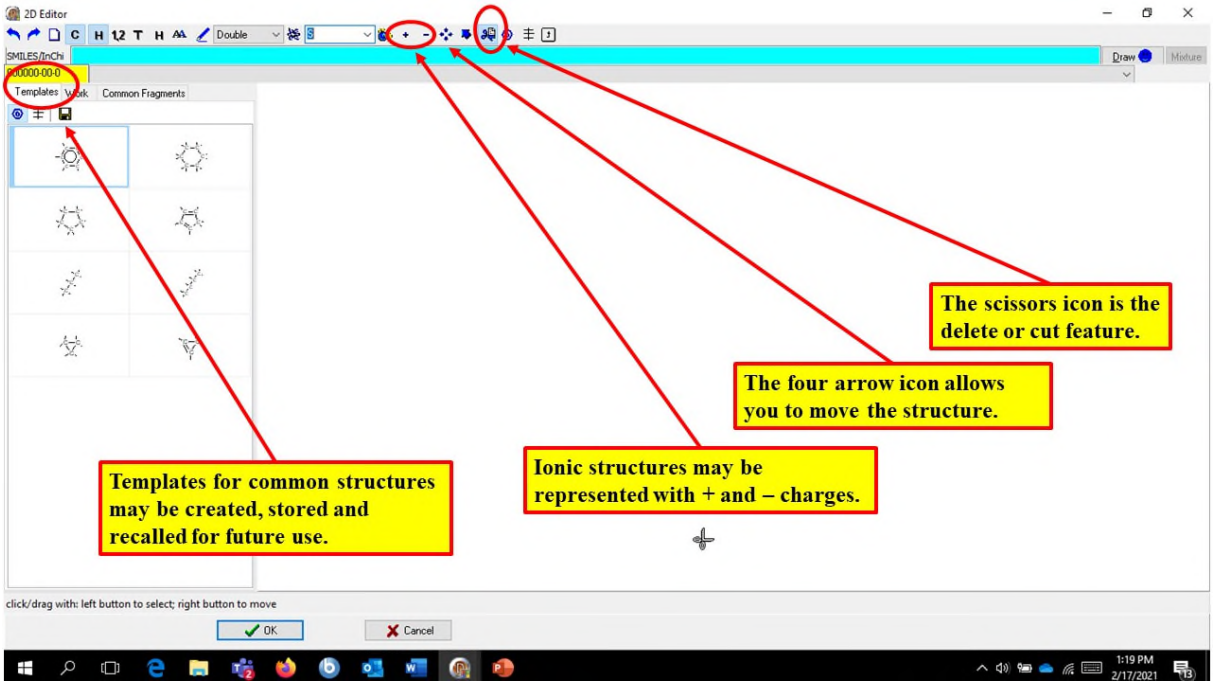
The table opens, click on atom choice, click yes to accept choice and the table goes away.



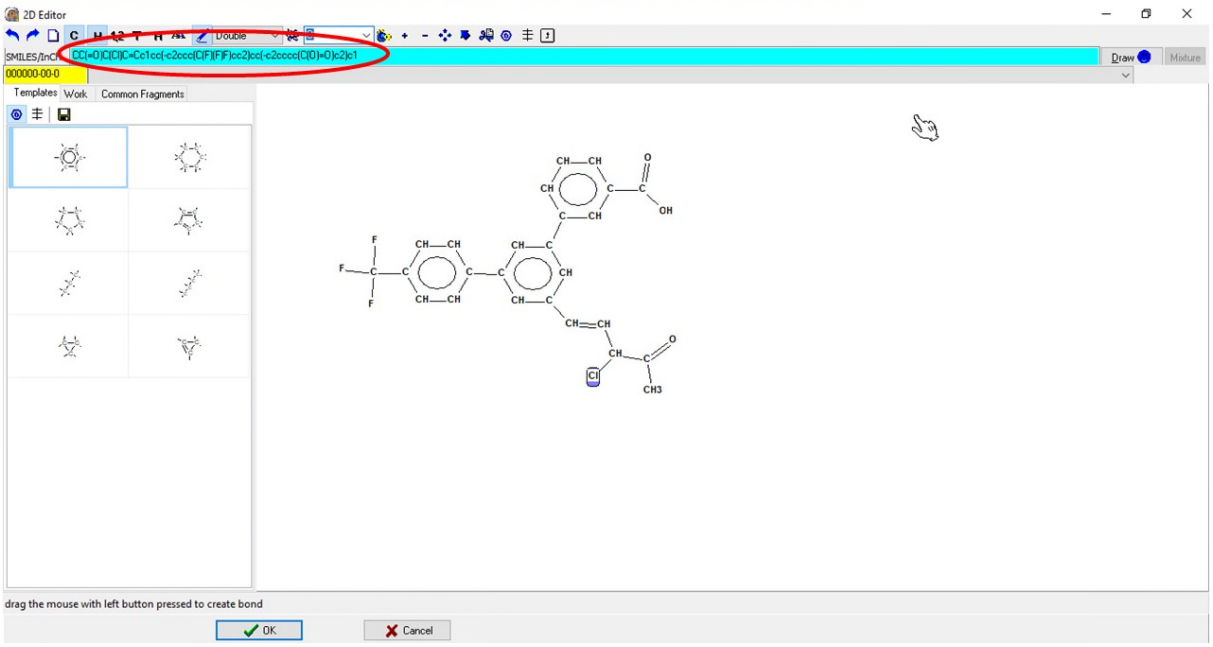
Simply click on the atom in the structure that you wish to replace and the substitution will be made.




2D Editor
The benzene ring icon allows you to put an aromatic ring on the drawing.
The atomic symbol icon allows for placement of a single atom.
click the mouse to create/modify atom
OK
Cancel


2D Editor
The scissors icon is the delete or cut feature.
The four arrow icon allows you to move the structure.
Templates for common structures may be created, stored and recalled for future use.
Ionic structures may be represented with + and – charges.
click/drag with: left button to select; right button to move
OK
Cancel

Once a structure is drawn, the SMILES string will be auto-generated for that structure.



3. Instructions for the Mandatory Data Evaluation Record (DER) Composer Fields.

Toxicokinetics studies should submitted in IUCLID by using "*Basic toxicokinetics – Endpoint study record*" template implementing OECD Harmonised Template (OHT) 58. DER Composer XML should be attached in the LITERATURE object of this template (EFSA MRL Manual v1).

EFSA recommends the applicant to fulfil all fields of the DER Composer. However, EFSA understands that some of the fields are common to OHT 58 template implemented in IUCLID for toxicokinectic studies and therefore implies duplication of the work. Therefore EFSA asked the applicant to provide at least the fields relevant for correct functioning for the metabolism layer of the MetaPath software. EFSA noted that implementation of the kinetic layer of the MetaPath software will be soon available and therefore results regarding toxicokinetic parameters are strongly recommended to fill in the DER Composer but are not currently mandatory.

Only those studies addressing metabolism are currently needed to be entered into the Composer. Other toxicokinetic studies investigating other Absorption Distribution and Excretion endpoints, e.g. oral absorption are not currently mandatory to be reported by using the DER composer.

The instructions for the DER Composer fields thar are mandatory for correct functioning for the metabolism layer of the MetaPath software are included in table 1.

Table 1: Instructions for the mandatory DER Composer Fields

Data entry flow	Type	Field	Instructions
1	**Header**	**I GENERAL INFO**	
	Field text	STUDY TYPE	Pre-filled: Metabolism rat; OPPTS 870.7485['85-1)]; OECD 417
	Field text. Picklist	AGENCY CODE(S):	Please select EFSA number

Field text	Agency Code value	Please insert EFSA PARAM term code for the active substance available in EFSA, 2020. doi. 10.5281/zenodo.4495166, e.g. for quinmerac the code is RF-0381-001-PPP. Please open the DCF catalogue " PARAM excel file", go to "term" tab and search for the active substance name.
Field text	TEST MATERIAL COMMON NAME:	e.g. quinmerac
Numerical value	TEST MATERIAL PURITY:	e.g. 97%
Field text	IUPAC NAME:	e.g. 7-Chloro-3-methylquinoline-8-carboxylic acid
Field text	SYNONYMS:	e.g. BASF 518 H
Field text	Citation	Please include study titles. Multiple references may be included in a single XML composer. Just needs to be the same active substance (where different labels might be included) and same species.
Field text	Sponsor:	e.g. BASF
Header	II. MATERIALS AND METHODS	
Header	A. MATERIALS:	
Header	1. Test compound:	
Button	ADD/DEL	To add or delete radio-labeled test materials. More than one radio-labeled can be included in a single XML composer but there is also the possibility to have separate XML composer for each radio-label.
Field text	Radio-labeled test material:	Follow Nomenclature recommended by ANSES, 2020
Numerical value	Radio-labeled purity:	
Numerical value	Specific activity:	
Field text	Lot/batch #:	
Smiles code	Structure:	Follow Drawing instructions for radiolabeled material as described by ANSES 2020.
Field text	Non-radio-labeled test material:	
Field text	Lot/batch #:	
Numerical value	Purity:	
Field text	CAS# of TGAI:	

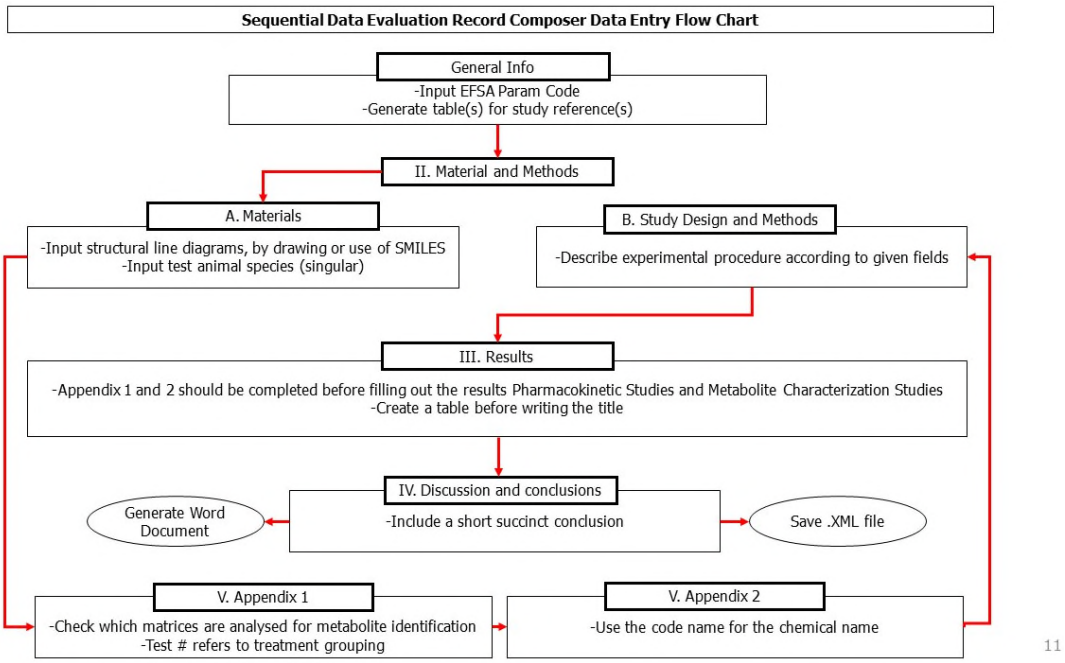
	Smiles code	Structure:	Follow Drawing instructions for non-radio-labeled material as described by ANSES 2020.
	Header	3. Test animals:	
	Text field: Piclist	Species:	e.g. rat (Please use singular)
4	**Header**	**B. STUDY DESIGN AND METHODS:**	
	Field text	1. Group arrangements:	Pre-filled text: Animals were assigned to the test groups noted in Table1
	HTML table	metaboliteStudies Table 1a	This table is auto populated with appendix 1a
5	**Header**	**III. RESULTS:**	
	Header	**B. Metabolite characterization studies:**	
	Matrix HTML table(s) (repeated block of fields)	Table(s) 8	The first column of a table is auto populated with information coming from Appendix 2. The auto populated names must not be changed. The tables should be summarising the occurrence of parent and metabolites in the different body fluids and tissues: i.e. the link to treatment groups should be established by using treatment groups from the appendix 1 for the column headers. Please note that where a numerical entry is present in the matrix (table) created between Columns (treatment groups) and Rows (metabolite names) establishes that correlation. Avoid use of non-numerical entries in the cells. If there is no correspondence – leave cell blank. If there is the need to add the standard deviation the coder would consider adding a custom column to the table next to that for the reporting value and label it as S.D.
2	**Header**	**V. APPENDIX:**	
	Header (repeated block of fields)	**APPENDIX 1a Summary of all treatment groups**	A matrix with all the different treatment groups and combination should be set.

	Text field	Test #	There is not fixed nomenclature for the test #. The nomenclature should clearly define groups in appendix 1 and the results tables treatment group identifier should be understandable. Examples: Lo-M-Urine for Low Dose in Male Rat Urine. Or low dose male rats as (1) and urine standardized as (a), feces (b), bile (c), thus: Thus 1a Low Dose Male Rat Urine 1b Low Dose Male Rat Feces 1c Low Dose Male Rat Bile 2a Low Dose Female Rat Urine 2b Low Dose Female Rat Feces 2c etc…….
	Text field. Picklist	sex	
	Numerical value	number	
	Text field	dose route	
	Numerical value	dose nominal	
	Numerical value	dose measured	
	Tex field. Picklist	dose type	
	Text field	matrix	
	Text field	descriptor	
	Text field	remarks	
3	**Header (repeated block of fields)**	**APPENDIX 2 Summary of parent and all identified/detected metabolites and their relationship**	**Follow Appendix 2 instructions**
	Text field	common name/code	Follow Nomenclature recommended by ANSES, 2020.
	Text field	chemical name	Follow Nomenclature recommended by ANSES, 2020.
	Smiles code	chemical structure	Follow Drawing instructions as described by ANSES 2020.
	Metabolic pathway description	parent	Link between parent and their metabolites should be established. Please start with the parent.

Text field. Picklist	expertise	none; assumed by the author(s); tolerance expression; residue of concern; expert specified.

4. Sequential Data Entry Flow Chart

The data entry flow in the DER Composer follows a specific order as given under Steps chapter. The sequential data entry flow chart is summarised in figure 1. The figure is based on a previous Flow Chart developed for the former version 3r21 of the livestock DER composer (Cory McCurry, July 2010).



The arrows and connectors of the flow chart are to represent the sequence by which a coder should input information.

Figure 1: Sequential Data Entry Flow Chart

5. Webinar: MetaPath – how to complete DER composers for pesticides mammalian toxicology metabolism studies - Q&A sessions

A webinar, hosted by EFSA, in cooperation with US EPA, took place 20th April 2021 and covered the following topic, Completing Data Evaluation Record (DER) for pesticide mammalian toxicology metabolism studies.

The webinar included a theoretical part and a live session to explain how to complete DER composers for pesticide mammalian toxicology metabolism studies[1].

Q&A sessions were also held to answer attendees' questions.

Number	Question	Answer
General information		
1.	*Will the DER Composer be replaced by a much more user-friendly MSS Composer?*	Through contracts NP/EFSA/PREV/2020/01 and NP/EFSA/PRES/2020/01 EFSA intended to update the DER Composer to a user-friendly MSS Composer.
2.	*Will the DER composer be freely downloadable, or will a paid license be required?*	Both MetaPath and DER composer could be downloaded for free at https://oasis-lmc.org/products/software/metapath.aspx .
3.	*Which citations should be included in the General Info?*	Reference of ADME experimental studies.
4.	*How will redaction of author names of mammalian studies be handled in DER Composers?*	The names of the authors should be reported by the applicant when submitting a study with the DER composer. Before sharing the final version of the file in a public database, EFSA prepare a sanitized version, removing the confidential information.
5.	*Is there a certain style that needs to be used for citations?*	There is no specific style, the citation should allow the risk assessor to identify properly the study or studies they are referring to.
Material and methods		
6.	*Will there be the possibility in near future to put in an InChI code or a structure?*	That capability is already included in the Composer. Options for structure generation include submission of InChI, submission of SMILES or use of the drawing tools included in the Structure Editor.
7.	*Open 2D Structure Editor - means - free tool (integrated into the MetaPath Program Supply); if only CAS number are inserted is it enough to have comprehended data on all molecule properties?*	Yes, the tool is free and included in DER Composer. Insertion of CAS number will not automatically produce a 2-D structure. Insertion of SMILES, InChI, or use of the drawing tools will produce the structure. The structure identification is derived from the experimental study. CAS numbers have been selected to track the active ingredient because they are very specific for the structure; however, they might not be available for metabolites.
8.	*Do you need to include e.g. % when filling in the environmental condition's fields?*	This is not a mandatory field. See Chapter 3 of the Manual.

[1] https://www.efsa.europa.eu/en/events/webinar-metapath-how-complete-der-composers-pesticides-mammalian-toxicology-metabolism

9.	*How to deal with isomers in this composer? Do active substances as well as metabolites must be included separately? Is the drawing program able to specify the different isomers?*	Within the structure drawing package there are features for drawing the stereoisomers, this is extensively explained in MSS composer manual by ANSES, 2020. Metabolites need to be included in the metabolic pathway. Please noted that the structure drawing package is the same in DER and MSS composers.
10.	*How can I add a new species to point 3 "Test animals"? I have only 3 species (i.e. Goat, Hen, Cow)* *Within # 3. Test animals, the pull down did not show Rat or Dog. It was possible to add the text however. Will that be okay?*	The name of the new species could be typed in. Please remember to use singular common name e.g., Rat, Mouse, Rabbit, Monkey, Dog, etc…
11.	*What about formatting within text blocks (narrative descriptions): e. g. Font type and size (of pasted text); e. g. italics for Latin names, bold words, underlining, etc. Does this formatting matter or stay as it has been inserted, or can it cause problems?*	Format does not matter. There are limitations in the ability to format that will be enhanced in the new MSS style Composer upgrade that is planned. The formats and special characters are available via copy and paste.
12.	*The DER composer is appropriate for in vivo metabolism studies, what about the in vitro comparative metabolism studies which are now required under the 1107/2009?*	To report comparative *in vitro* metabolism studies by using the DER composer is not mandatory. See Chapter 3 of the Manual.
Appendix 1 and 2		
13.	*Is it possible to prepare a separate table for the quantification of stereoisomers, e.g enantiomers of the parent compound (which is also quantitated in total)?*	It is possible.
14.	*Should the data be included study by study or should the studies for one species be summarised in one entry?*	Studies for one species can be summarised in one Composer file unless clear differences in metabolism are observed between two or more studies. A separate XML/entry should be created for one species and one active ingredient.
15.	*Is there a certain way to name the Appendix1 Test#, e.g. similar to the one used in the MSS Composers?*	For the treatment group nomenclature there is no fixed criteria. See Chapter 3 of the Manual.
16.	*Presumably you define different radiolabel positions in Appendix 1 as well to generate data entry tables for different labels in III, results. Is this correct?*	Yes. See also replies under 17 and 19.
17.	*If the molecule is radiolabelled in 2 different parts, should I report it only once? Or should we fill in two composer xml files?*	It is possible to separate XML for each radio-label and each XML will import as a separate folder/map into MetaPath. However, the DER Composer also allows for the option of combining more than one radio-label. Also, remember that MetaPath allows the user to combine the two later if so desired by using the map merge function.
18.	*Is there a limit to the number of treatment groups?*	There is no limit.
19.	*I guess in Appendix V 1st Table two different labels must also be included separately? Which means two times the same matrices/single oral etc.?*	A second radiolabelled molecule could be added under > II. Material and methods > 1. Test Compound > click on "ADD" button to include a second molecule. In this specific case, in the "Radio-labelled test material" box, it is important to specify the site of label. Then two options are available:

		1. In the *Appendix Ia* the *Test#* could be adapted to specify the Radio-labelled test material they are referring to. 2. An additional reference could be added in the citation box (under *General Info*) to cover the different radiolabelling. In this case a second *Appendix Ib* will appear under *V. Appendix.*
20.	*While inserting the information in the appendix regarding the treatment groups, do we need to insert information regarding organs and tissues under the filed Matrix?*	The information to be included in Appendix 1 are the ones related to body fluids and tissues in which metabolite identification has been done. At a minimum the guideline study looks at excreta, but some will include Bile studies as well as investigation of metabolites in tissues.
21.	*How to handle generic structures?*	It should be described under Appendix 2 Editor > Expertise > Expertly Specified. Two approaches could be taken: 1. Include the more logical position (based on chemistry, hindrance). 2. Draw both metabolites' structures and include all the position in the drawing.
22.	*What about "generic structures" of metabolites, e. g. the position of hydroxylation (or conjugation) has several possibilities?*	See reply n.21.
23.	*What to do if position of hydroxylation is not identified and there is no information to make expert judgment?*	See reply n.21.
24.	*In your example of ring hydroxylation, you suggested to use expert judgement. Is there a way to use generic structures instead, since for many chemicals the hydroxylation probability is high at multiple positions, and it is likely that all occur to a limited extend?*	See reply n.21.
25.	*If you add all possible structure where you don't know the exact position of the OH, how do you make it clear in the pathway that you did not have all of these metabolites as discrete metabolites (i.e. that they are all possibilities for the same metabolite?)*	See reply n.21.
26.	*What about isotope labels without radioactivity (e. g. 13C, ...) ?; List them under "radiolabel" ?*	Stable isotope labels (e.g., 13C) could be included under the radio-label test material(s). They could also give rise to specific treatment groups where the stable isotope was utilized as test material.
27.	*What if you do not know the exact structure of the metabolite. For example, the position of an OH group on your metabolite so there are several possible isomers and there is no expert view on which is most likely, or all are equally likely. How would you enter a structure when you do not know exactly what it is, but you know it is a metabolite?*	See reply n.21.
28.	*I noticed there were templates in the structure editor - will these be covered in the session after the break?*	In this regard, please see the information reported under "2.10. Drawing tools, Structure Editor"
Results – Pharmacokinetic studies		
29.	*Can you paste data from excel or word tables into the result tables?*	Currently not. Through contracts NP/EFSA/PREV/2020/01 and NP/EFSA/PRES/2020/01 EFSA intended to update the DER Composer and this useful functionality can be indeed explored.

30.	*Can you import whole tables from excel?*	No. See reply n. 29.
31.	*If at one timepoint no value is given, is there a special type to fill-in "not given" or "not detected"? And where to put such abbreviations? In the text above?*	Yes, for the results under "Pharmacokinetic studies". In this section there is the possibility to include "ND" or "NS", adding guidance (e.g. ND= not detected) in the free text box below. It should be avoided in the case of "Metabolites characterization studies".
32.	*Is there any template that can be used to upload all the data rather than doing this manually?*	No. See reply n. 29.
33.	*Is there a possibility to paste tables or table rows from a file as a whole, without the necessity to insert each cell separately?*	No. See reply n. 29.
34.	*Would the 'other' tab be used also for distribution data?*	Yes, the "Other" tab under III. Results > A. Pharmacokinetic studies > Other, can be used e.g. for distribution data, bio-accessibility and enzyme activity if needed..
35.	*Will it be possible to save table layouts if there are many such tables to include?*	Currently not. Best option is to use the copy/paste table function. Through contracts NP/EFSA/PREV/2020/01 and NP/EFSA/PRES/2020/01 EFSA intended to update the DER Composer and this useful functionality can be indeed explored.
36.	*Is there a possibility to import tables from e.g. xls files?*	See reply n. 29.
37.	*Is there any consideration given to build templates so that data can be uploaded, or we can implement simple copy paste functions in the future?*	Currently not. See reply n. 29 & 35.
Results - Metabolite characterization studies		
38.	*If all of these have been done for one active substance; can this can be re-utilised subsequently (a few months later) for another active as a template to save re-entering the general info, tables, etc.. as the study designs are often the same?* *If we cannot re-use the work of a previous active substance as a template, does this mean we have to start from scratch for a new active substance ?*	By using the SAVE AS command an XML created for one active could be re-used for another but there would be changes to be made considering the new active, structures & descriptors, test animal characteristics, all of the metabolites, values in tables would be different, etc… Our assessment would be that for all the required modifications it would be best to start from scratch. Rather than a template we would consider the first study as an example for completing subsequent studies. Whereas, in the case of a different species with the same active substance the document could be slightly modified accordingly. However, through contracts NP/EFSA/PREV/2020/01 and NP/EFSA/PRES/2020/01 EFSA intended to update the DER Composer and the inclusion of predefine tables can be indeed explored.
39.	*Do the rows update automatically when updating the Appendix?*	No, therefore a specific way to enter the data (specific flow) is needed. A new metabolite could be included in the

		"Metabolite Characterization Tab" table; however, the name should be entered into the table exactly the same as the name of the metabolite reported in the Appendix.
40.	*Does MetaPath include "identified" metabolites under "characterization" ? - while the guidelines differentiate between "identified" and "characterized" ?*	In MetaPath they are treated as synonymous. If a metabolite is identified but not quantified, a code might be used in the results table to explain it (e.g. 111111) and then explanation could be given in the free text box below as a footnote (e.g. 111111 = metabolites observed but not quantified) .
41.	*What if, if the metabolites are only postulated by the author but not find in the samples? Can we left the table related to these empty?*	Yes, to allow for a clean look to the Table, delete the rows (metabolites) with no value to report.
42.	*Does the metabolite quantitation table accept range values?*	No.
43.	*Can you use less than (<) symbol?* *Do the symbols "<" and ">" cause irritations in Metapath because they could mean "open/close brackets" in xml?*	The < symbol does make the value disappear as well as the ≤ symbol. In addition to this, for metabolites that were observed but not quantified it was initially recommended that a "+" be used in the characterization table(s). In addition to numbers, the "+" allows for maintaining correspondence between treatment and metabolite. However, the "+" symbol is not imported into MetaPath on the depicted table. Therefore, as already explained under reply n. 40, the use of a code, e.g. 11111 to denote presence of metabolite with an explanation as footnote, is currently the option recommended.
44.	*In the example table ppm and TRR are provided together. Would you propose to sperate theses values in different tables? And what are the rules to separate information, like treatment groups or matrices or labels?*	The preferred option is to be separated; however, it is a matter of preference. Both can be entered as long as the units are described in each column. By using the "General Column Label".
45.	*Is there a restricted number of tables (only three?)?*	No.
46.	*I notice that there is no build pathway option as with MSS. So, there is no way to check visually that your pathway looks right before it ends up in Metapath?*	There is no immediate screen of the proposed metabolic pathway. Through contracts NP/EFSA/PREV/2020/01 and NP/EFSA/PRES/2020/01 EFSA intended to update the DER Composer to a user-friendly MSS Composer on which this functionality will be available.
47.	*Is it possible to include < LOQ or <0.001 ppm?*	See reply n. 43.
48.	*If you added a table by mistake, how can you delete it?*	There is a delete function.
49.	*How to handle coeluting minor metabolites?*	Keep them separate, maybe add a footnote to explain it. Possible to split the reported quantity between the two (or more) co-eluting metabolites; just indicate as a footnote.
50.	*Is it possible to prepare a separate table for the quantification of stereoisomers, e.g enantiomers of the parent compound (which is also quantitated in total)?*	There is no need to add a separate table if the stereoisomers are included as separate metabolite.

51.	*Is there a word limit on the discussion?*	There is not a limit.
52.	*What would happen if the metabolite name or codes changed through the lifetime of the active substance?*	Even during the assessment of a substance different applicants could name the metabolite differently. This is countered in MetaPath by using the structure represented by SMILES of the metabolite instead of the name of the metabolite for searching for a specific metabolite.

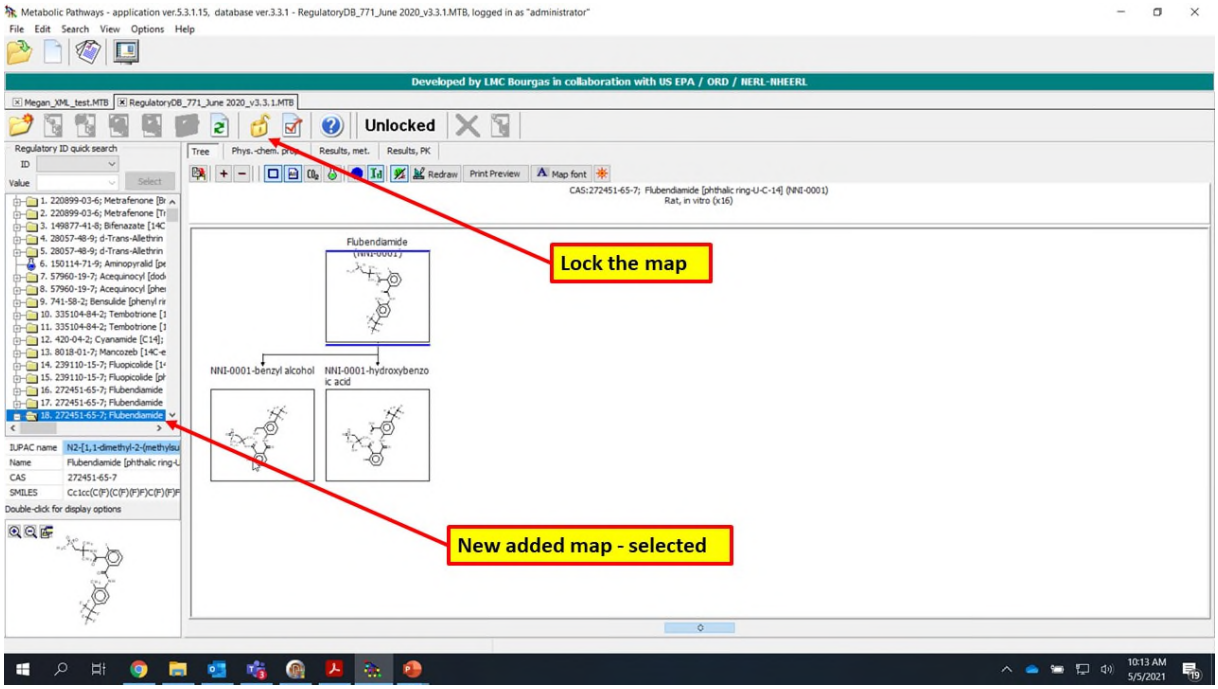
6. *In vitro* metabolism studies

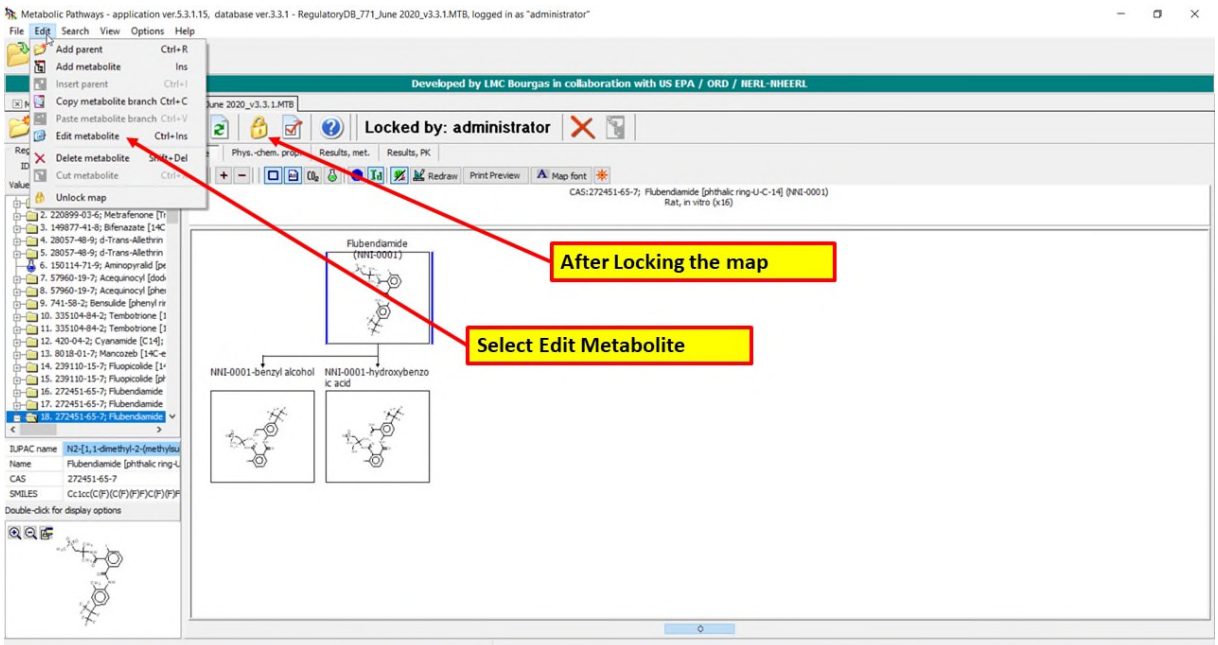
Comparative *in vitro* metabolism are requested according to the EU data requirements on pesticides[2]. Currently, comparative *in vitro* metabolism studies should be reported under 5.8 Other toxicological studies (ENDPOINT_STUDY_RECORD.AdditionalToxicologicalInformation -v.6.3) as described in the EFSA MRL Application Manual (EFSA, 2021).

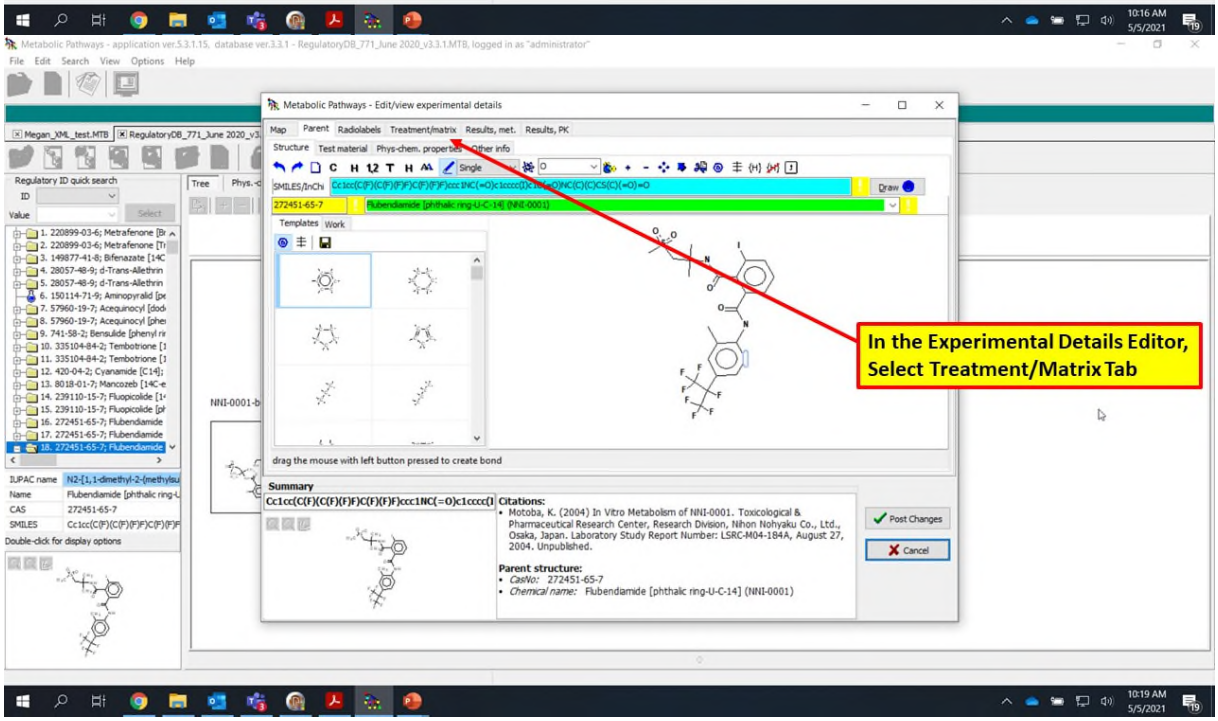
If metabolite identification has been conducted in the comparative *in vitro* metabolism study it is possible to use the DER composer to report the metabolic pathway in these studies. However, the DER composer currently does not allow distinction at the *in vitro* /*in vivo* level. When setting up treatment groups in Appendix 1 the coder should describe the matrix appropriately, e.g., liver microsomes. Once the XML is imported into MetaPath, there would be the need to edit and modify the treatment groups to reflect the *in vitro* study in MetaPath. The following step-by-step guide describes instructions on how to do it, however, currently it is not mandatory to report *in vitro* metabolism studies by using the DER composer and further editing in MetaPath.

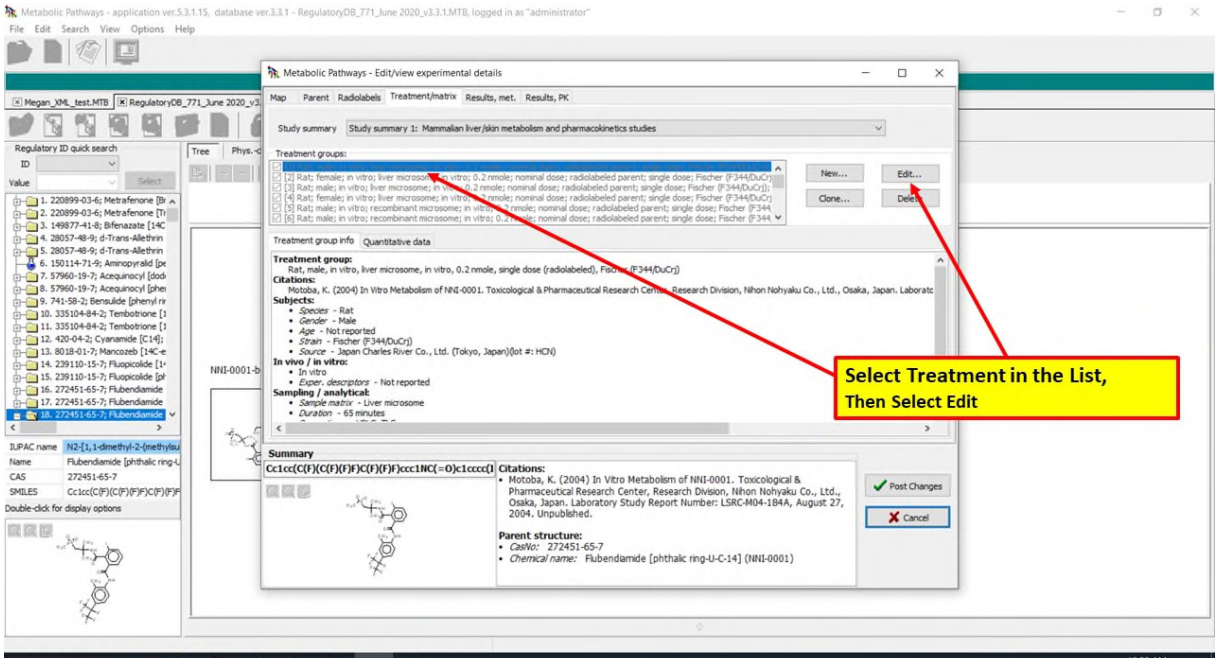
[2] Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Text with EEA relevance
OJ L 93, 3.4.2013, p. 1–84

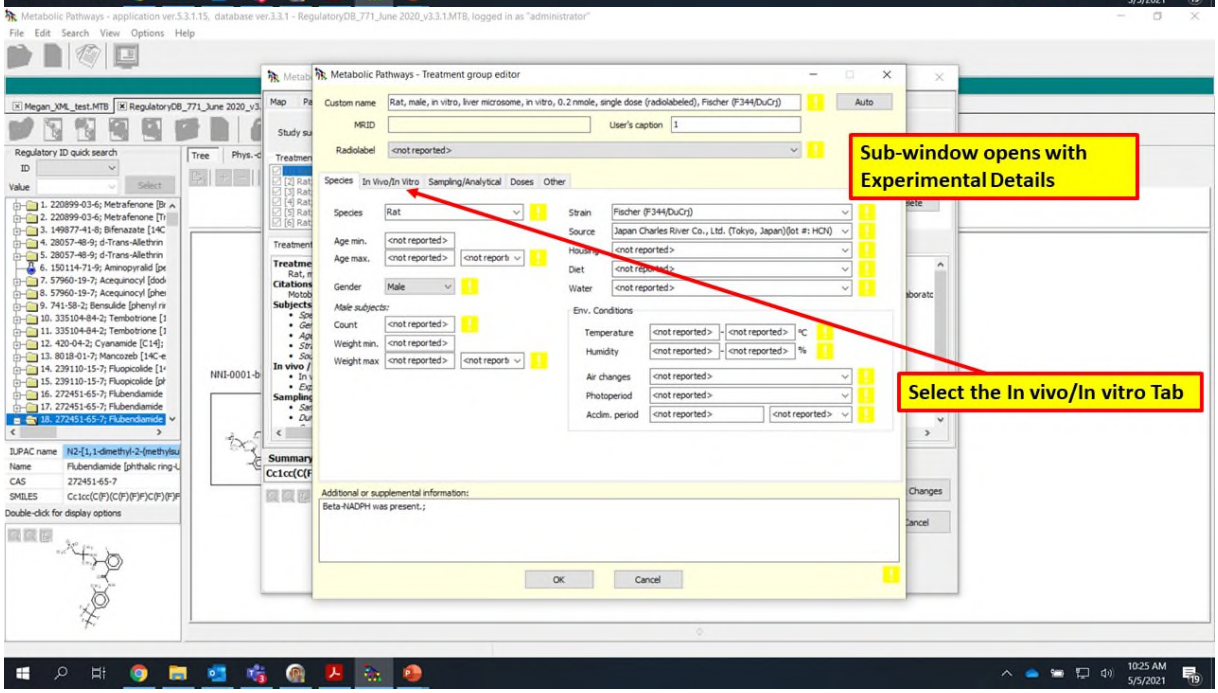
Steps for Finishing Entry of In Vitro Studies within MetaPath – Need to Apply Descriptors for (1) In Vitro (2) Experimental System (3) Organ/Tissue After the Import of the DER Composer XML

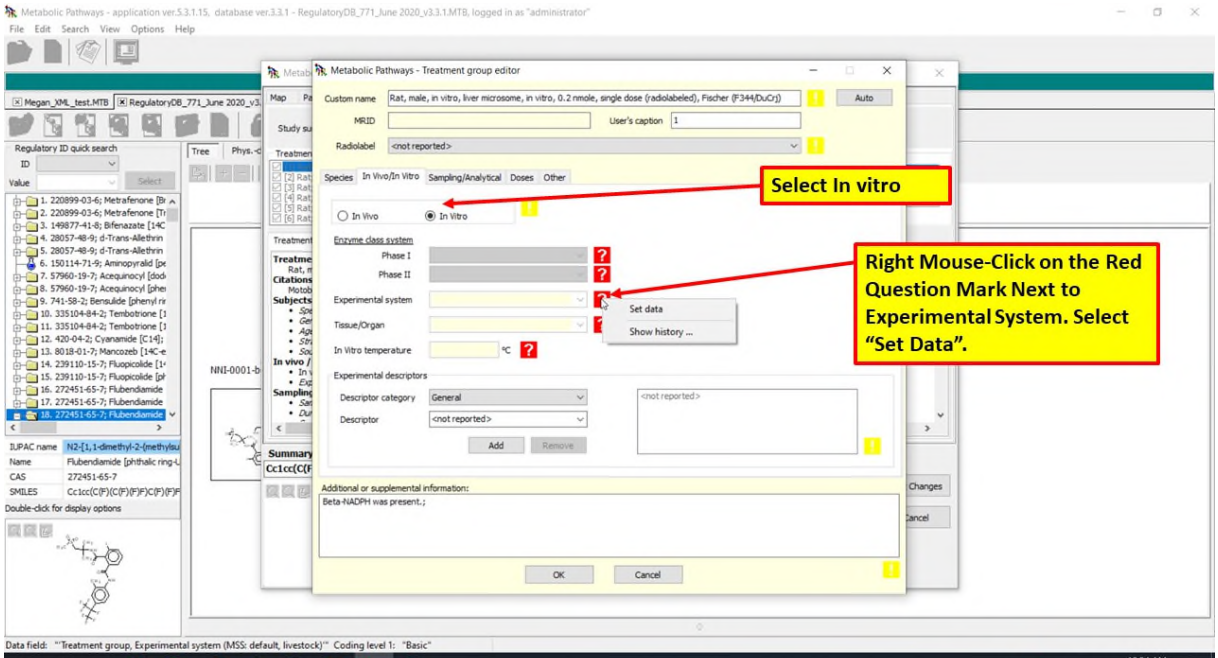


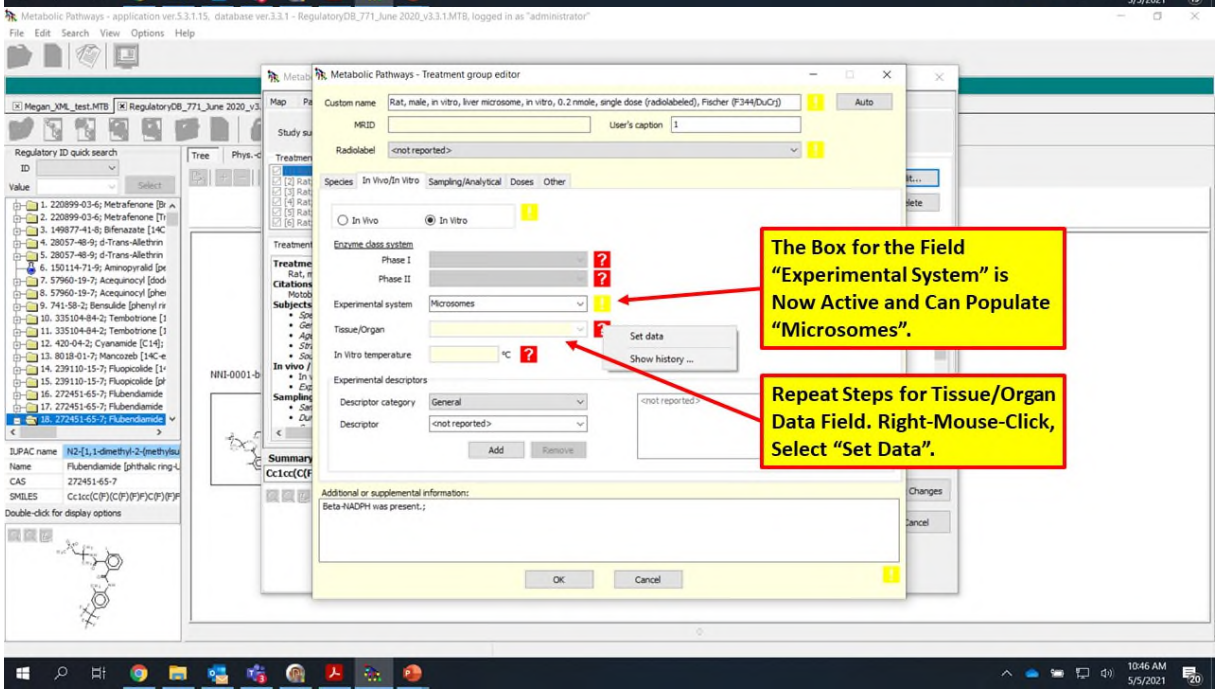

Developed by LMC Bourgas in collaboration with US EPA / ORD / NERL-NHEERL
Locked by: administrator
Add parent Ctrl+R
Add metabolite Ins
Insert parent Ctrl+I
Copy metabolite branch Ctrl+C
Paste metabolite branch Ctrl+V
Edit metabolite Ctrl+Ins
Delete metabolite Shift+Del
Cut metabolite Ctrl+X
Unlock map
After Locking the map
Select Edit Metabolite
Flubendiamide (NNI-0001)
NNI-0001-benzyl alcohol
NNI-0001-hydroxybenzoic acid

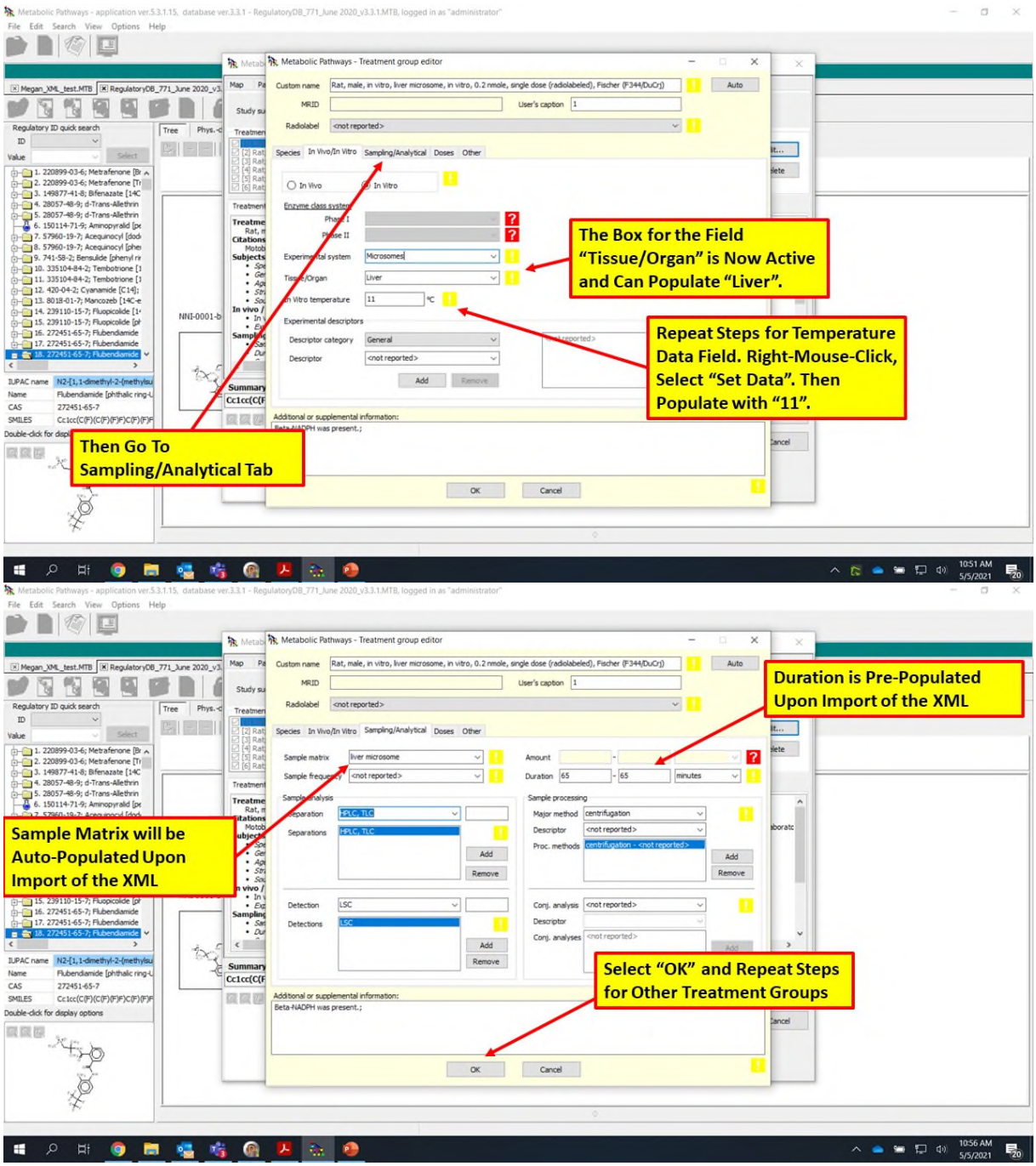

Metabolic Pathways - Edit/view experimental details
In the Experimental Details Editor, Select Treatment/Matrix Tab
drag the mouse with left button pressed to create bond
Summary
Citations:
Parent structure:
Post Changes
Cancel


Metabolic Pathways - Edit/view experimental details
Select Treatment in the List,
Then Select Edit


Metabolic Pathways - Treatment group editor
Sub-window opens with
Experimental Details
Select the In vivo/In vitro Tab


Metabolic Pathways - Treatment group editor
Select In vitro
Right Mouse-Click on the Red Question Mark Next to Experimental System. Select "Set Data".
Set data
Show history ...


Metabolic Pathways - Treatment group editor
The Box for the Field "Experimental System" is Now Active and Can Populate "Microsomes".
Repeat Steps for Tissue/Organ Data Field. Right-Mouse-Click, Select "Set Data".
Set data
Show history ...



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