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European Food Safety Authority

WEBINAR: METAPATH

How to complete MSS composers for pesticides metabolism studies

-

Metabolism in rotational crop studies

Wednesday 31 March

Time	Topic
9h35 – 10h30	Livestock MSS composer
10H30 – 10h45	Coffee break
10h45 - 11h30	**Rotational crops MSS**
11h30 – 12h	Q&A session and Conclusion

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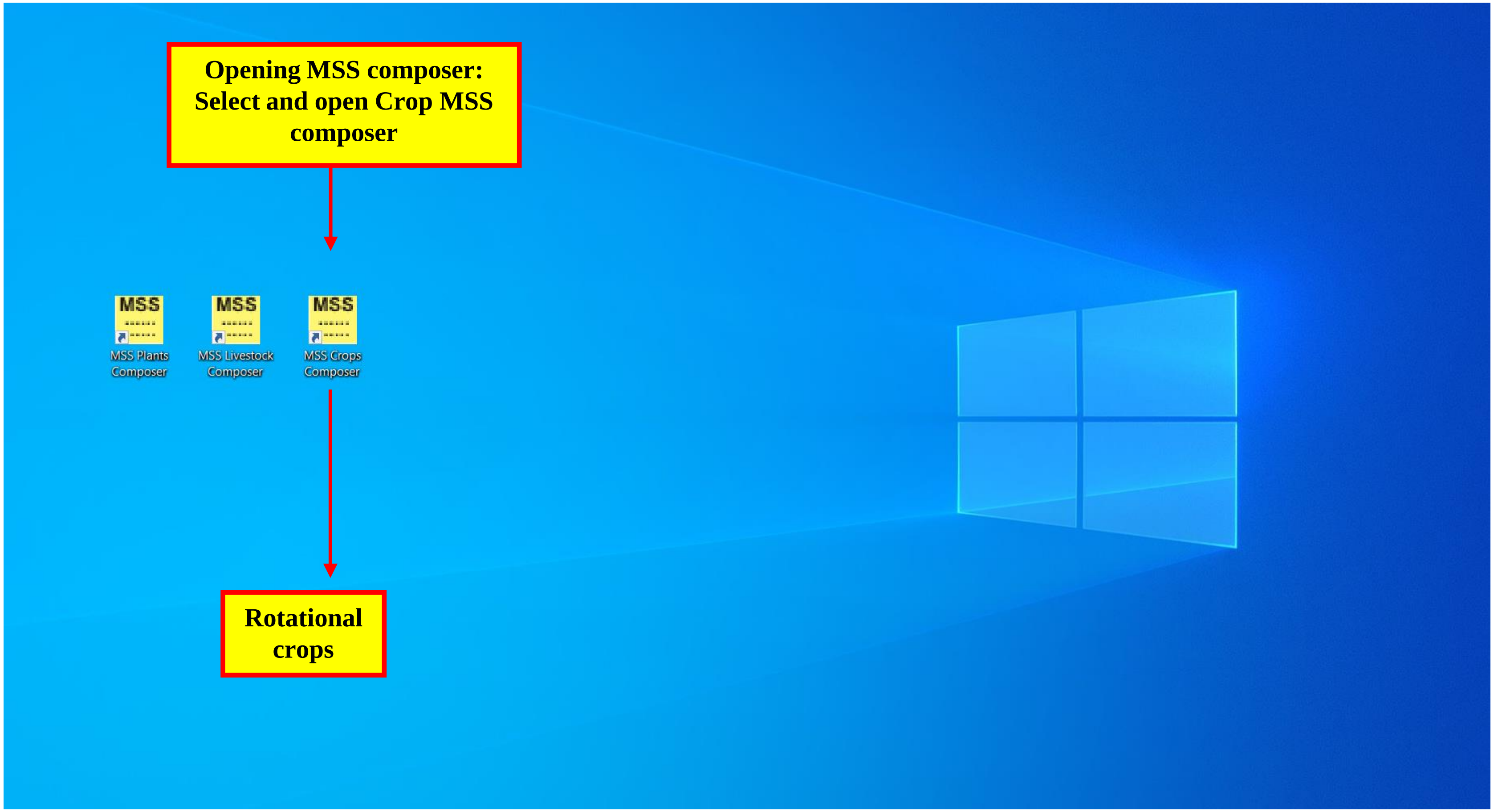
Opening MSS (1/2)



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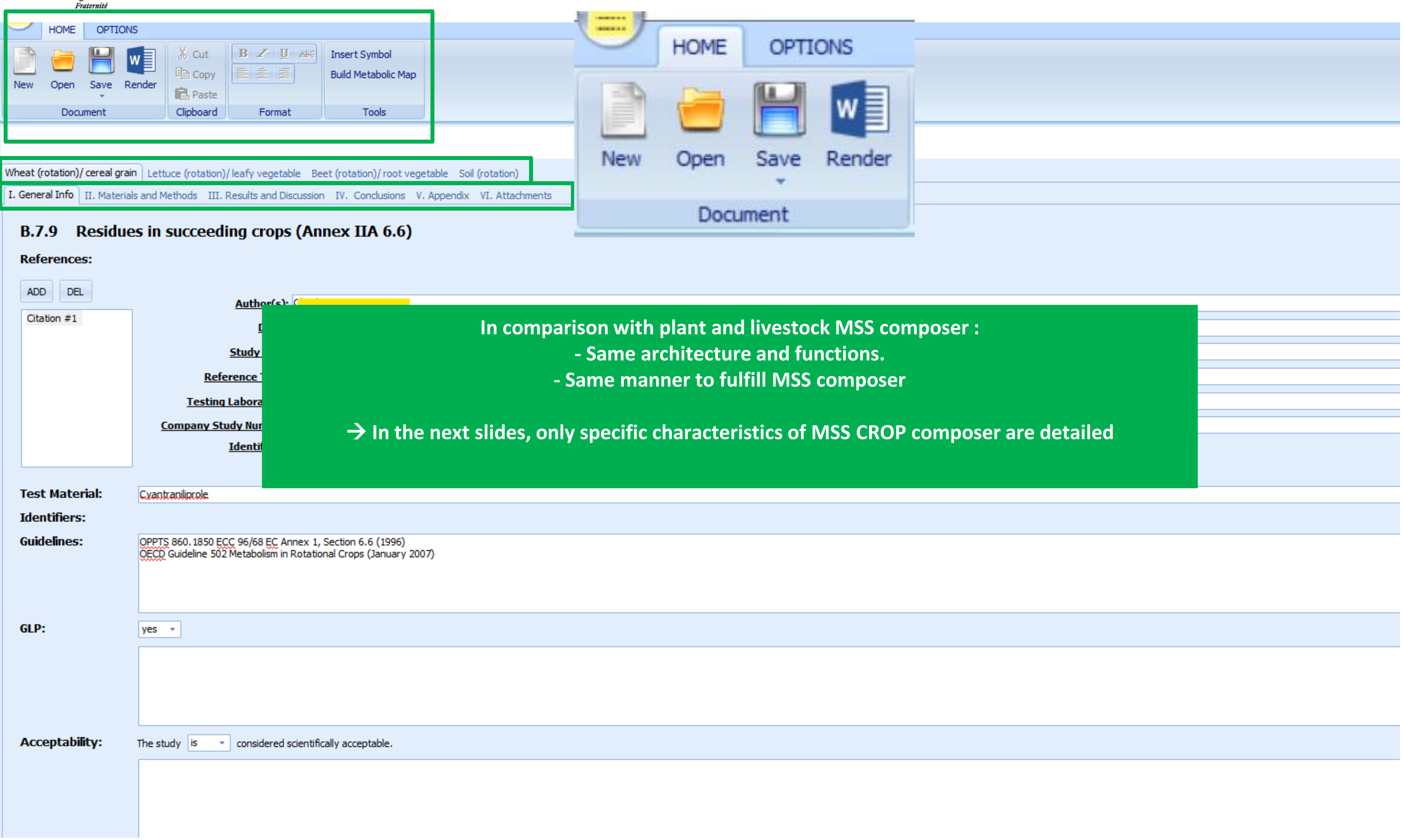
Opening MSS (2/2)

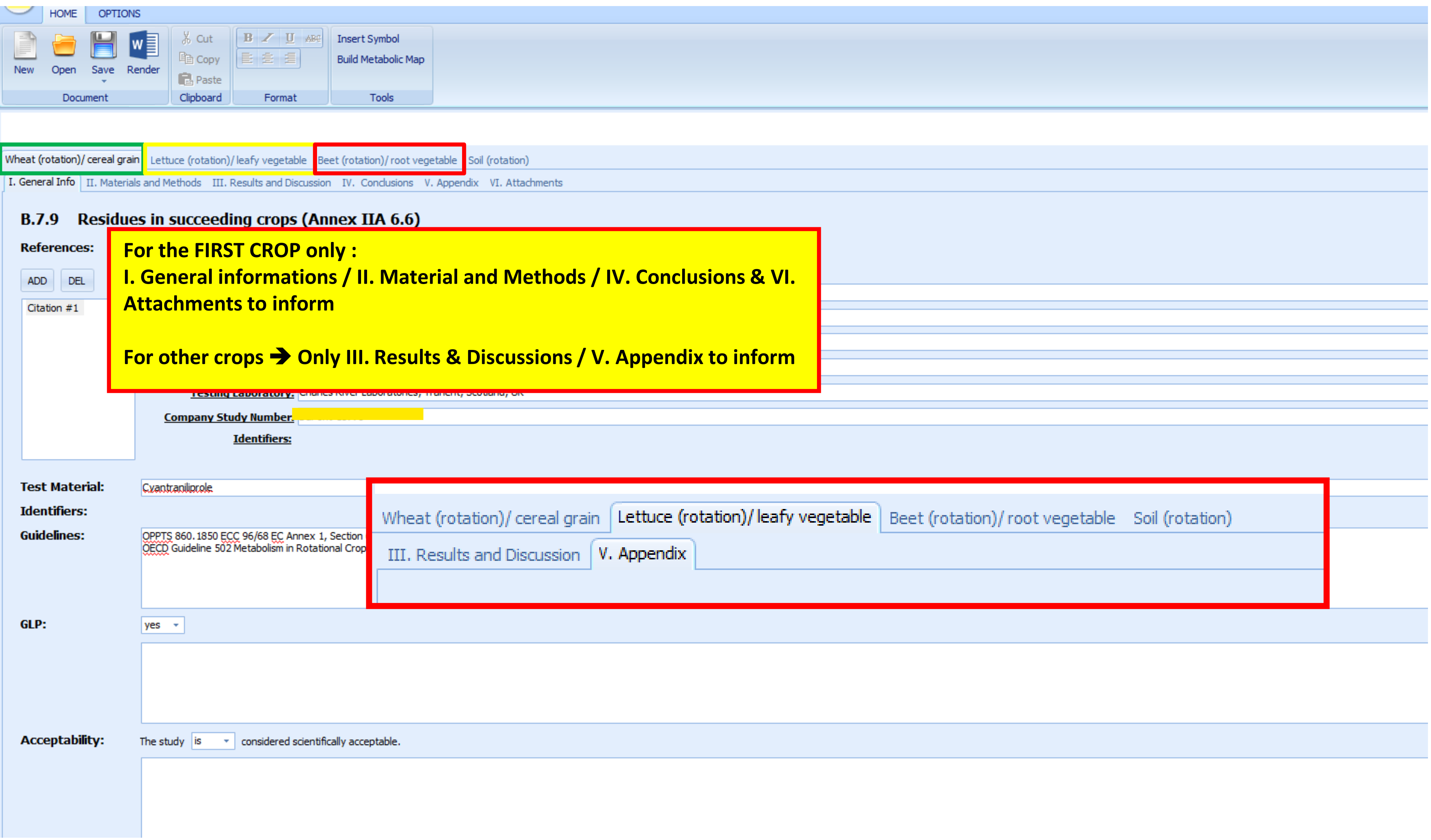


In comparison with plant and livestock MSS composer :

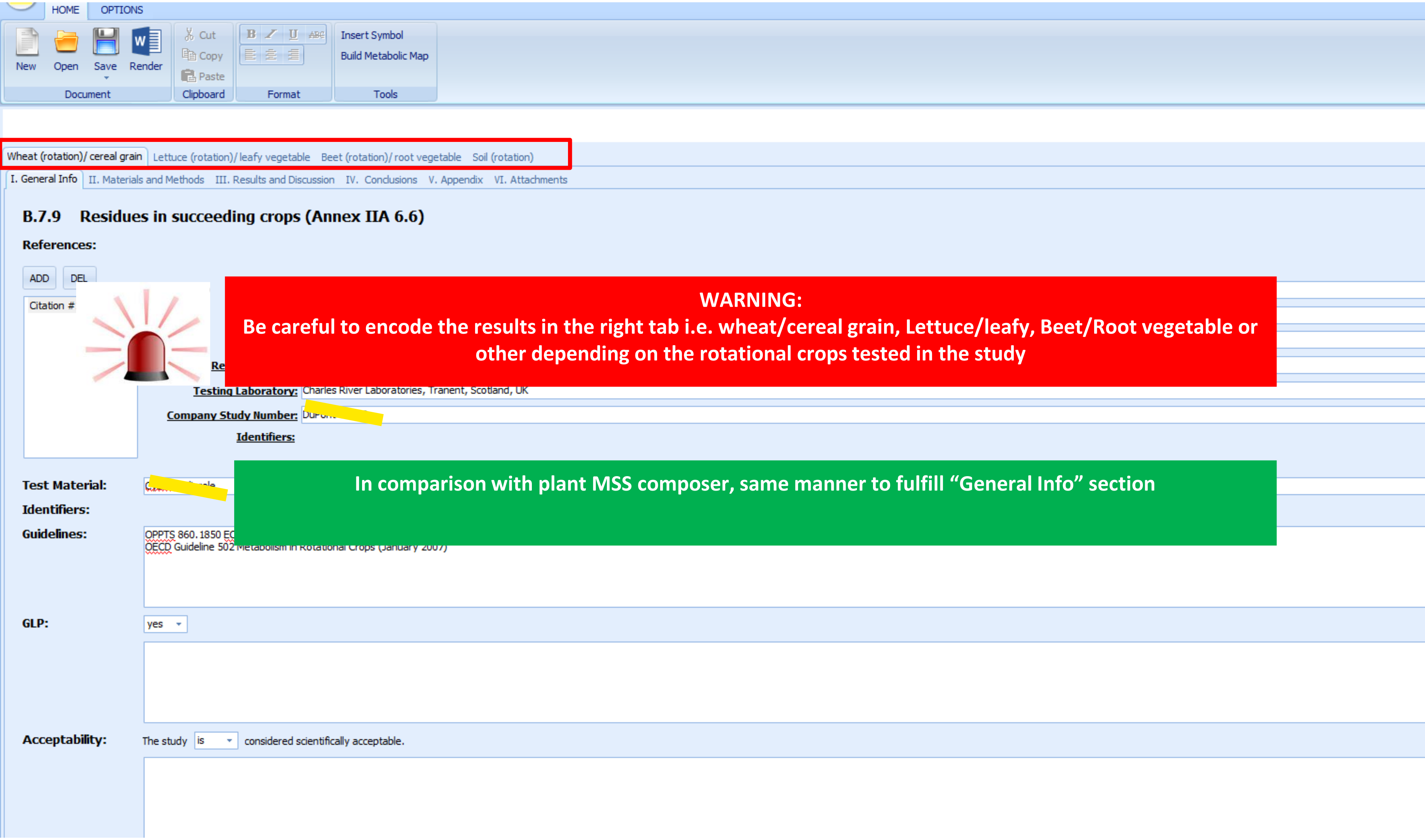
- Same architecture and functions.
- Same manner to fulfill MSS composer

→ In the next slides, only specific characteristics of MSS CROP composer are detailed

Opening MSS (preamble)



I. General info



WARNING:
Be careful to encode the results in the right tab i.e. wheat/cereal grain, Lettuce/leafy, Beet/Root vegetable or other depending on the rotational crops tested in the study

In comparison with plant MSS composer, same manner to fulfill “General Info” section

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I. General info

HOME OPTIONS

New Open Save Render | Cut Copy Paste | Insert Symbol Build Metabolic Map

Document Clipboard Format Tools

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

B.7.9 Residues in succe

Same manner to fill "General Info" section than Plants MSS

References:

ADD DEL

Citation #1

Author(s): [redacted]A.

Date: 2009 **Pages:**

Study Title: Confined rotational crop study using [1[redacted]

Reference Type: IIA, 6.6.2/01

Testing Laboratory: Charles River Laboratories, Tranent, Scotland, UK

Company Study Number: [redacted]

Identifiers:

Test Material: [redacted]

Identifiers: EDIT

Guidelines: OPPTS 860. 1850 ECC 96/68 EC Annex 1, Section 6.6 (1996)
OECD Guideline 502 Metabolism in Rotational Crops (January 2007)

GLP: yes

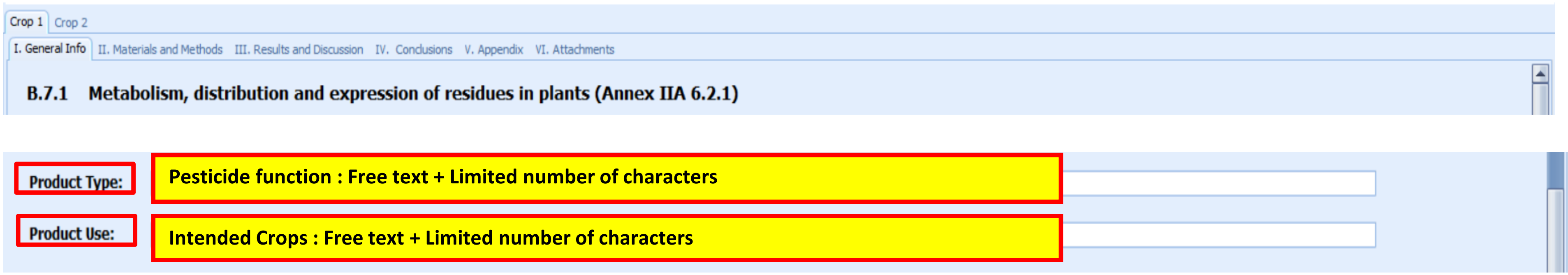
Acceptability: The study is considered scientifically acceptable.

BACKGROUND INFORMATION

EXECUTIVE SUMMARY

I. General info

Same manner to fill "General Info" section than Plants MSS



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II. Materials and Methods

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

A. Materials | B. Study Design

CAS Chemical Name	3-bromo-1-(3-chloro-2-pyridinyl)-N-[4-cyano-2-methyl-6-[(me
CAS no.	736994-63-1
Company experimental name	DPX-HGW86
Other synonyms (if applicable)	
Molecular Formula	C19H14BrClN6O2
Analytical Purity	
Impurities	
Physical State	
Stability Under Test Conditions	
Expiration Date	
Lot/Batch #	

Radiolabeled Test Material

ADD DEL

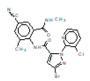
[Cyano-14C]-c
[Pyrazole carbonyl-...
[Cyano-14C] #3
[Pyrazole carbonyl-...

Radiochemical purity: 99.0 %

Specific activity as received: 0.63 MBq/mg

Specific activity of dose: units

Structure:



Same manner to fill "Materials and methods" section than Plants MSS

Table PhysChem Physicochemical Properties.

Parameter	Notes	Value	Units	Reference
		[217-224]	°C	
pH				
Density				
Water solubility (20°C)		12.33 * 0.61	ppm	
Solvent solubility (mg/L at __°C)				
Vapour pressure at 20°C		5.133 * 10-15	Pa	
Dissociation constant (pKa)	At 20°C	8.80 * 1.38		
Octanol/water partition coefficient Log(Kow)	At 22°C	1.97 ± 0.01		
UV/visible absorption spectrum				

2. Test Crops

Table B.7.9-1. Crop Information

Crop/Crop Group	Variety	
Wheat (rotation)/ cereal grain	Spring wheat (Triticum aestivum cv Paragon)	ays a
Lettuce (rotation)/ leafy vegetable	Lettuce (Lactuca. sativa cv Green Salad Bowl, which is a non-hearting cultivar)	fter t
Beet (rotation)/ root vegetable	Red beets (Beta vulgaris cv Detroit Crimson Globe 2109)	fter t
Soil (rotation)	Soil samples sown with wheat	

WARNING:
You have to fill these crop groups carefully.
Those rows are establishing the tested crops for which results will have to be informed

Test Site Type: greenhouse

3. Soil Type

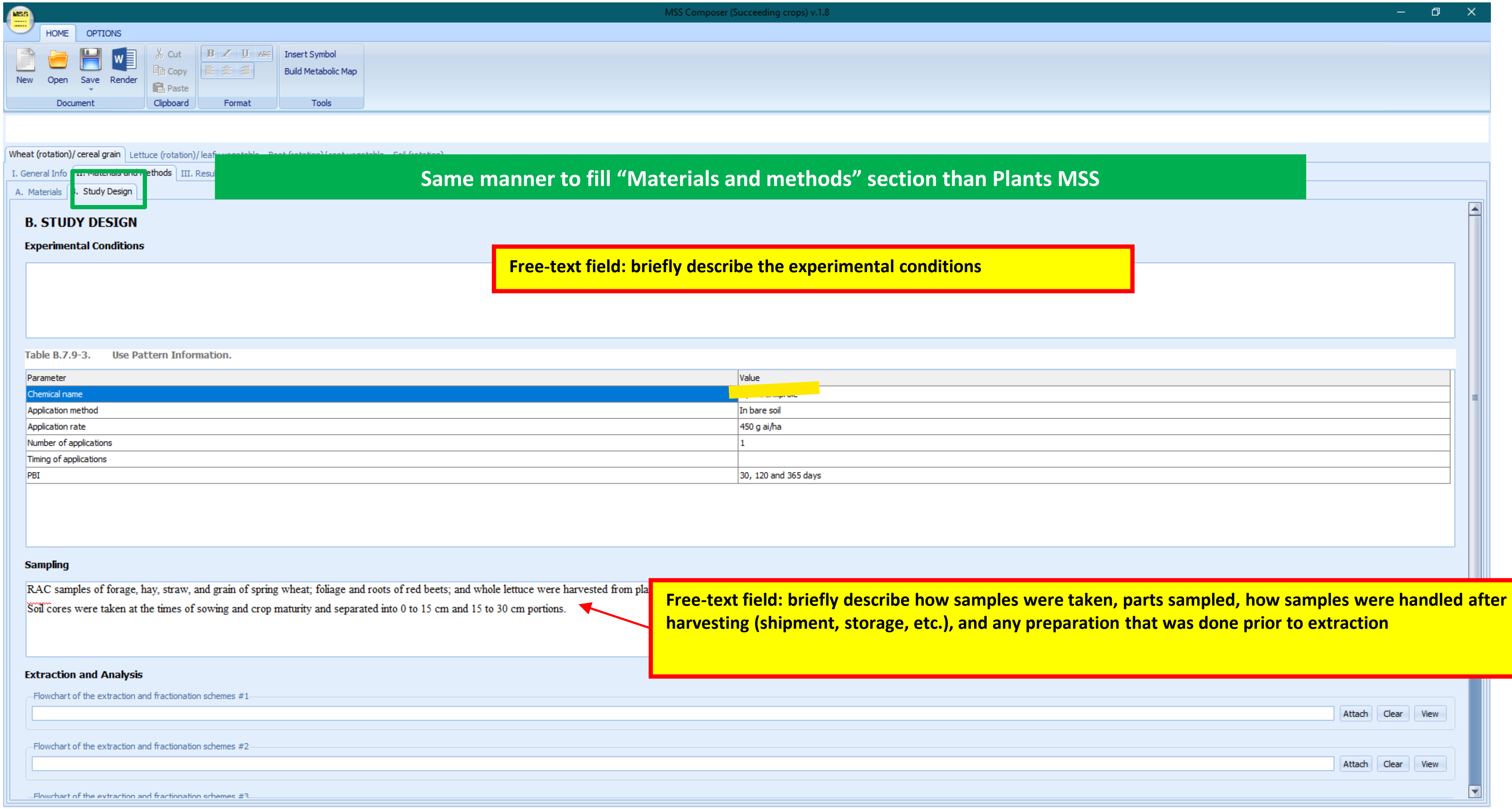
Table B.7.9-2. Soil Physicochemical Properties.

Soil Type	pH	OM %	Sand %	Silt %	Clay %	Moisture Holding Capacity (at 1/3 bar)	CEC meg / 100 g
Sandy loam soil	6.2 (in water)	1.4 (OC)	70	15	15	35.7 (maximum)	9.3

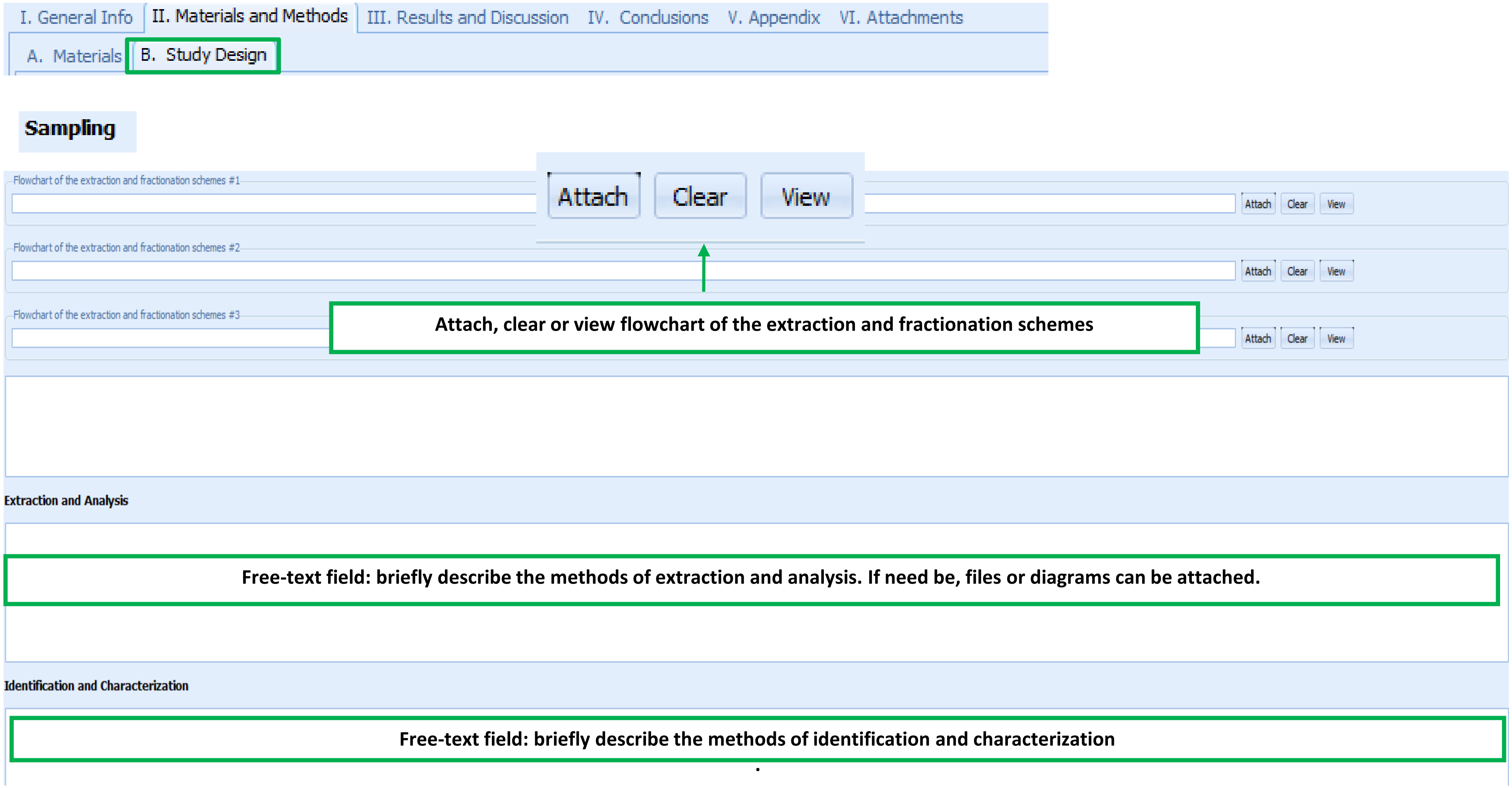
Environmental Conditions

Temperature	
Rainfall	
Lighting	
Potential for Photodegradation of Substance	

II. Materials and Methods



II. Materials and Methods



III. Results and discussion

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

One tab for each rotational crop tested

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

A. Total Radioactive Residues | B. Extraction, Characterization, and Distribution of Residues | C. Storage Stability of Residues | D. Identity of Residues in Rotational Crop | E. Proposed Metabolic Pathway

5 SUBTABS

tive Residues

Extraction efficiency of radioactive residues from plant metabolism study using residue enforcement method

WARNING/REMEMBER:

For the rotational crops tested informed in II. Material and Methods (wheat/cereal grain, Lettuce/leafy, Beet/Root vegetable or other depending on those tested in the study)

General information and study design are the same for all the tested crops but single results have to be informed in this III. Results and Discussion tab

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III. Results and discussion

Just click on the other tab to fill results for other rotational crop testeds

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

A. Total Radioactive Residues | B. Extraction, Characterization, and Distribution of Residues | C. Storage Stability of Residues | D. Identity of Residues in Rotational Crop | E. Proposed Metabolic Pathway

Same manner to fill this section than Plants MSS

Please refer to the 1st Day presentation for basic functions : add a row (matrices), change rows and columns names

A. Total Radioactive Residues

Extraction efficiency of radioactive residues from plant metabol

	Recovered equivalents (mg/kg)	Overall extraction efficiency (%)
Enforcement method		
Extraction method used in study		100

Fill in table if extraction efficiency data available

Quantitation

Human food commodities: TRRs in crops grown in soil treated with both labels ranged from <0.01 to 0.06 mg/kg for wheat grain
Animal feed commodities: The TRRs in wheat commodities harvested from the 30, 120, and 365 day sowings into soil treated with both labels ranged between 0.09 to 0.31 mg/kg for early forage, 0.31 to 1.62 mg/kg for hay, and 0.27 to 0.97 mg/kg for straw

Fill in text Informations on the methods for determining TRR values

Table B.7.9-4. TRRs in Matrices.

Matrix	Timing and Application	Preharvest Interval (days)	PBI	[Cyano-14C		[Pyrazole carbonyl-14C	
				% TRR	ppm	% TRR	ppm
Human food commodities (grain)			30		0.056		0.054
Human food commoditi			120		0.004		0.008
Human food commodities (grain)			365		0.014		0.018
Animal feed commodities (forage)			30		0.313		0.287
Animal feed commodities (forage)			120		0.129		0.103

In rows : Fill the matrice names corresponding to the rotational crop tested and the different Plant Back Intervals (PBI)

Fill in table for TRR's in various matrices

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III. Results and discussion

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

A. Total Radioactive Residues | B. Extraction, Characterization, and Distribution of Residues | C. Storage Stability of Residues | D. Identity of Residues in Rotational Crop | E. Proposed Metabolic Pathway

B. Extraction, Characterization, and Distribution of Residues

[Cyano | [Pyrazole carbonyl-1 | Cyano-14C] #3 | [Pyrazole carbonyl-14C] #4

Prefilled: Separate tab for each radiolabel

in this example there are two labels but 4 tabs (grain 2 PBIs/ Forage 3 PBIs, Hay 3 PBIs, Straw 3 PBI s = 11 tabs)

Characterization of residue 30, 120 or 365 days after soil treatment.

The grain extracts from the 120 DAT [14C]-cyantraniliprole treatment contained negligible amounts of radioactivity (0.004 mg eq/ ...
losses of TRR during preparation of samples and analysis by HPLC were calculated and considered having no impact on the values.

In wheat forage, the unextracted residues accounted for <0.03 mg/kg and 10 %TRR. Extraction was therefore deemed sufficient. The losses of TRR during preparation of samples and analysis by HPLC were calculated and considered having no impact on the values. In samples from ...
... liprole.

Free text for description of results (footnotes, abreviations…)

Metabolite Fraction							Forage - 120 DAT		Forage - 365 DAT	
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR	ppm
TRR		0.056		0.014		0.313		0.129		0.128
Extracted residues	85.9	0.048	56.5	0.008	92.4	0.289	90.2	0.113	91.5	0.117
Extract 1	23.5	0.013	32.7	0.005	64.7	0.202	69.3	0.089	64.1	0.082
Extract 2	14.2	0.008	23.8	0.003	21.4	0.067	20.9	0.027	22.8	0.029
Extract 3					6.4	0.020		NC		NC
Extract 4:	26.5									0.004
Organic fraction	16.6									
Aqueous fraction	8.0									
Gel fraction	1.9	0.001								
Extract 5	10.6	0.006		NC		NC		NC	1.2	0.002
Extract 6	11.1	0.006		NC						
Unextracted residues	14.1	0.008	43.6	0.006	7.6	0.024	9.8	0.013	8.5	0.011
Extracted residues after processing	91.5	0.05	56.5	0.008	84.4	0.263	95.3	0.122	86.5	0.111
Loss during processing	-5.6	-0.003		<0.01	8.1	0.026	-5.1	-0.006	5.1	0.006

This tab summarises the extraction, characterisation and distribution of the TRR among the differents matrices

WARNING:
You can rename columns but do not delete column headings: empty headings cause irreversible merger of columns

WARNING:
More-than (>) sign authorised but not less-than (<) sign! Using a less-than sign makes information disappear

III. Results and discussion

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | **III. Results and Discussion** | IV. Conclusions | V. Appendix | VI. Attachments

A. Total Radioactive Residues | B. Extraction, Characterization, and Distribution of Residues | **C. Storage Stability of Residues** | D. Identity of Residues in Rotational Crop | E. Proposed Metabolic Pathway

C. Storage Stability of Residues

^{14}C-Residues in crop samples were extracted and initial analysis conducted by HPLC within 4 weeks of harvest. After a period of storage (64 to 73 weeks at -20°C) milled tissue from selected crops sown 3C
cyantraniliprole), beet foliage, and hay ([PC-^{14}C]-cyantraniliprole) were analysed.

The composition of the residue from wheat grain was similar to the original residue after prolonged storage, with cyantraniliprole being the major component, and metabolites IN-J9Z38, and IN-MLA84 bei

Free-text field: describe storage conditions, discuss whether the petitioner demonstrated that residues are stable during storage.

Table B.7.2.1-8. Summary of Storage Conditions.

Matrix (RAC or Extract)	Storage Temperature °C	Actual Storage Duration (Days or Months)	Interval of Demonstrated Storage Stability [specify crop/matrix if different] (Days or Months)

Tab to summarize storage stability data in study and demonstrated storage stability

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III. Results and discussion

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

III. Results and Discussion | V. Appendix

A. Total Radioactive Residues | B. Extraction, Characterization, and Distribution of Residues | C. Storage Stability of Residues | D. Identity of Residues in Rotational Crop | E. Proposed Metabolic Pathway

D. Identity of Residues in Rotational Crop

[Cyano-14C] | [Pyrazole carbonyl-14C] | [Cyano-14C] #3 | [Pyrazole carbonyl-14C] #4

Nature of residue 30 or 120 days after soil treatment.

The majority of the extracted radioactivity from lettuce planted 30 and 120 days after soil treatment was identified as 0.01 to 0.08 mg/kg, 39.6 to 69.1% TRR). Other lettuce metabolites in
more than 0.01
mg/kg. No unidentified component individually accounted for more than 0.01 mg/kg.

Table B.7.9-7. Summary of Characterization and Identification of Radioactive Residues in Rotational Crop Matrices Following Application of [Cyano-14C]-cya

Compound	30 PBI a		120 PBI a		Matrix 3		Ma
	%TRR	ppm	%TRR	ppm	%TRR	ppm	%TRR
Cyantraniliprole	6						
IN-J9Z38	4						
IN-JCZ38	3.7	0.005	1.6	0.001			
IN-MLA64	4.3	0.005	2.3	0.001			
IN-MYX9							
Total uni							

This tab summarises the identification of compounds in tested matrices

WARNING:
Pay attention to the number of column required to report all the data available in the study (one column per matrix). Knowing that each subtab contains a 7-column table, if you need 8 columns, you have to create a second radiolabelled test item to get the extra columns you need

WARNING:
You can rename columns but do not delete column headings: empty headings cause irreversible merger of columns

WARNING:
More-than (>) sign authorised but not less-than (<) sign! Using a less-than sign makes information disappear

WARNING:
When filling header column, always start with the parent compound and carry on with identified metabolites

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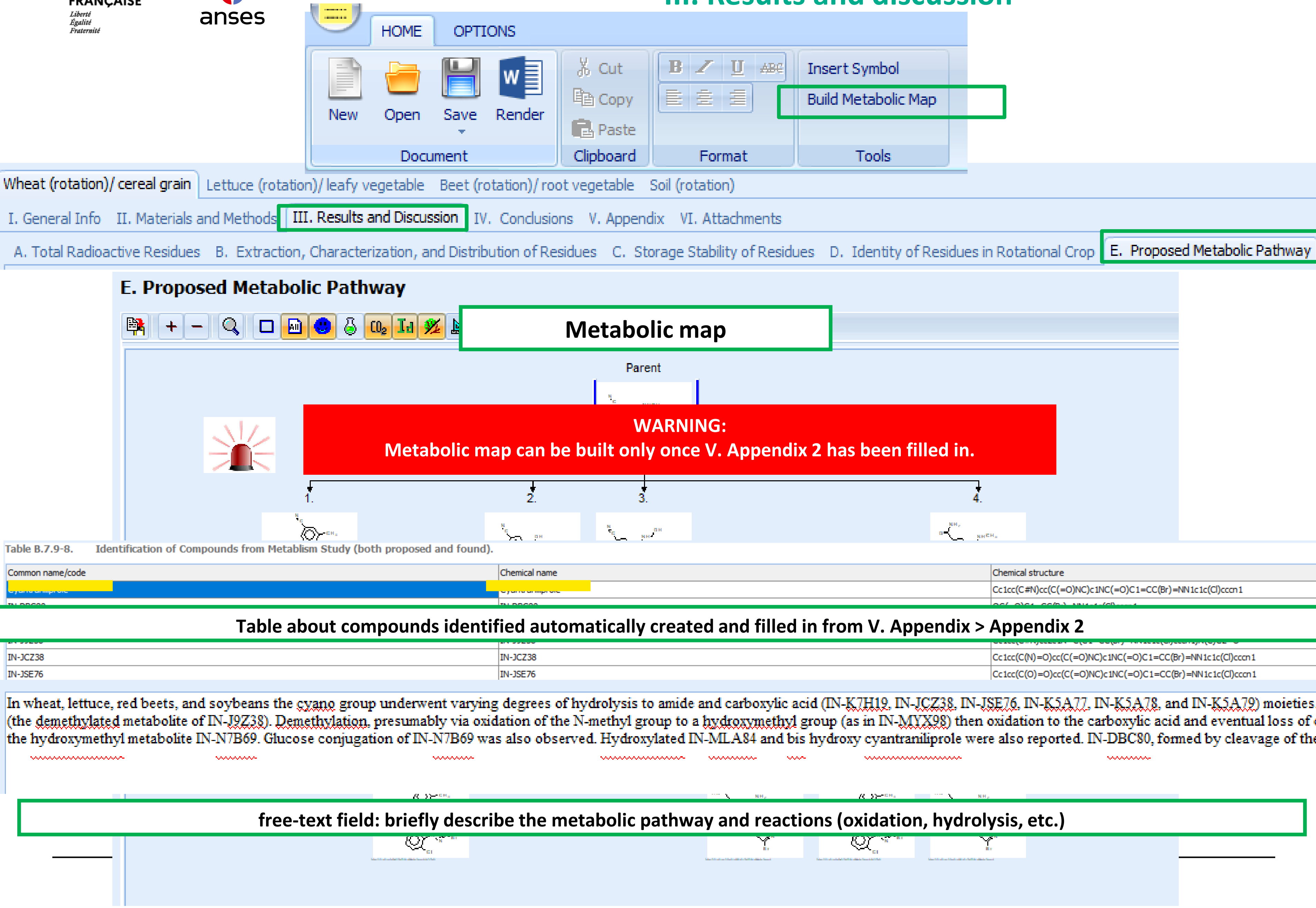
III. Results and discussion



IV. Conclusions

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

I. General Info | II. Materials and Methods | III. Results and Discussion | IV. Conclusions | V. Appendix | VI. Attachments

Same manner to fill “Conclusion” section than Plants MSS

CONCLUSIONS

In human food commodities, TRRs in crops grown in soil treated with both labels ranged from <0.01 to 0.06 mg/kg for wheat grain, 0.01 to 0.03 mg/kg for red beet roots and 0.02 to 0.11 mg/kg for lettuce. In animal feed commodities the TRR into soil treated with both labels ranged between 0.09 to 0.31 mg/kg for early forage, 0.31 to 1.62 mg/kg for hay, and 0.27 to 0.97 mg/kg for straw. TRRs in red beet foliage sampled from the 30 and 120 day sowings into treated soil ranged fro

The TRR in the upper 15 cm of soil collected 30 DAT with [CN-^{14}C] and [PC-^{14}C] [redacted] as 0.38 and 0.21 mg/kg, respectively. Residues in soil treated with [CN-[redacted] declined by 120 DAT (0.20 mg/kg), but not in DAT, in the [CN-^{14}C[redacted] and [PC-[redacted] soils, were 0.30 mg/kg and 0.16 mg/kg, respectively.

Soil residues at 493 DAT (wheat maturity from 365 sowing) did not decline significantly from those at 30 DAT. Since this soil had received two wheat sowings this indicated that total radioactivity in soil remained similar throughout the study.

Concentrations of total radioactivity in soil from the lower 15 to 30 cm horizon were <0.01 mg/kg.

REFERENCES

[redacted]. Confined rotational crop study using [redacted] Laboratories, Tranent, Scotland, UK, study [redacted]

Free-text field: cite references for other metabolism studies (if applicable); if available, include the EPA MRID# and PMRA# of both the study and the review.

V. Appendix

Wheat (rotation)/ cereal grain | Lettuce (rotation)/ leafy vegetable | Beet (rotation)/ root vegetable | Soil (rotation)

III. Results and Discussion | V. Appendix

Appendix 1

Test#	Number	Application Method	Application Rate	Number of Applications	Timing of Applic	PBI	Matrix	Experimental Descriptor	Remarks	Citation	RLTM	Test Crop	Soil Type
Cy_lettuce_30_PBI		In bare soil	450 g ai/ha	1		30 day	Foliage			Citation #1	[Cyano-14C]-MTP_W-2!	Lettuce (rotation)/ leafy	Sandy loam soil
Cy_lettuce_120_PBI		In bare soil	450 g ai/ha	1		120	Foliage			Citation #1	[Cyano-14C]-MTP_W-2!	Lettuce (rotation)/ leafy	Sandy loam soil
Cy_lettuce_b_30_PBI		In bare soil	450 g ai/ha	1		30	Foliage			Citation #1	[Pyrazole carbonyl-14C]	Lettuce (rotation)/ leafy	Sandy loam soil
Cy_lettuce_b_120_PBI		In bare soil	450 g ai/ha	1		120	Foliage			Citation #1	[Pyrazole carbonyl-14C]	Lettuce (rotation)/ leafy	Sandy loam soil

Summarize of all tested matrices from metabolism study. Each line represents a matrice

Appendix 2

ID	Common Name / Code	Chemical Name	SMILES	Parent(s)	Expertise
1	[redacted]	3-Bromo-1-(3-chloro-2-pyridinyl)-N...	Cc1cc(C#N)cc(C(=O)NC)c1NC(=O...		
2	IN-J9Z38				
3	IN-NBC94				
4	IN-MLA84				
5	IN-N7B69				
6	IN-MYX98	3-Bromo-1-(3-chloro-2-pyridinyl)-N...	Cc1cc(C#N)cc(C(=O)NCO)c1NC(=...	1	
7	IN-HGW87	N-[2-(Aminocarbonyl)-4-cyano-6-...	Cc1cc(C#N)cc(C(N)=O)c1NC(=O)...	6	

Summarize of all identified and/or detected compounds from metabolism study and relationships between compounds.
Each line represents a compound.
ALWAYS begin with parent compound and carry on with metabolites.

Appendix 3

	Cy_lettuce_	Cy_lettuce_	Cy_lettuce_	Cy_lettuce_
MTP_W-29-31	linked	linked	linked	linked
IN-J9Z38	linked			
IN-JCZ38	linked	linked		
IN-MLA84	linked	linked	linked	linked

Summarize about the detection or not of compounds in tested matrices.
Generated automatically once appendix 1 and 2 are fulfilled

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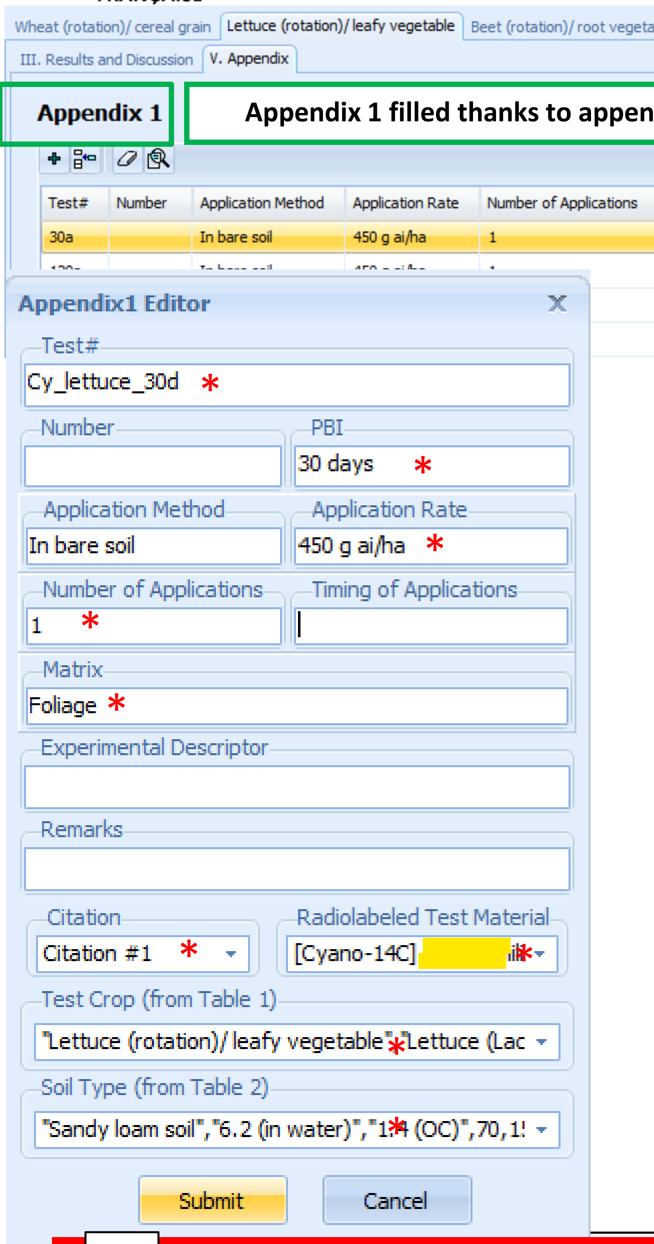
V. Appendix

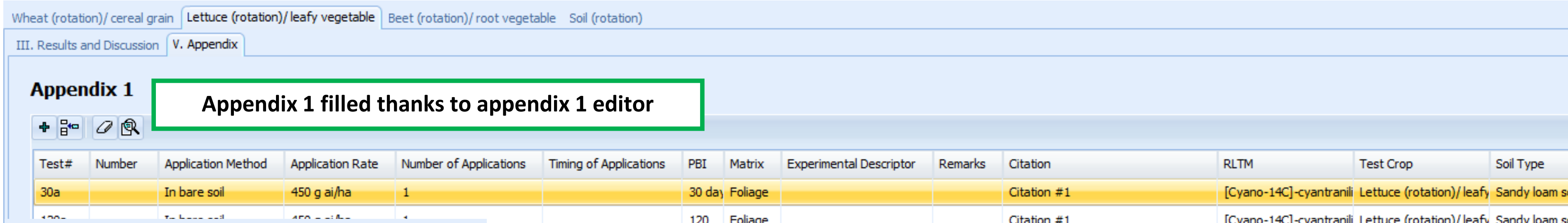
Appendix 1 | **Appendix 1 filled thanks to appendix 1 editor**

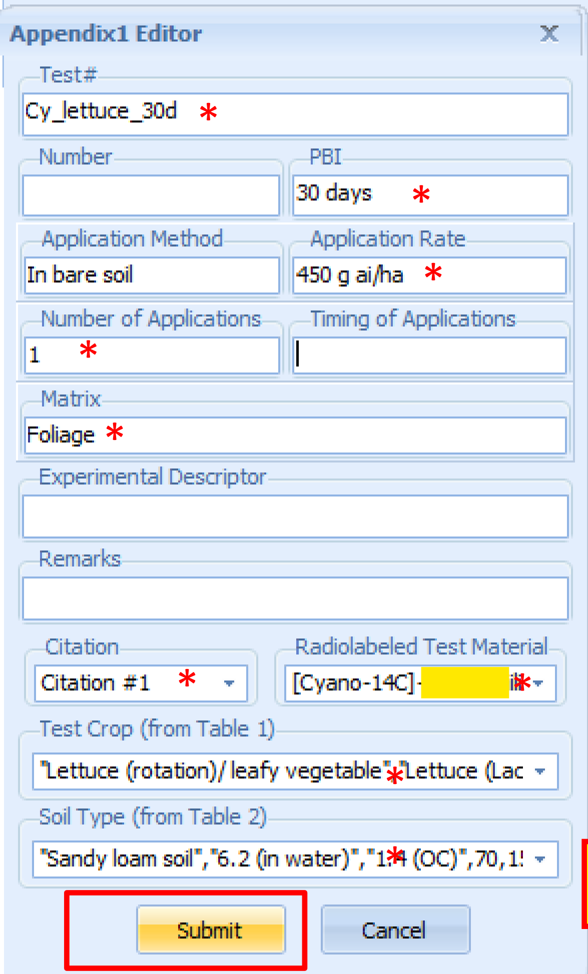


Test#*	→ Matrices should be named briefly but unambiguously so that they can be easily distinguished 1. first letters of the labelling (mandatory) 2. portion analysed (mandatory) 3. dose applied (optional) 4. PHI (optional) → Every information separated from the next with an underscore (_)
Number	→ number of plants by radiolabelled test material
Plants:* PHI ***Rotational Crops:*** **PBI***	**/!\ The value must be separated from the unit by a space otherwise the information will not appear once the MSS xml file imported into MetaPath.**
Application Method	
Application Rate*	**/!\ The dose rate must be separated from the unit by a space otherwise the information will not appear once the MSS xml file imported into MetaPath.**
Number of Applications*	Value

* **field must be filled in to allow importation of the MSS xml file into MetaPath**

V. Appendix





Matrix*	
Remarks	→ free-text field. To explain terms and abbreviations
Citation*	→ select corresponding citation (according to the radiolabelled test material or the administered dose)
Radiolabeled Test Material*	→ select corresponding radiolabelled test material
Test Crop (from Table 1)*	→ select corresponding test crop
Soil type (from Table 2)*	→ select corresponding soil type

Click on Submit to validate created matrice

* field must be filled in to allow importation of the MSS xml file into MetaPath

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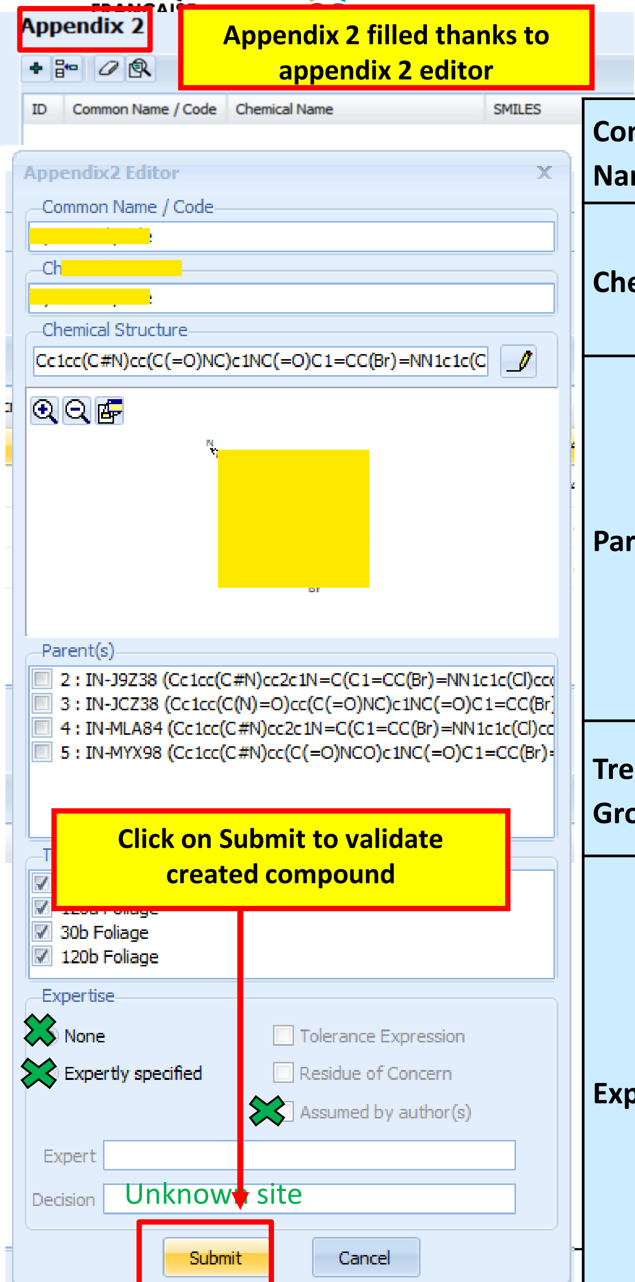
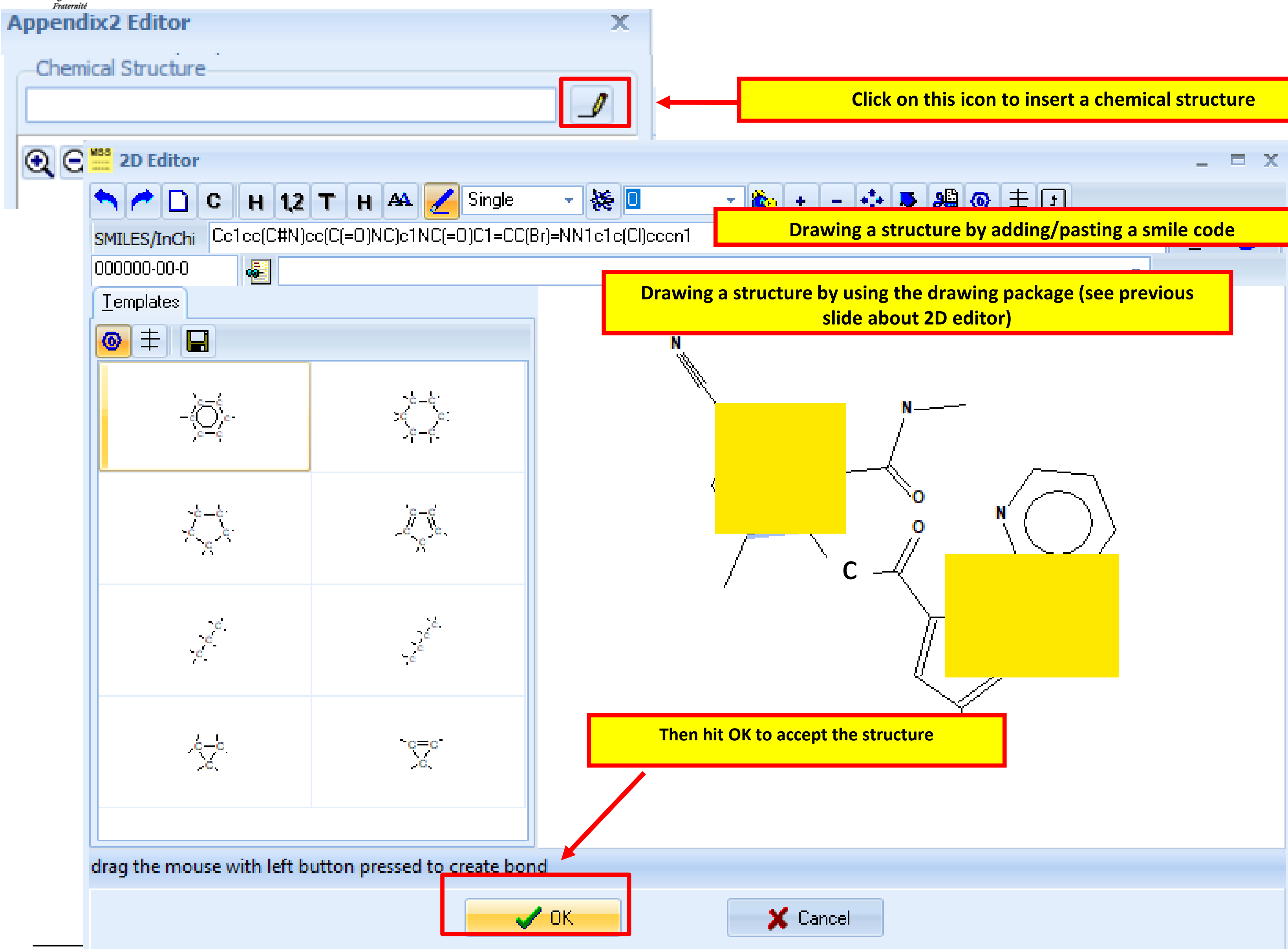
V. Appendix



Common Name/Code	**common name / company experimental name**
Chemical Name	**common name** (**company experimental name)** Do not write down the full chemical name of the molecules
Parents	Describe **relationship(s) between compounds** by ticking the box(es) that correspond(s) to **compound(s) from which the metabolite can be generated.** Relationships specified for all metabolites, except parent compound. *N.B.: The metabolic pathway is built based on the information encoded in this field.*
Treatment Groups	Tick the box(es) that correspond(s) to matrix(ces) in which the compound has been identified.
Expertise	If **no issue** drawing the compound , select "**None**" Select "**Expertly specified**" and "**Assumed by author(s)**" for <u>compounds that were not identified in the study</u> but are assumed intermediates between identified metabolites. In case of **uncertainties** while drawing a compound (e.g.: position of a chemical group not clearly determined), select **"Expertly specified"** and specify in the "**Decision**" field which assumptions were made when drawing the compound (e.g.: Unknown site of conjugation)

V. Appendix


Appendix2 Editor
Chemical Structure
Click on this icon to insert a chemical structure
2D Editor
Single
Drawing a structure by adding/pasting a smile code
SMILES/InChi
Cc1cc(C#N)cc(C(=O)NC)c1NC(=O)C1=CC(Br)=NN1c1c(Cl)cccn1
000000-00-0
Templates
Drawing a structure by using the drawing package (see previous slide about 2D editor)
Then hit OK to accept the structure
drag the mouse with left button pressed to create bond
OK
Cancel

V. Appendix

Appendix 3

	Cy_lettuce_	Cy_lettuce_	Py_lettuce_	Py_lettuce_
	linked	linked	linked	linked
IN-J9Z38	linked	linked	linked	
IN-JCZ38	linked	linked		
IN-MLA84	linked	linked	linked	linked
IN-MYX98	linked	linked		linked

This table is <u>filled in automatically</u> using the information available in Appendix 1 and 2.

You can link and unlink matrices and compounds by right-clicking in the cells. This can also be done by scrolling **but it is very sensitive**.

Recommendation:
we strongly recommend updating this table using the "Treatment group" fields of Appendix 2.

MSS Crop composer

Live filling