BfR

Risiken erkennen – Gesundheit schützen

How to Tackle Extraction Efficiency – A Proposal

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Presentation outline

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3. Cases where proof of extraction efficiency not necessary
4. A straightforward example
5. Cross validation
6. A not so straightforward example
7. The final decision tree
8. Summary and conclusion
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Objective of food monitoring

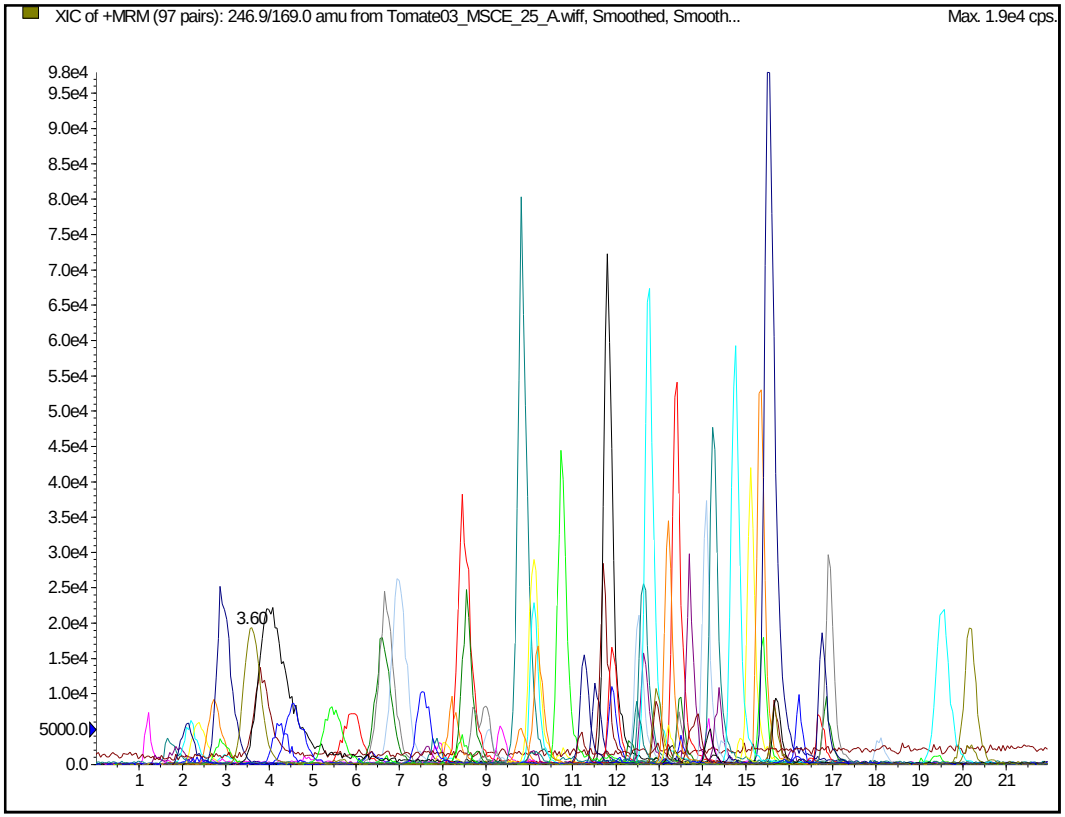
MRL Compliance

Consumer exposure

For testing of MRL compliance high sample throughput is necessary!

Multiresidue methods

- ❑ Analysis of a high number of pesticides within one method
- ❑ Increasing number of published methods



Behaviour of incurred residues often unknown!

Solvents used in high throughput multi-residue methods

- QuEChERS: extraction with **acetonitrile/water**
- ChemElut: extraction with **methanol/water**
- L 00.00-34 (DFG S19): extraction with **acetone/water**, **acetonitrile** (or hexane/acetone**)**
- Validation: determination of recovery and precision from fortified samples
- Normally no use of incurred residues
- However, examples exist where laboratory fortifications did behave differently from incurred residues

➢ **No information about extraction efficiency from method validation**

Factors influencing extraction efficiency

Particle size

Matrix characteristics

Extraction time

Temperature

Solvent type

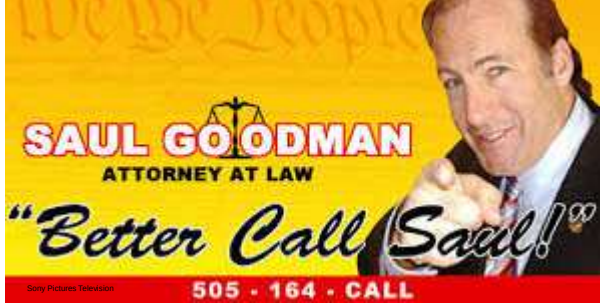
Plant physiology

Physicochemical properties

Lipophilicity of compounds

➢ **Many parameters can influence extraction efficiency. While some can be optimized during method validation, information from studies conducted with radiolabelled pesticides is required for others!**

Legal basis



Guidance document SANTE/11945/2015

"Where practicable, samples containing incurred residues can be analysed using varying extraction conditions to obtain further information on extraction efficiency"

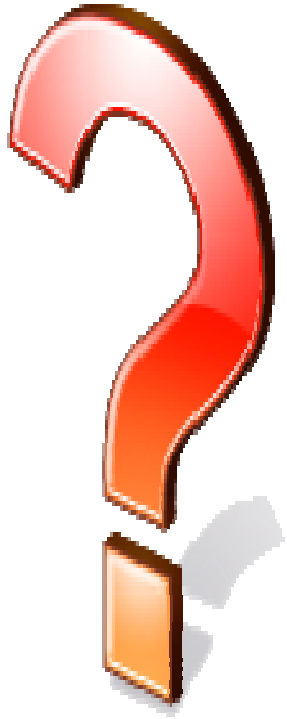
Guidance document SANCO/825/00 rev .8.1

"The extraction procedures used in residue analytical methods … should be verified … using samples with incurred residues from radio-labelled analytes."

Commission Regulation (EU) No 283/2013, Annex Section 6.2 (Data requirements for active substances)

"To quantify the major components of the residue and to show the efficiency of extraction procedures for these components"

Several questions and a starting point



- When is extraction efficiency necessary?
- Which data are available and helpful?
- Which solvents should be used?
- In which cases is it possible to “bridge” between solvents?
- In which cases is it possible to “bridge” between commodities?

- Development of a systematic, stepwise approach
- Consideration of data from DAR/RAR, EFSA Scientific Report, EU MRL Data base

MRLs are not established

Examples:

- Compounds listed in Annex IV of Regulation (EC) No.396/2005

- Compounds without MRLs for animal matrices

With no MRLs set, monitoring is not required and proof of extraction efficiency is not needed!

Significant residues (> 0.01 mg/kg) do not occur

Examples:

- Most sulfonylurea herbicides, (e.g. Azimsulfuron, Ethametsulfuron, Ethoxysulfuron, Flupyrsulfuron, Foramsulfuron, Halosulfuron, Iodosulfuron, Imazosulfuron, Mesosulfuron, Metsulfuron, Oxasulfuron, Prosulfuron, Rimsulfuron, Tribenuron… and others)

- Some other pesticides, e.g. Carfentrazone-ethyl, Florasulam, Fluazinam, Flurtamone, Isoproturon, Mecoprop (P), Picolinafen, Tembotrione, ...(all regulated MRLs at LOQ!)

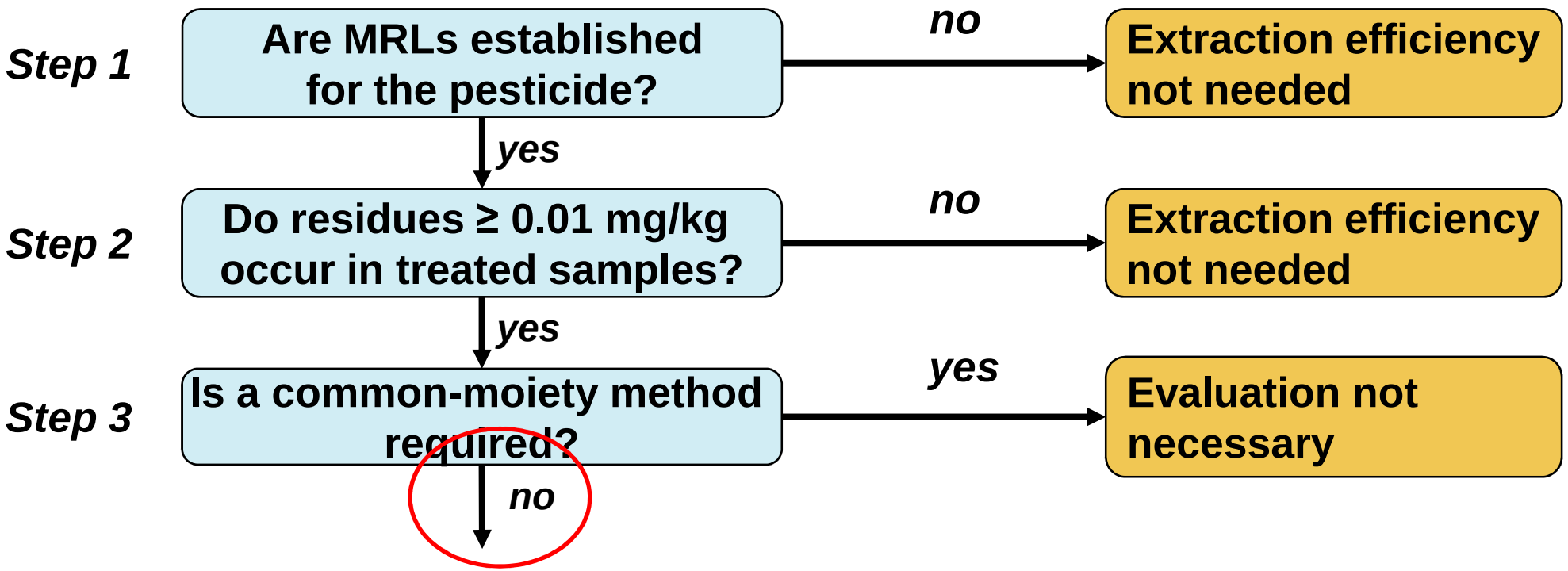
Without crops containing incurred residues the determination of extraction efficiency is neither possible nor needed!

Use of a common moiety method

- Residue definition includes a common moiety and conversion is conducted in the presence of the homogenate, e.g.
  - Amitraz (2,4 -dimethylaniline moiety)
  - Clofentezine (2-chlorobenzoyl moiety)
  - Cycloxydim (determined as 3-(3-thianyl)glutaric acid S-dioxide and/or 3-hydroxy-3-(3-thianyl)glutaric acid S-dioxide or methyl esters thereof)
  - Flufenacet (N fluorophenyl-N-isopropyl moiety)

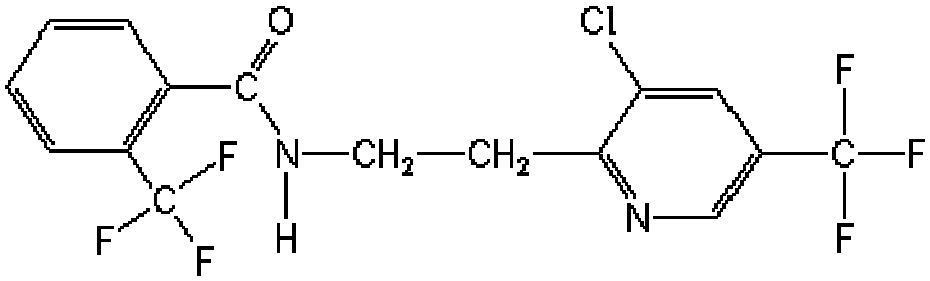
Results are related to the extraction efficiency and conversion.

The decision tree so far…



… but where to go from here?

Fluopyram in grapes / experimental part of metabolism study



- Regulated analyte: fluopyram (parent)

Study design

- Test substance: phenyl-UL-^{14}C fluopyram
- Three spray applications, harvest 18 days after last application (DALA)

Extractions solvent used

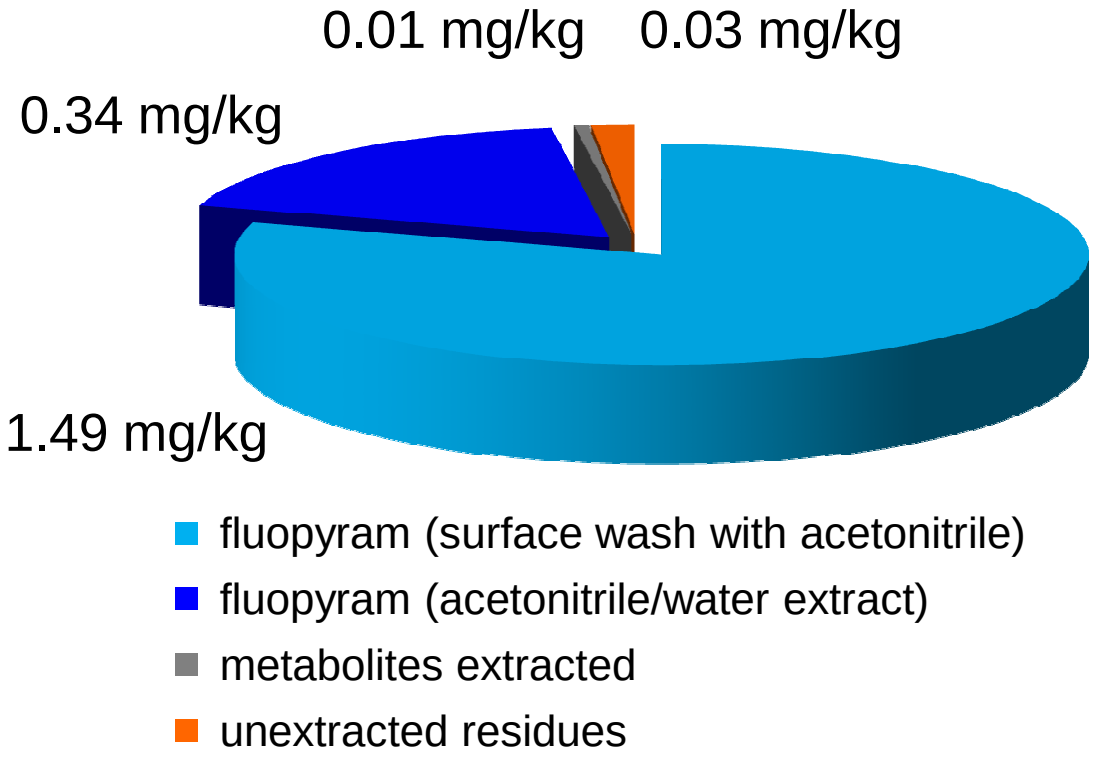
- Washing with acetonitrile
- Extraction of washed fruit homogenate with acetonitrile/water (8+2), three times

Determination of residues

- Total radioactive residue (TRR) in grapes and extracts by liquid scintillation counting (LSC)
- Identification of fluopyram and metabolites by HPLC-UV, LC-MS and NMR

4. A straightforward example
Fluopyram in grapes / results of the metabolism study

Total residue: 1.86 mg/kg



Radioactivity corresponding to 80 % TRR was washed off with pure acetonitrile. Additional 18 % TRR was extracted with acetonitrile/water (8/2, v/v).

A monitoring method with acetonitrile/water as solvent (e.g. QuEChERS) will completely extract the regulated analyte!

Fluopyram in grapes / generalisation

Step 4

(1) >70 % of TRR extracted?

(2) Sum of components of DoR >50 % of TRR?

yes

Step 5

Solvent in metabolism study and monitoring method identical?

yes (acetonitrile/water)

Step 6

Extraction efficiency is demonstrated.
No further data needed.

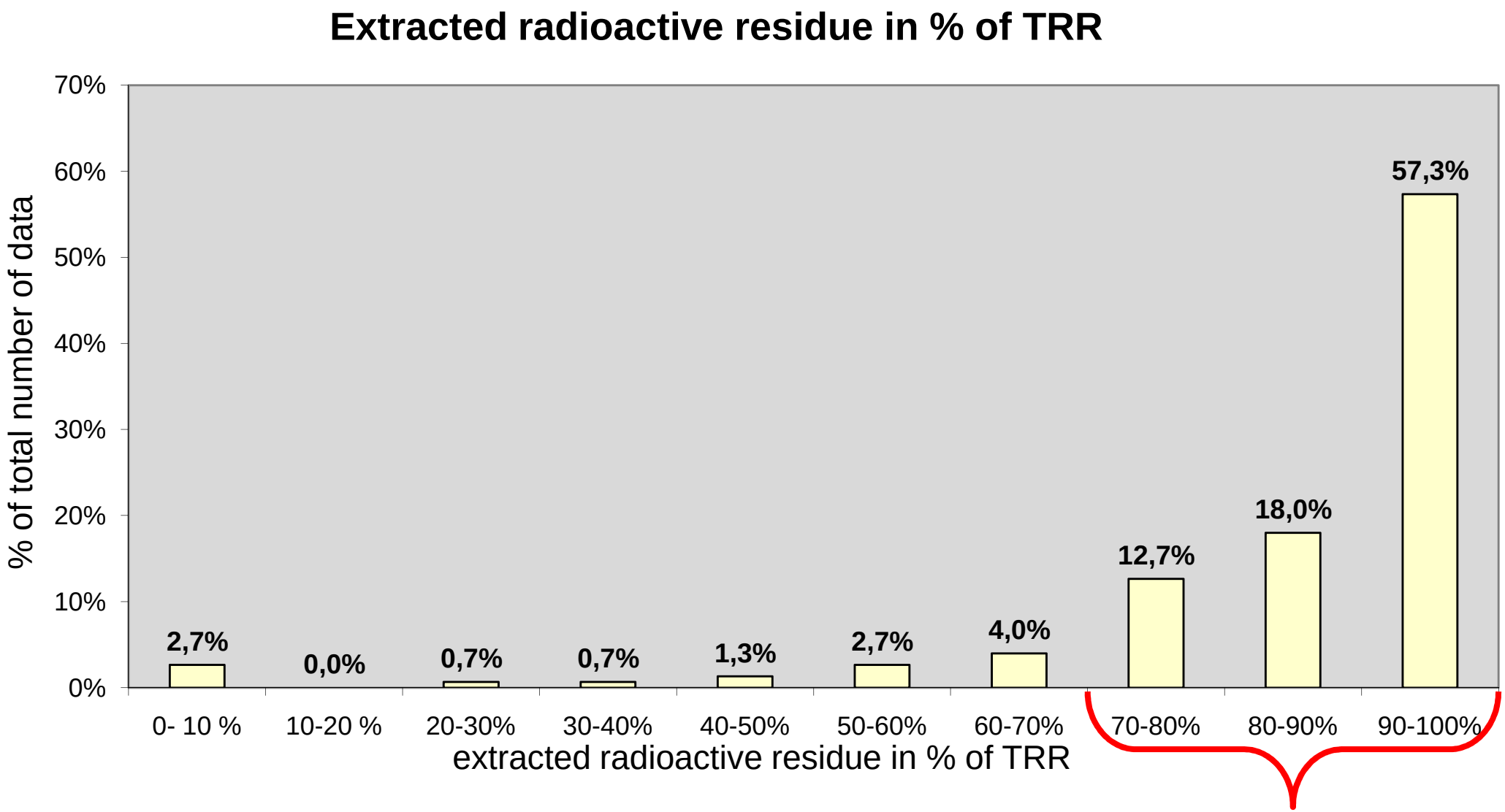
i.e. less than 30 % of TRR not extracted.

In such case not extracted radioactivity corresponds to < 40 % of the extracted components of the regulated residue definition! No further information on identity of un-extracted radioactivity needed.

Calculation:

50 % / (50 % + 30 %)

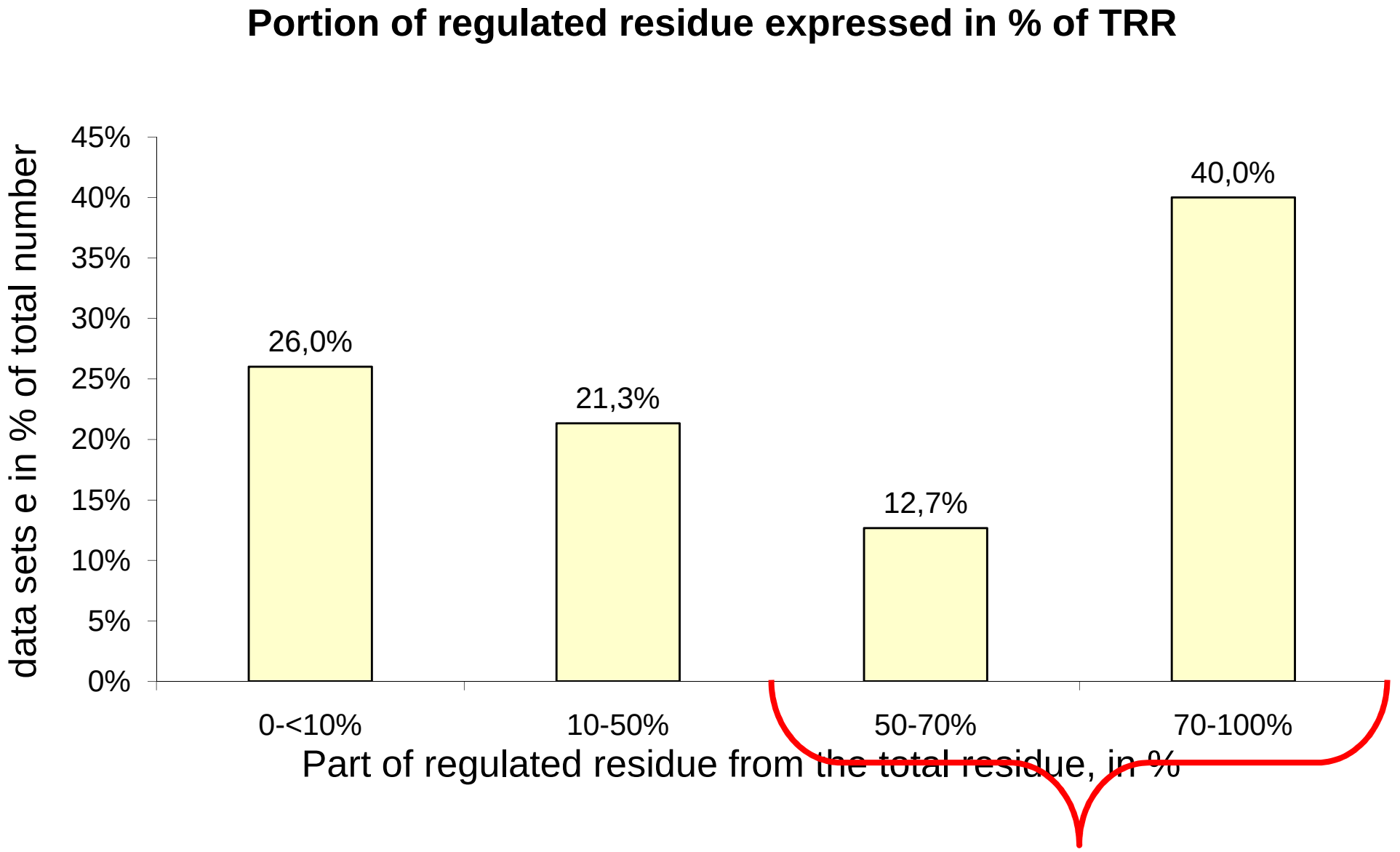
How often is criterion (1) fulfilled?



88 % fulfill the first criterion of step 4!

50 active substances, 150 data sets

How often is criterion (2) fulfilled?



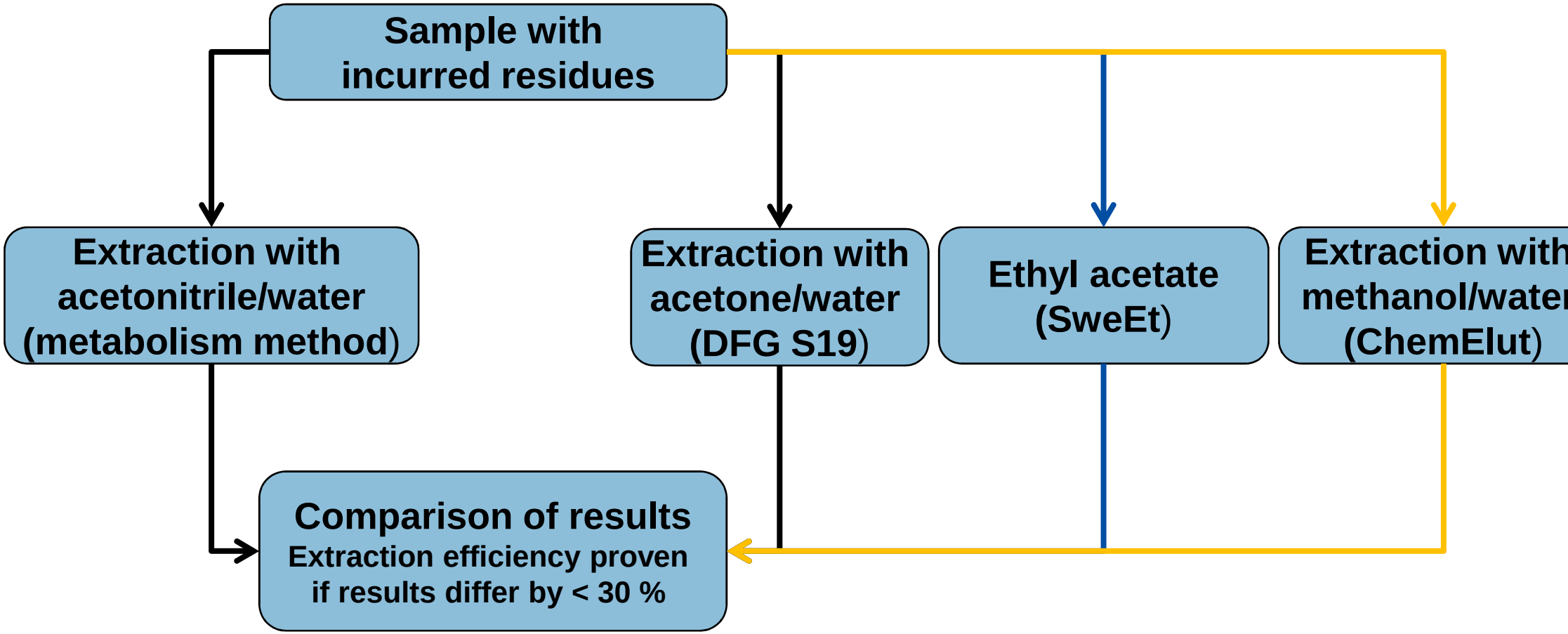
>50 % fulfill the second criterion of step 4!

50 active substances, 150 data sets

What to do if solvents are not identical?

Additional fluopyram monitoring method using acetone/water (S19 method)

Cross validation



An example

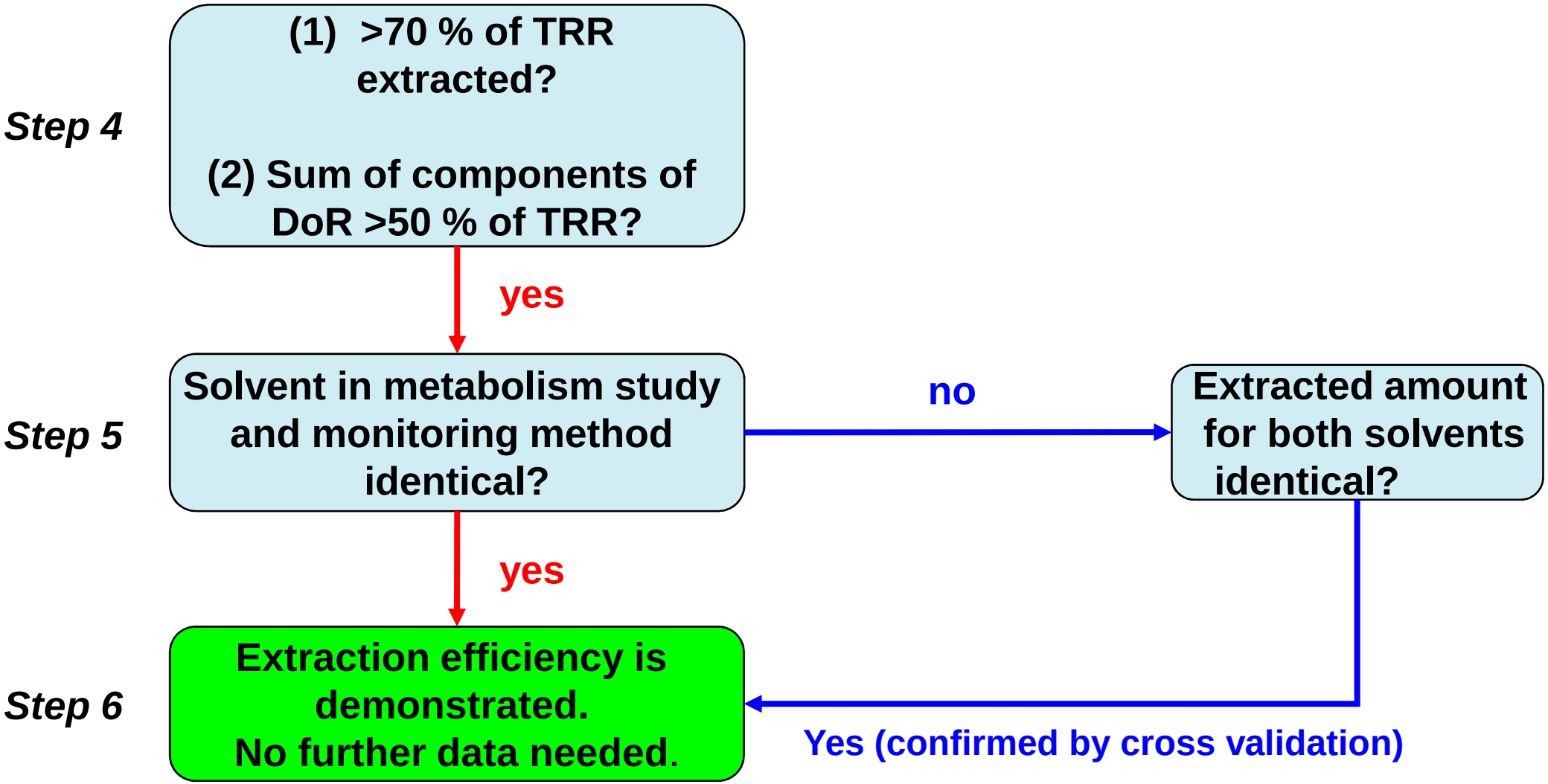
Pendimethalin

Residue definition monitoring: Parent

		Metabolism study [1]		QuEChERS (solvent: acetonitrile)			DFG S19 (solvent: acetone/water, 2/1, v/v)		
	TRR [mg/kg]	ERR [2] [mg/kg] (% of TRR)	Parent [mg/kg]	ERR [mg/kg] (% of TRR)	Parent [mg/kg]	**Extraction efficiency (%)**	ERR [mg/kg] (% of TRR)	Parent [mg/kg]	**Extraction efficiency (%)**
Wheat straw	60	17.2 (29)	0.335	5.56 (9)	0.181	**54**	8.74 (15)	0.076	**23**
Carrot root	0.232	0.173 (75)	0.038	0.037 (16)	0.02[illegible]	**[illegible]**	0.121 (52)	0.03[illegible]	**[illegible]**
Lettuce	0.297	0.243 (82)	0.119	0.202 (68)	0.136	**114**	0.181 (61)	0.108	**91**

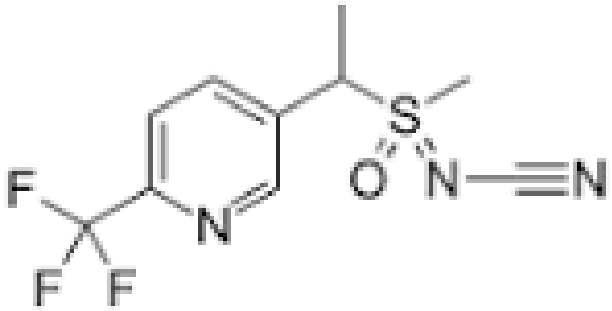
[1] extraction solvent: 3 x methanol followed by 2 x water
[2] ERR: extractable radioactive residue

What to do if solvents are not identical?



What to do if both criteria of step 4 are not fulfilled?

- Regulated analyte: sulfoxaflor (parent)
- Crop: lettuce



Study design

- Test substance: ^{14}C-sulfoxaflor
- Three foliar spray applications, harvest 7 days after last application (DALA)

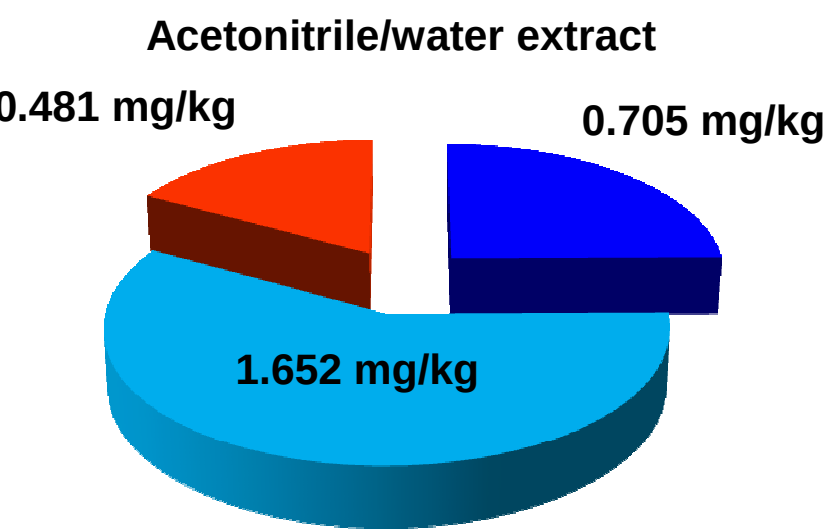
Extraction solvent used

- Acetonitrile/water (1/1, v/v)
- Further extraction with alkaline methanol and acidic hydrolysis

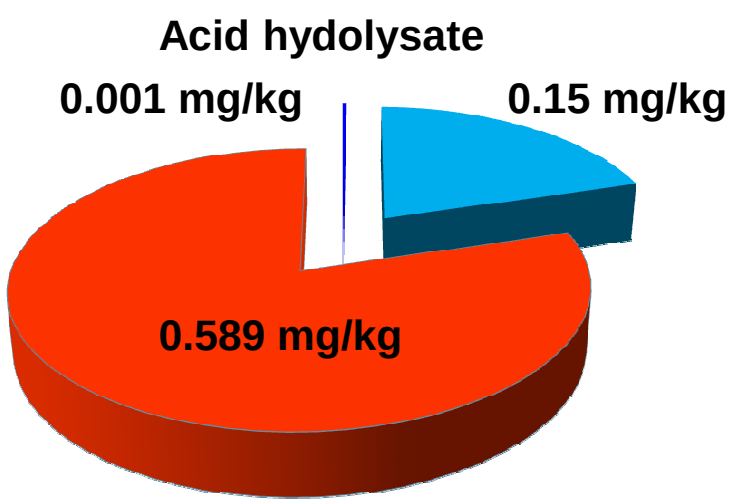
Determination of residues

- Total radioactive residue (TRR) in lettuce and extracts by liquid scintillation counting (LSC)
- Identification of sulfoxaflor and metabolites by HPLC and LC-MS/MS

Sulfoxaflor in lettuce / results of the metabolism study

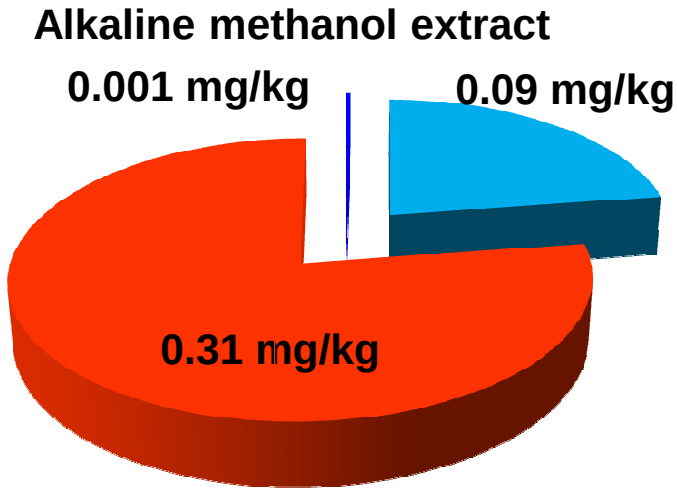


Total residue: 4.39 mg/kg



Only 65% of the TRR was extracted with ACN/water, parent accounted for 16% TRR.

Both criteria of step 4 are not fulfilled!



No parent detected in non-extractable residues

Sulfoxaflor (RD-Mo) Metabolites Other compounds

Sulfoxaflor in lettuce

Step 4

(1) >70 % of TRR extracted?

(2) Sum of components of DoR >50 % of TRR?

(1) No: 64.5 % of TRR extracted
(2) No: parent: 16 % of TRR

no

Are compounds of DoR for monitoring present in non-extractable radioactive residue?

yes

no

Step 5

Solvent in metabolism study and monitoring method identical?

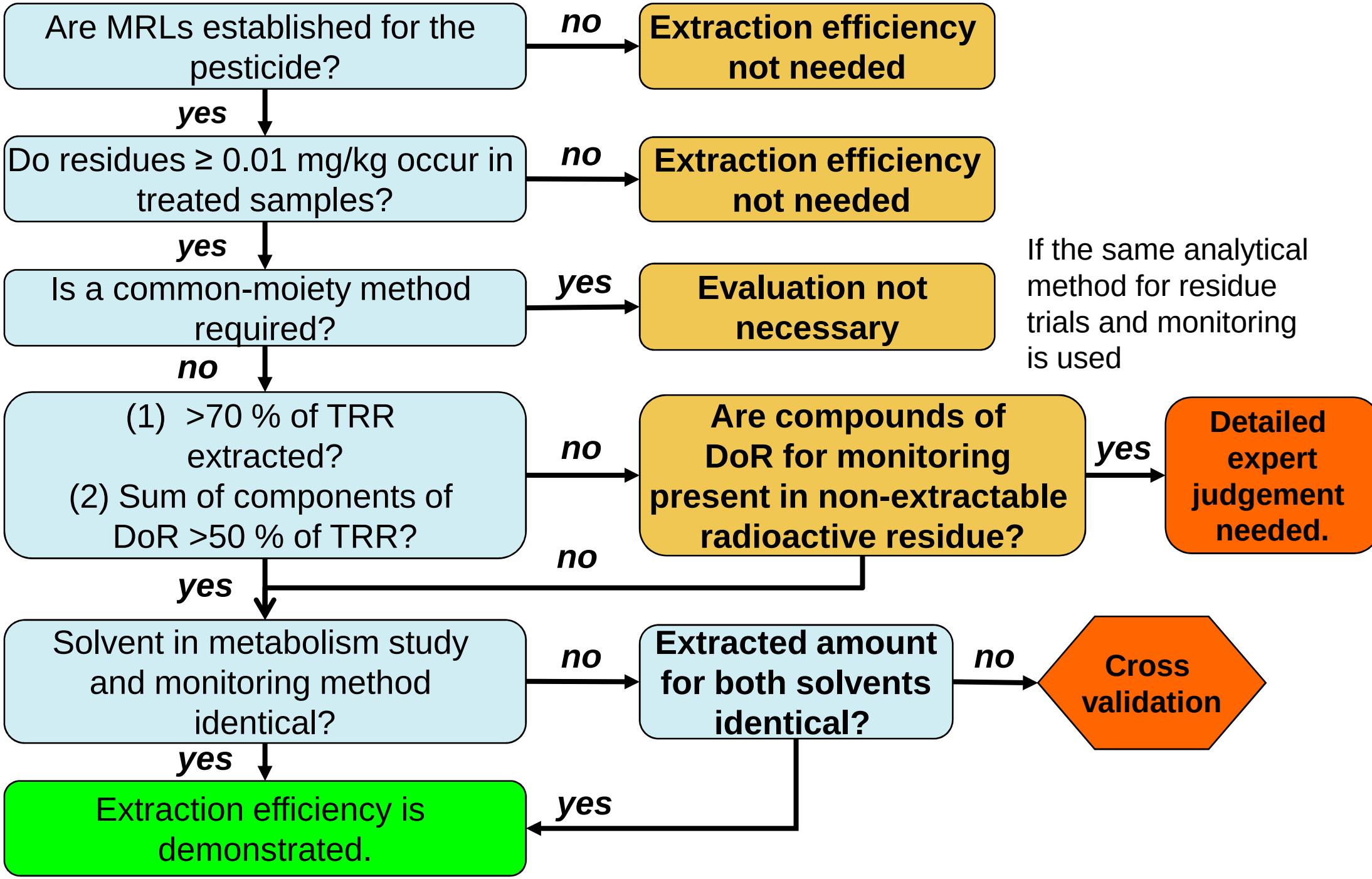
yes: acetonitrile/water (1/1, v/v)

Step 6

Extraction efficiency is demonstrated. No further data needed.

If yes, detailed expert judgement is needed

7. The final decision tree



Common problems when evaluating extraction efficiency

- Unresolved problem: conjugates that are regulated, e.g. Bentazone, Dichlorprop, Fluazifop-P-butyl, MCPA, MCPB Pyridate, Spirotetramat

- Analytes included in the residue definition are not observed in primary metabolism:

  ♦ inclusion of further studies is necessary (processing studies, metabolism in rotational crops …)

- Switching from single residue methods to multi-residue methods:

  ♦ Consideration of extraction efficiency may be required.

- Samples with incurred residues are not available.

8. Summary and conclusion

- Extraction efficiency data are not required in all cases.
- In many situations a large amount of radioactivity is extractable with solvents and refers to the regulated residue. If extraction solvents in metabolism study and monitoring method are identical, no further studies are needed.
- Less ideal situations (e.g. many metabolites, different solvents used, unidentified radioactivity, etc.) require case-by-case decisions.
- If the extraction solvents in metabolism study and residue method are not identical, cross validation with samples containing incurred residues might be possible.
- Due to the limited access to the required studies and/or incurred residues, surveillance laboratories will often have to rely on assistance by industry or regulatory agencies.

8. The road ahead

- Draft of the guidance document „Guidance Document on the Evaluation of the Extraction Efficiency of Residue Analytical Methods” was reviewed on EU level.

- Discussion in upcoming SCoPAFF meeting (16./17. June 2016)

- We propose to start a database in containing the relevant information from radiolabeled studies (e.g. matrices, extraction solvents, %TRR extracted etc.) for easier application of the decision tree.

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Thank you for your attention

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