Application of the problem formulation approach to model case studies

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Several factors have contributed to the perception of a need for better tools for GMO risk assessment

With the increasing size of the dossiers, it becomes desirable to simplify.

With new, more complex traits, such as resistance to drought or to salinity, an evaluation based on the equivalence between a GMO and non-GMOs may be less effective.

It is necessary to improve the quality of communication with the stockholders, and to engage them better in the process.

A brief history of problem formulation

A concept that has developed over several years:

US EPA (1998) Guidelines for ecological risk assessment.

Johnson KL, Raybould AF, Hudson MD, Poppy GM (2007) How does scientific risk assessment of GM crops fit within the wider risk analysis? Trends Plant Sci 12, 1-5.

Hokanson KE, Ellstrand NC, Ouedraogo JT, Olweny PA, Schall BA, Raybould AF (2010) Biofortified sorghum in Africa: using problem formulation to inform risk assessment. Nature Biotech. 28, 900-903.

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2010) Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Res. 19, 425–436.

Tepfer M, Racovita M, Craig W (2013) Putting problem formulation at the forefront of GMO risk analysis. GM Crops & Food 4, 10-15.

Risk assessment based on problem formulation

Instead of accumulation all possible information concerning, for instance plant-gene-environment interactions, to show that nothing is changed by the GMO, start out by examining the potential negative effects that are of concern.

These potential negative effects are then reformulated as risk hypotheses.

Then, through detailed scrutiny of the causal chain that could link the GMO and a negative effect (harm), you can determine of scientific knowledge make it possible to invalidate the causal chain (refutation of the risk hypothesis).

How to proceed, using the problem formulation strategy

1. Identify protection goals

- protect environment, human and animal health
- Societal goals

2. Based on these goals, create a catalogue of risk hypotheses

3. Prioritization: rank hypotheses according to importance

4. For the hypotheses to be examined, create a "pathway to harm"

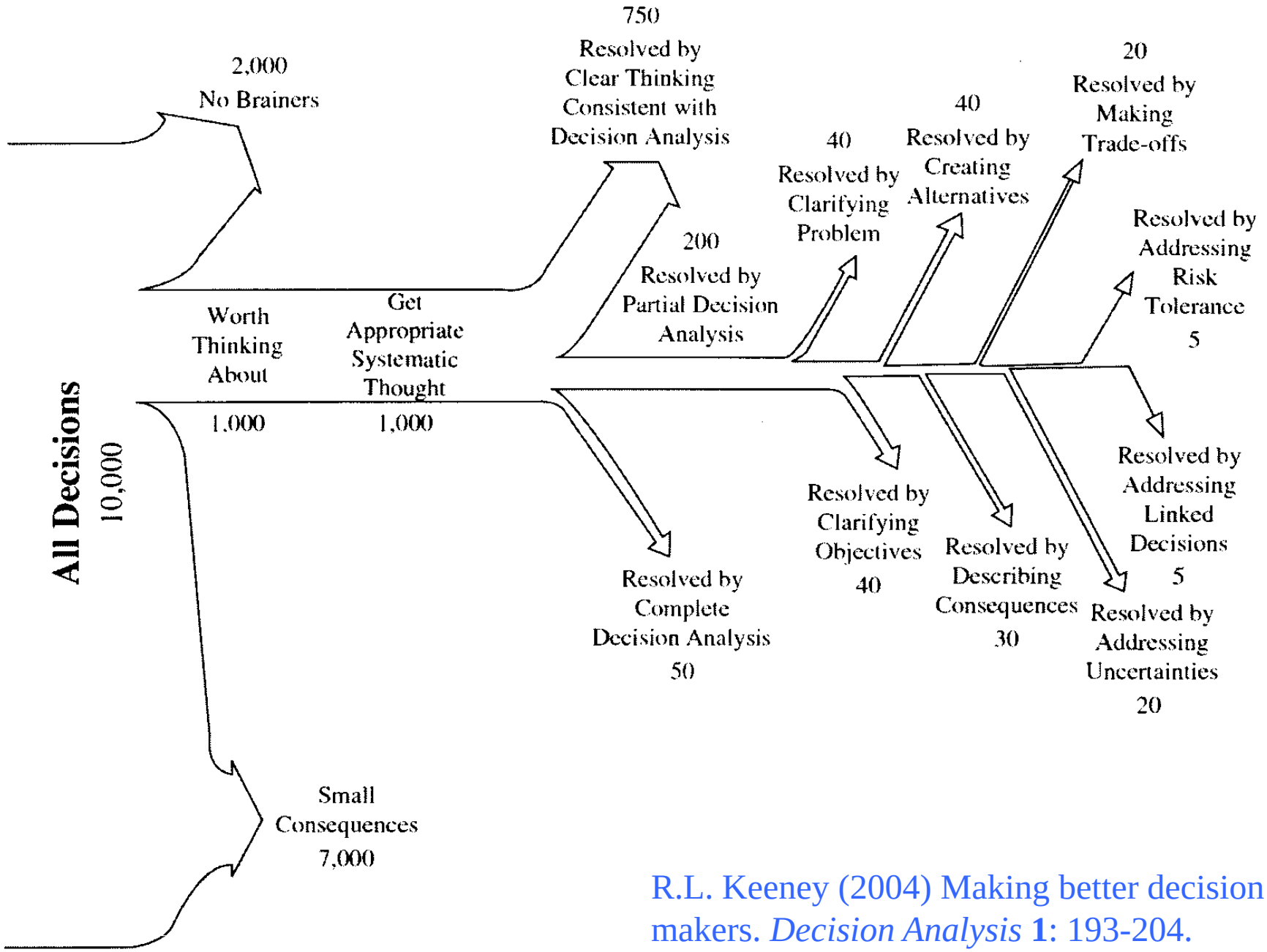
5. Test of risk hypothesis

- Identify key steps in the pathway, and fit available data to it.
- If necessary, gather new data.

6. If there are still concerns, consider mitigation measures

7. Draw conclusions regarding the potential risk

Prioritization: how 10 000 decisions should be resolved



R.L. Keeney (2004) Making better decision makers. *Decision Analysis* **1**: 193-204.

First case study, a retrospective view of an old story:

Maize that expresses the Cry1Ac *Bt* toxin is resistant to European corn borer (ECB).

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To consider for commercial release of this GMO in the USA.

- Bt toxin gene: *p35S-Cry1Ab-t35S*
- Kanamycin resistance gene: *p35S-Npt2-t35S*

- Protection goals: protect the environment, human and animal health
- Catalog of concerns translated into risk hypotheses
- Prioritization
- Test of risk hypotheses
- Risk assessment

Creating a catalog of risk hypotheses

Example: maize that expresses the Cry1Ac *Bt* toxin is resistant to European corn borer (ECB). What are the potential risks associated with commercial release of this GMO in the USA?

- Emergence of resistant ECB will lead to being unable to use *B. thuringiensis* as a biological control agent.
- *Bt* maize will lead to decline in populations non-target insects that have important ecological functions (pollinators, predators of pest insects, soil microbes, soil organisms that degrade plant detritus, etc.).
- *Bt* maize will lead to decline in populations of Monarch butterflies.

Any others?

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The Monarch butterfly controversy can be regarded as a failure in prioritization -or in risk communication? The public viewed this as the most important issue.

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Pathway to harm

1. Bt maize will be grown in the US Midwest

2. Monarch larvae only eat milkweed leaves

3. Milkweed is common in/near maize fields in the region where monarchs occur

4. *Bt* maize pollen will fall on milkweed leaves

5. *Bt* maize pollen on milkweed leaves is toxic to monarch larvae

6. Monarch larvae will be poisoned by *Bt* toxin

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Risk Hypothesis

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Pre-existing knowledge in 1997, when Bt maize was commercially released in US

Pathway to harm

1. Bt maize will be grown in the US Midwest — Yes

↓

2. Monarch larvae only eat milkweed leaves — Yes

↓

3. Milkweed is common in/near maize fields in the region where monarchs occur — Yes

↓

4. *Bt* maize pollen will fall on milkweed leaves — To some extent

↓

5. *Bt* maize pollen on milkweed leaves is toxic to monarch larvae — Perhaps

↓

6. Monarch larvae will be poisoned by *Bt* toxin — Perhaps

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7. Monarch butterfly populations will be reduced — Perhaps

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Losey et al. 1999 (laboratory experiments)

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7. Monarch butterfly populations will be reduced — Perhaps

Losey et al. have not falsified the risk hypothesis: do field studies?

Risk Hypothesis

Bt *maize will reduce the populations of monarch butterflies*

Sears et al. 2001 (field studies)

Pathway to harm

1. Bt maize will be grown in the US Midwest — Yes

↓

2. Monarch larvae only eat milkweed leaves — Yes

↓

3. Milkweed is common in/near maize fields in the region where monarchs occur — Yes

↓

4. *Bt* maize pollen will fall on milkweed leaves — Very little

↓

5. *Bt* maize pollen on milkweed leaves is toxic to monarch larvae — No

↓

6. Monarch larvae will be poisoned by *Bt* toxin — No

↓

7. Monarch butterfly populations will be reduced — No

Sears et al. have shown that exposure is very low, therefore no appreciable risk

Last step: evaluate the quality of the risk assessment

How rigorously has the risk hypothesis been tested?

What are the uncertainties, limits to our understanding?

Sears et al. did not refute the risk hypothesis; they showed that exposure was very low, and this led to acceptable levels of risk (insignificant monarch mortality).

For instance, do the conclusions apply broadly, e.g. to other similar genes or other ecosystems?

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For instance, do the conclusions apply broadly, e.g. to other similar genes or other ecosystems?

Conclusion: no more demonstrations with people dressed in Monarch costumes (I couldn't even find a photo on the web!).

Is this the end of the story?

Perhaps not, risk assessment is an iterative process, if new information appears, it may be necessary to revisit the risk assessment.

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But:

Has the size of milkweed populations become limiting for monarchs?

What are the limiting factors to their population size?

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Crop Protection 29 (2010) 1542–1544

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Loss of grassland milkweed habitat due to shift to biofuel production?

4134–4139 | PNAS | March 5, 2013 | vol. 110 | no. 10

Recent land use change in the Western Corn Belt threatens grasslands and wetlands

Christopher K. Wright[1] and Michael C. Wimberly

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Loss of grassland milkweed habitat due to shift to biofuel production?

Management: protect over-wintering sites, encourage growing milkweed


Questions?

Second hypothetical case study:

GM camelina ‘SuperCam’ with increased oil accumulation in the seeds

What is Camelina?

- Camelina (*Camelina sativa*) is an oilseed crop in the family Brassicaceae
- Widely grown in the Middle East and Europe, from the Neolithic to the early 20th century
- A crop with high genetic diversity
- Has undergone little genetic improvement
- Is beginning to attract renewed attention, particularly in North America.

Camelina, a re-emergent crop

It is beginning to attract renewed attention, particularly in North America.

- Grows on marginal soils, adapted to semi-arid conditions
- Requires few treatments (herbicides, pesticides, fertilizer)
- Good yield of seed: 10-25 q/ha
- Good yield of oil: 20-30%

Biodiesel

Human consumption: food, cosmetics

- Oil rich in linoleic acid (omega-6), linolenic acid (omega-3), tocopherol

‘SuperCam’ GM camelina accumulates 30% more oil than controls

Transgenes :

p35S-EPSPS-tNos (confers glyphosate tolerance)

p35S & *t35S* are from CaMV

CP4 EPSPS is from *Agrobacterium tumefaciens*

tNos is from *Agrobacterium tumefaciens*

pNapin-L1L-tNos

pNapin & *L1L* (Leafy cotyledon-like1) are from arabidopsis

(L1L is a transcription factor that boosts lipid synthesis)

(L1L: Tan et al. 2011. Plant Physiol. 156: 1577-1588.)

SuperCam risk assessment using problem formulation

1. Identify management goals (for a CFT for proof of principle)

- protect environment, (human and animal health)
- (Societal goals)

2. Based on these goals, create a catalogue of risk hypotheses

3. Prioritization: rank hypotheses according to importance

4. For the hypotheses to be examined, create a "pathway to harm"

5. Test of risk hypothesis

- Identify key steps in the pathway, and fit available data to it.
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A risk hypothesis simply joins a cause and un undesired effect

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Plant	Genes	Environment
Camelina	L1L	France
	EPSPS	

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Any others?

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Catalog of risk hypotheses

Plant	Genes	Environment
Camelina	L1L EPSPS	France

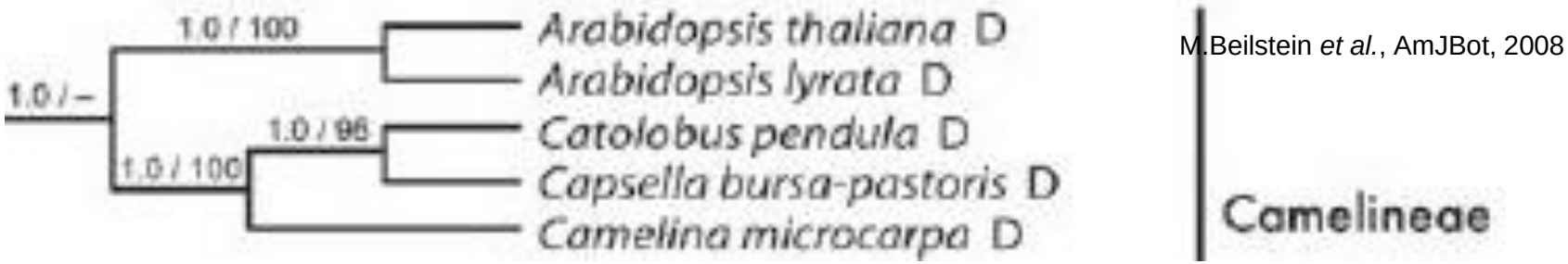
Growing SuperCam camelina will make capsella insensitive to glyphosate

Using glyphosate to eliminate weeds in camelina fields will lead to loss of an endangered butterfly that depends on weed species X.

Any others?

Phylogeny of camelina

- Family: Brassicaceae
- Tribe: Camelinae
- Genus: *Camelina*
- Species: *sativa*
- Close to *Arabidopsis thaliana* and *Capsella bursa-pastoris* (Shepherd's purse)



M.Beilstein *et al.*, AmJBot, 2008

Arabidopsis and capsella are abundant wild species in temperate regions.

So you might imagine that camelina could cross with arabidopsis and capsella.

Risk hypothesis: **Growing SuperCam camelina will make capsella insensitive to glyphosate**

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Pathway to harm

SuperCam camelina is grown in the field

↓

↓

HT capsella becomes insensitive to glyphosate

Risk hypothesis: Growing SuperCam camelina will make capsella insensitive to glyphosate

Pathway to harm

1. SuperCam camelina is grown in the field
2. Camelina and capsella flower simultaneously
3. Pollinators visit flowers of both species
4. Capsella X camelina hybrid seeds are produced
5. The hybrids generate progeny in back-crosses to capsella
6. HT capsella becomes insensitive to glyphosate

Risk hypothesis: Growing SuperCam camelina will make capsella insensitive to glyphosate

Pathway to harm

1	SuperCam camelina is grown in the field		
	↓		
2	Camelina and capsella flower simultaneously	←	**Yes: observed in the field**
	↓		
3	Pollinators visit flowers of both species	←	**Yes: observed in the field**
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Pathway to harm

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2	Camelina and capsella flower simultaneously	←	**Yes: observed in the field**
	↓		
3	Pollinators visit flowers of both species	←	**Yes: observed in the field**
	↓		
4	Capsella X camelina hybrid seeds are produced	←	**Unlikely in the field:** Very few hybrid seeds were produced in the greenhouse
	↓		
5	The hybrids generate progeny in back-crosses to capsella	←	**No:** the hybrids were male and female sterile
	↓		
6	HT capsella becomes insensitive to glyphosate		

Conclusions regarding SuperCam and capsella

Within the scope of current knowledge, the risk of creating herbicide-tolerant capsella is low. So this should not be an issue for carrying out CFTs in Versailles with SuperCam.

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BUT if you want to think forward about commercial development:

•Is the herbicide-tolerance transgene necessary for SuperCam? If not, don't use it.

•If the developer wants to include the herbicide tolerance gene, is the present scientific information sufficient? Are there mitigation measures to consider?

•If we assume that herbicide-tolerant capsella would result from growing Super Cam, exactly what would be harmed?

Transgenic Res
DOI 10.1007/s11248-013-9722-7

ORIGINAL PAPER

Evaluation of the potential for interspecific hybridization between *Camelina sativa* and related wild Brassicaceae in anticipation of field trials of GM camelina

Stéphane Julié-Galau · Yannick Bellec · Jean-Denis Faure · Mark Tepfer

Received: 5 April 2013 / Accepted: 8 June 2013


Questions?

"Something's wrong with the broccoli. Please take it back to the kitchen and have it genetically modified."

• • •


©1990 BY NICOLE HOLLANDER
I DREAMT THAT AFTER I ATE SOME GENETICALLY ALTERED TOMATOES, THEIR TRAITS WERE TRANSMITTED TO ME.
I STAYED YOUNG AND FIRM FOREVER, AND IN ADDITION, I WAS RESISTANT TO BUDWORM.
11-1

OPINION/LETTERS

Cartoon by Rob White