Tables (1-5) for

Off-target Effects of Plant Transgenic RNAi: Three Mechanisms Lead to Distinct Toxicological and Environmental Hazards

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Presented at GMO-Free Regions, BERLIN May 2015

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Table 1. Types of interfering RNAs mentioned in the text. All the RNAs described below can cause the destruction of an mRNA.

Name and abbreviation of RNA	Description
miRNA: microRNA	Naturally-occurring small (25nt or less) RNAs may direct mRNA cleavage or translational inhibition
siRNA: short interfering RNA	Small (25nt or less) RNA complementary to its target that directs mRNA cleavage
hpRNA: hairpin RNA	Foldback RNA of artificial origin. Large: 100-2,000nt
dsRNA: double stranded RNA	Duplexed long RNAs usually perfectly complementary in sequence
amiRNA: artificial miRNA	An artificial sequence substituted into a natural miRNA

Table 2. The evidence for off-target effects of RNAi within plants and for effects of RNAi on non-target invertebrates.

Nature of OTE	*Consequence of OTE*	*Reference*
Confirmed OTEs in plants:	RNAi caused pollen defects in multiple lines	Xing and Zachgo 2007
	RNAi caused late flowering and downregulation of various mRNAs	Xu et al. 2006
	Overexpression of a natural miRNA led to unexpected downregulation of the *OPT1* mRNA	Schwab et al. 2005
	Overexpression of an artificial miRNA (amiRNA-mads-1) led to unexpected floral organ defects. A second artificial miRNA unexpectedly downregulated the *FIL* mRNA	Schwab et al. 2006
Suspected OTEs in plants:	Expression of a hairpin RNAi transgene in the TREUS™ commercial soybean line is associated with the presence of two novel fatty acids.	Delaney et al. 2008

Confirmed effects of a plant RNAi transgene on a non-target organism:	The reproduction of three root knot nematode species, in addition to the target species, were significantly inhibited by a hairpin transgene. The reproduction of a related non-target cyst nematode species was significantly inhibited by a hairpin transgene.	Huang et al. 2006 Sindhu et al. 2009
Confirmed effects of ds RNAs on a non-target organism:	Lethality and other effects in two related non-target beetle species	Baum et al. 2007

Table 3. Important *in vivo* studies on dsRNA toxicity in mammals.

Consequence	Species	References
Fever	Guinea pig, Cow, Goat, Rabbit	Lindsay et al. 1969; Cooper et al. 1988; McVicar et al. 1973
Defects in liver function	Mouse, Dog	Phillips et al. 1971; Morahan et al. 1972
Leukopenia	Dog	Phillips et al. 1971
Autoimmunity	Mouse	Steinberg et al. 1969
Growth rate defects/Weight loss	Rat, Dog, Mouse	Leonard et al. 1969; Phillips et al. 1971
Hypoglycemia	Mouse	Vignaux and Gresser 1981
Ocular toxicity	Rabbit	Ostler et al. 1970
Embryo toxicity	Rabbit, Mouse	Adamson and Fabro 1969; de Fougerolles and Baines 1987; Lin et al. 2006; Shimada et al. 2003
Inhibition of mitosis	Mouse	Serota and Baserga 1970; Jahiel et al. 1971
Thymus degeneration	Mouse, Rat	Leonard et al. 1969
Lethality	Mouse, Dog, Goat, Rat, Rabbit, Monkey	Absher and Stinebring 1969; Ostler et al. 1970; Phillips et al. 1971; Homan et al. 1972; McVicar et al. 1973; Vignaux and Gresser 1981

Table 4. Proposed preconditions for regulatory approval of plant transgenic RNAi events. The following molecular conditions should be fulfilled before approval of any RNAi transgene is considered.

Requirements
Avoid perfectly duplexed sequences over 28nt in length
Avoid complementarity with known human DNA or RNA sequences over 19nt
Avoid complementarity with known host DNA or RNA sequences over 19nt
Ensure minimal expression levels by a transgene promoter
Recommendations
RNAi-triggering sequences should be as short as possible
Avoid near-complementarity with known human sequences over 19nt
Use a naturally-occurring miRNA promoter

Table 5. Summary of principal concerns associated with RNAi technology

Hazard	Mechanism	Notes
Off-target effects (OTEs)	RNAi affects endogenous genes in addition to the intended target. In plants such OTEs could result in altered nutrition, toxicity, agronomic performance, pest resistance etc.	Much the same risks as with unintended effects caused by transformation-induced mutations (Wilson et al. 2006). Discussed in this review.
OTEs in non-target organisms	RNAi molecules silence homologous and non-homologous genes in non-target organisms, causing deleterious effects.	As with Bt transgenic toxins, relatedness to the target is a risk factor but not the only one. Discussed in this review.
dsRNA toxicity	dsRNA molecules of perfect homology over 29 nt in length are toxic to mammals because they are PAMPs.	Effects of dsRNAs in mammals include neurotoxicity, behavioral defects, immune system effects, reproductive effects and liver toxicity. Discussed in this review.
Saturation of RNAi machinery	Saturation can lead to perturbations of cellular metabolism and even death.	Perhaps of most concern if saturation is conditional or unpredictable. Not discussed in this review.
Instability of RNAi	RNAi may unexpectedly fail due to viral infection, mutation or epigenetic change	Of particular interest because RNAi is under consideration for the manipulation of nutrients, toxins and allergens in the diet. Not discussed in this review (Small 2007).

Hazard	Mechanism	Notes
OTEs caused by transitivity	Transitivity is where RNAi spreads to neighbouring sequences on the target mRNA. Such spread might increase OTEs.	Transitivity is found in plants but seems to be rare. Not discussed in this review.

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