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ACB'S OBJECTIONS TO MONSANTO'S APPLICATION FOR FIELD TRIAL RELEASE OF HERBICIDE-RESISTANT CANOLA (BRASICA NAPUS RT73)

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CONTENTS

Overview of application

***Brassica napus* RT73 Canola plants produce CP4 5-enolpyruvylshikimate-3-phospate synthase (EPSPS) from *Agrobacterium* spp. and glyphosate oxidoreductase (GOX) from the *Ochrobactrum anthropi.* The CP4 EPSPS is naturally less sensitive to inhibition by glyphosate while GOX hydrolyses glyphosate to aminomethylphosphonic acid (AMPA) and glyoxyclic acid. Both the EPSPS and GOX transgenes confer resistance to the glyphosate herbicide, Roundup™ (Monsanto). These transgenes are being used together in an attempt to combat the emerging resistance to glyphosate. This application is for the field-trial release of RT73 in the summer-rainfall areas of South Africa in order to evaluate the feasibility of counter-season seed production.**

Introduction

Canola is one of two cultivars of oilseed rape (*Brassica napus* L. and *Brassica campestris* L.). Canola seeds are used to produce edible oil for humans and to produce Canola meal as livestock feed because it has lower levels of the toxic erucic acid and glucosinolates than traditional oilseed rape. Canola was originally bred from oilseed rape in Canada in the early 1970s and the name "Canola" was derived from "Canadian oil, low acid".

The current application is for the field release of Genetically Modified (GM) RT73 Canola that has been engineered to be resistant to the common herbicide glyphosate (Roundup™, Monsanto). Since Canola is typically grown in the winter rainfall areas of the Western Cape, the purpose of these field trials is to test the production of RT73 in the summer-rainfall areas (Lichtenburg, Witbank, Reitz) that will provide physical isolation from the main Canola growing areas of the Western Cape. All seed produced will be exported. However, it is unclear from the application *when* the Canola will be planted, *how much* will be planted and harvested and *for how many seasons* this application covers.

According to the dossier, an application for export of the RT73 will be made nearer the date of harvest “as information on the exact amounts will only be known at that stage”. Surely the expert agronomists from Monsanto will have a good estimation of the amount of seed that can be expected from this harvest? The dossier states that 80kg will be imported for the field trial. Sowing Canola is typically carried out at a density of 6kg per hectare and the yield is typically 590-1740 kg/ha; which varies depending on the variety, geographic location and amount of rainfall (Walton 1997; Si and Walton 2004). This suggests that the applicant will

sow 13.3 hectares and with an average yield 1198 kg/ha, will expect to harvest 15 933 kg. Since Canola seeds have a weight of approximately 3.5mg, this means that 4 552 285 714 (4.6 billion) seeds will be available for harvest during this field trial.

Lack of molecular characterization of RT73

RT73 Canola has been engineered to be resistant to the herbicide glyphosate (Roundup^{TM}) through the use of two transgenes. The cp4 epsps transgene codes for the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), and the *goxv247* transgene coding for a modified version of glyphosate oxidase (GOX). The transgenes were engineered into Canola using the bacterium, *Agrobacterium tumefaciens* to produce the transgenic GMO, RT73. The *cp4 epsps* and *goxv247* transgenes are each driven by the 35S promoter from a modified figwort mosaic virus (FMV) and terminated with the 3' (terminal) end of the pea rbcS E9 gene. Both transgenes contain a chloroplast transit peptide sequence to direct the proteins to the plant chloroplast. According to the application, single copies of these two transgenic cassettes (transgenes with associated regulatory elements) were present in RT73 while sequences from the plasmid used in the transformation procedure, such as the plasmid origin of replication and a gene for streptomycin resistance (*aad*) were absent from RT73. However, we have not been provided with any data to support this claim and the site of insertion is not documented. This absolutely vital information has been withheld from us presumably because Monsanto considers the data of Southern blots and PCR to be confidential!

We note that the dossier submitted to the European regulatory authority, EFSA, in 2004(http://www.biosafety.be/gmcropff/EN/TP/Opinions_EFSA/opinion_gmo_05_en_final.pdf) revealed that there are molecular changes at the site of insertion: a 40 bp region of the host genome has been deleted, while 22 bp of unknown extraneous DNA is present at the 5' end of the transgene insertion site. This represents an unknown risk.

The applicant also states that the transgenes are stable in the field and across generations, yet there is no molecular data to support this. There were molecular analyses reported to have been carried out (pg 10 section 4.4), but neither the details nor the data are provided to substantiate this. Furthermore, the expression levels were determined to be in the range of 0.027-0.034 ug/mg for CP4 EPSPS and 0.154-0.211 ug/mg which represents 0.025 to 0.07% of total protein in the seed, respectively (data mentioned in text but raw data not provided by the applicant). While this expression level is relatively low, the levels in other plant tissues have not been determined. This important aspect needs to be considered since the transgenes are expressed constitutively and in every tissue of the Canola R73 and this may represent a hazard to biodiversity since many species will interact with the Canola plant. Additionally, the difference in expression level (nearly fourfold) of the two transgenes is surprising given that they utilise the same promoter (35S-FMV from Figwort Mosaic Virus), and no explanation is offered for this finding.

The methods stated to test for batch consistency (section 4.7, page 11) rely on the quantitative PCR detection method that targets only a small portion (108bp) of the transgenic cassette at the 3' region. This approach will not detect any rearrangements or

deletions that can take place in the transgenic cassettes and should be combined with more appropriate PCR detection methods that span the transgenic cassettes. This is an important point since transgene stability has been observed (Collonier *et al.*) and transgenes have been shown to increase the rate of gene flow indicating that they are more likely to spread Bergelson et al. 1998)

In addition to the lack of characterization of the transgenic cassettes, the alterations in the transgenes are poorly described. There have been changes in codon usage for both *goxv247* and *cp4 epsps* (compared to the natural bacterial counterparts), but the data is not presented by the applicant, nor are any changes in amino acid sequences of these modified proteins documented by the applicant. This is important in the biosafety assessments since novel amino acid sequence of these proteins can alter the toxicity and/or allergenicity.

Toxicology and allergenicity tests inappropriate

The transgenic proteins have been tested for mammalian toxicity, however, these were not isolated from the RT73 transgenic plant but as recombinant proteins from bacteria. The bacterial surrogate enzymes were assumed to be identical to the enzymes produced in RT73 and used for toxicity studies on mice. In light of the well documented effects of secondary modifications on protein structure and function, these tests are inappropriate. Moreover, N-terminal analysis identified the presence of four anomalous amino acids for the bacterial GOX. Additionally, the digestibility and degradability of GOX and EPSPS were tested with the bacterially-expressed proteins in simulated gastric fluid *in vitro*.

There are several known toxins produced by *Brassica napus*. Indeed, much of the traditional Canola breeding to date has focused on reducing the levels of these toxins (mainly erucic acid and glucosinolates), so that the oil is safe for human consumption and the seed meal is safe for animal consumption. The conclusions by the applicant that the RT73 is “not materially different” from the non-GMO parental Canola line (Westar) (section 5.2 page 30) and is therefore compositionally equivalent is unfounded since compositional equivalence has not been demonstrated. Evidence from the more detailed dossier submitted to EFSA indicate statistically significant compositional differences between RT73 and the non-GMO parent (Westar). The level of toxic glucosinolates was found to be significantly higher in RT73 compared to Westar (http://www.biosafety.be/gmcropff/EN/TP/Opinions_EFSA/opinion_gmo_05_en_final.pdf). These findings do reveal increased risks to biodiversity, particularly farm animals (sheep, cattle, chicken, trout) whose diet can contain up to 25% Canola meal. However, the fact that compositional analysis is not available for independent analysis means that we are severely hampered in our independent assessment of the application.

The allergenicity testing is also unreliable, since it depends on theoretical evaluations based on assumptions that have been extensively questioned. The conclusions that the transgenic proteins are unlikely to have allergenic properties is based upon the following assertions made by the applicant: the newly introduced protein originates from a non-allergenic source; there is no significant sequence homology to known allergens; the protein will be rapidly digested in the intestine; the expression level of protein in the GMO is low; and the protein is not new to the human diet. Appropriate allergenicity testing should take place in mice, cattle or sheep feeding trials with blood samples taken to monitor animal immune response (IgE

levels and cytokines) and physiological parameters measured, but this has not been carried out.

Even with the complete the lack of *in vivo* allergenicity testing, there are real concerns that the GOX may be allergenic since it shares sequence similarity to tropomyosin (responsible for persons suffering from shrimp-allergy) (Kleter and Peijnenburg, 2002). This has been emphasized by EFSA: "Since cross-reactivity between GOX and tropomyosin is not ruled out completely, persons allergic to shrimp meal should be aware of the possibility of hypersensitivity reaction when working with GT73 oilseed rape" (http://www.biosafety.be/gmcropff/EN/TP/Opinions_EFSA/opinion_gmo_05_en_final.pdf). Although EFSA granted approval of RT73, it was the of the opinion that the risks were low since their application was for import only for food and feed and so pollen flow, hybridization and out-crossing were not significant issues. However, the current application in South Africa is for field trials and will result in pollen dispersal over many kilometers; thereby increasing risks of RT73 pollen exposure and allergenicity. For example, Reiger et al. (2003) studied the movement of Canola pollen and detected pollen-mediated gene flow nearly 3 km from a source field. This also presents several hazards to biodiversity that are exposed to the Canola RT73 in the field.

Feeding trials reveal unexplained adverse effects

Feeding studies have been carried out for rats, sheep, and trout. In a rat feeding study there was a significant increase in liver weight (12-16%) for the RT73 compared to the non-GM Canola (Westar) when the diets consisted of 15% Canola meal. Both EFSA and ACRE and ACAF (Advisory Committee on Animal Feed) raised these concerns and Monsanto maintained they were probably due to the higher glucosinolate concentration in the RT73 diets compared with the corresponding control diets (the differences were nearly twice that of the control). If this is the case, then supporting evidence needs to be provided. Additionally the linolenic acid content of RT73 was found to be significantly lower compared to the non-GMO parent (Westar) and, despite the fact that linolenic acid is an important essential fatty acid, these difference were not considered biologically relevant! Together, this evidence indicates that RT73 is an inferior feed compared to the non-GMO parent Canola (Westar), and Monsanto's claims that RT73 is substantially equivalent to the non-GMO Canola ("There are no specific differences when RT73 is compared to conventional oilseed rape except for its tolerance to glyphosate. RT73 has been shown to be substantially equivalent, with exception of the introduced trait…".) is clearly unfounded.

Despite the lack of substantial equivalence and the important findings in animal feeding trials, these studies have not been pursued with the expected experiments involving histological observation and tests of liver function to ascertain if there are any physiological changes that would present an unacceptable risk to farmed animals. A subsequent study was flawed in design since it did not analyse histology and physiology and used several non comparators (vastly different Canola varieties grown under very different conditions). There were other earlier feeding studies with rat and trout that also did show an effect on organ weight as a result of RT73 (compared to the non-GM Canola parent), but definitive conclusions cannot be drawn from these since RT73 was contaminated or co-mingled with another Canola GM event, RT200 (see box below).

Contamination of Canola RT73 transgene is inevitable

There are several routes for gene escape of RT73 into the environment:

- Seed spilled during transit and dispersed by human error or malicious intention;
- Crossing of RT73 with other *Brassica* spp. in the field (pollen flow);
- Seed dispersed from the field by animals (birds, rodents, goats, cattle, sheep), and by wind and water; and
- Horizontal gene transfer to other plants,animals, fungi and microorganisms

Seed is spilled during transit

Countries that have not approved field release of a GMO, but import the said GMO for food and feed have documented cases of feral populations such as GM maize and Canola around ports, on transport routes and on farms such as around chicken-feeding houses in Japan and Korea. In Japan, the port of Kashima (and possibly also Kobe port) imported oil GM Canola and there are feral populations in 25 out of 48 checking points at locations within 5 km radius of the port (Aono 2006). It is highly likely that escaped seed will germinate, flower and potentially cross-pollinate with Canola crops, feral populations or wild relatives in South Africa. These escaped feral populations may persist for several years unnoticed and may also persist and penetrate other Brassica spp populations because they are (of course) resistant to the common herbicide, Roundup^{TM}. For example escaped feral Canola persisted for at least 8 years to 10 years after its last cultivation, and seeded and populated other areas giving rise to dispersed feral populations that persisted. The dispersal pattern can be partially related to the transport traffic at harvest from the field to the silo (D'Hertefeldt, et al. 2008).

There is, in fact, already a problem with RT73 Canola production in Canada since it appears to be contaminated by an unapproved transgenic, RT-200 (see GENET news, box below). This contamination has perhaps stimulated Monsanto to seed-bulk RT73 in South Africa (the start of which is this application for field trial). The worldwide seed production of Canola is set to increase since it has approx. 40% oil, has wild and weedy characteristics and is therefore being developed and promoted as a source of biofuel (biodiesel) http://cmsdata.iucn.org/downloads/biofuels_and_invasives_background_paper.pdf)

From GENET news

According to the St. Louis POST-DISPATCH, Monsanto, the St. Louis chemical and biotechnology giant, last month announced it had recalled "small quantities" of a genetically engineered Canola seed containing an unapproved gene that had gotten into the product by mistake. Canola is a crop grown for livestock feed, and for oil consumed by humans. The canola-recall story, only 84 words long, was buried in the POST-DISPATCH April 18, under a confusing headline, deep in a news-wrap up column on the business page.[1] Putting the wrong gene into a commercial product by mistake is precisely the kind of error that opponents of genetic engineering have been predicting for a decade. Proponents of genetic engineering have said it could never happen because of rigorous quality-assurance by the industry itself and tight regulation by governments. The recall was reportedly initiated by Monsanto Canada Ltd., and by Limagrain Canada Seeds, Inc., of Saskatoon, Saskatchewan, which was selling the seed under license from Monsanto. The recalled canola seed was "Roundup ready" --meaning it had been genetically engineered to withstand dousing with Monsanto's herbicide, glyphosate, which is marketed under the trade name Roundup. Since February, 1996, Monsanto has been marketing various genetically-engineered crops that are "Roundup-ready" in an effort to boost sales of Roundup, the herbicide responsible for a large proportion of Monsanto's annual profits.[2] (See REHW #521.) The idea is to douse Roundup-ready crops with Roundup to kill weeds, leaving the genetically-engineered crop intact.

According to the Associated Press, Monsanto refused to disclose how much genetically engineered canola seed is being recalled, but said the amount was "small."[1] Canadian government officials say the quantity being recalled is not small. Brewster and Cathleen Kneen, publishers of the RAM'S HORN, a Canadian

newsletter devoted to analysis of the food system, said that, in mid-April, Monsanto reported to the Evaluation Branch of the Biotechnology Strategies and Coordination Office of the Canadian government that it was recalling 60,000 bag units of two types of canola seeds (types LG3315 and LG3295) because one or both types contained the wrong gene.[3] Thus the amount recalled is sufficient to seed 600,000 to 750,000 acres of land. According to RAM'S HORN, some of the seed had already been planted when Monsanto discovered the mistake. The MANITOBA CO-OPERATOR, a Canadian agricultural newspaper, quoted Ray Mowling, a Monsanto spokesperson, saying, "In some recent quality assurance testing by us, we've identified that there's a possible variety contamination."[4] Brewster Kneen of RAM'S HORN points out that it takes a long time to produce enough Roundup Ready seed for 600,000 acres, so this error went undetected for a substantial period. Under Canadian law, there are three levels of approval for genetically engineered crops: environmental (meaning the crop can be planted), livestock (the resulting crop can be fed to livestock), and human (the resulting crop can be fed to humans). Two Roundup-resistant canola genes, RT-73 and RT-200, had been approved for planting, but only RT-73 was approved for livestock and humans. It was the unapproved RT-200 that somehow ended up in the seed that had to be recalled. "The preliminary testing showed it to be the wrong configuration, as opposed to the one approved," Monsanto's Mowling said. Canola oil is used in low-fat foods, pharmaceuticals, nutritional supplements, confectionery products, margarine and shortening, personal care products, lubricants, soaps, and detergents. The presence of the unapproved canola gene in a commercial product reveals, at a minimum, that Monsanto's quality-assurance programs failed in this instance, and that the biotechnology regulatory system in Canada is ineffective. The regulatory system in the U.S. is more lax than Canada's. Limagrain's Gary Bauman said his company will try to discover how the mistake occurred. However, he said it will be difficult to trace exactly where in the process it happened because the seeds available for testing now are progeny (offspring) of the original seeds. "We may never know how it happened," he said. Bauman later seemed to lay the blame squarely on Monsanto. He said only Monsanto has the expertise to detect genetic differences between seeds. "The apparent contamination, discovered by Monsanto, is something only they are able to detect. We are not even allowed to try to investigate how to look at and discover this gene within our own varieties," Bauman said.[5]

GENET. http://www.gene.ch/genet/2002/Apr/msg00056.html

Crossing of RT73 with other Brassica spp. and related species in the field

Canola pollen can flow nearly 3 km from a source field (Reiger et al. (2003). There are 154 species of Brassicaceae present in South Africa that can potentially cross fertilise with RT73 (Germishuizen and Mayer 2003). The species most closely related to Brassica napus RT73 and therefore most likely to cross are:

- *Brassica spp. (Brassica rapa, Brassica campestris, Brassica junceae, Brassica enlongata, Brassica nigra, Brassica oleraceae, Brassica tornefortii,)*
- *Rapahanus spp. (Rapahanus rapahanistrum, Rapahanus sativus)*
- *Erucastrum spp. (Erucastrum arabicum, Erucastrum griquense,Erucastrum strigosum)*
- *Sinapsis spp. (Sinapsis alba and Sinapsis arvensis)*
- *Erucastrum spp. (Erucastrum arabicum, Erucastrum griquense, Erucastrum strigosum*
- *Eruca sativa*
- *Crambe hispanica*
- *Diplotaxis muralis*
- *Hirschfeldia incana*
- *Rorippa nudiuscula*
- *Rapistrum rugosum*

Despite the fact that the sites were chosen to be far removed from any other official Canola cultivation (500km) there are probably many other Brassica species cultivated in the surrounding areas that are in much greater proximity. Surprisingly, the applicant has not carried out studies to determine the presence of these species in neighboring areas and farms and so these threats to these related species cannot be assessed. Is any Brassica

spp. present in the vicinity? The survey should encompass 50km^2 and include farmland, roadsides and gardens around houses. Many of the Brassicaceae are grown throughout South Africa, including cabbage, broccoli and kale (Brassica oleracea), radish (Raphanus sativus), rocket (Eruca sativa) and mustard (Hirschfeldia incana; formerly Brassica geniculata). Others represent wild weedy relatives such as wild-mustard (Sinapsis alba and Sinapsis arvensis) bastard-cabbage (Rapistrum rugosum) dog-mustard (Erucastrum), Rorippa nudiuscula and Diplotaxis muralis. The Brassicaceae include many important crop plants that are grown as vegetables (Brassica, Nasturtium, Raphanus), sources of vegetable oils (Brassica) and condiments (Armoracia, Brassica, Eutrema, Sinapis) and ornamentals in the genera Erysimum, Iberis Linnaeus, Lobularia, Malcolmia, and Matthiola. The Brassicaceae family also includes more than 120 species of weeds. Also, in South Africa the wild Nasturtium (*Tropaeolum majusor* and *Rorippa nasturtium-aquaticum*) and watercress (Nasturtium officiale) are prevalent members of Brassicaceae, but have not been considered by the applicant.

There is reference to lists submitted by SANBI (Attachment 3) indicating RT73 canola may cross with over 400 species related to Brassica which are found within 58km^2 of the proposed RT73 field trials. There is no monitoring plan to address these possible outcrossing events and the applicant makes unsubstantiated judgment (based on a subjective degree of relatedness) that many of these species will not outcross with RT73 Canola and that the most likely species that could outcross are: *Lepidium africanum, Lepidium transvaalense, Lepidium boneriensis, Rorippa fluviatilis* and *Heliophila rigidiuscula*. The pollen flow from Canola has been estimated in several studies and is affected by local weather conditions and abundance of pollinators. Since Canola is pollinated by bees (*Apis* spp.) and spotted beetle (*Astylus* spp.), the pollination can extend for several kilometers and follows a leptokurtic distribution. This can result in the establishment of hybrids far from the source of RT73 pollen. An additional factor is that the Canola seed is small (<2mm) and can be dispersed by vehicles, water or other animal vectors over great distances (see below).

The trials are said to be surrounded by 2m weed free area, 3m fallow alley and 12m conventional Canola to act as a buffer for dispersal and pollen flow. It is not documented what will become of this surrounding conventional Canola since it will be cross-hybridize with RT73 and therefore be unsuitable for harvest by Monsanto and also represent a persistent threat to RT73 gene escape if not removed and destroyed (incineration is recommended).

Rigorous standards for the production of certified Canola seed in Canada have failed to maintain seed purity after transgenic Canola was introduced in Canada. The contamination of pedigree Canola seed lots or commercial crops with herbicide resistance traits can occur by either pollen-mediated gene flow or whole seed dispersal. The Canola whole-seed contaminants may be homozygous for the herbicide resistance trait while contaminants resulting from pollen-mediated gene flow will be heterozygous for the resistance trait in the initial F1 populations (progeny from GM x non-GM cross). Canola seedlings heterozygous for the herbicide resistance traits can survive and thrive following recommended commercial dosages of these herbicides in the field (Hall et al., 2000; Rieger et al., 2002). By 1998, after only two seasons of commercial cultivation of GM herbicide-tolerant Canola in western Canada, volunteer Canola plants carrying GM resistance traits were found in many fields where farmers were not intentionally growing these GM varieties (Hall et al. 2000). Downey and Beckie (2002) tested a total of 70 certified Canola seed lots drawn from 14 different conventional, open-pollinated Canola (Brassica napus) cultivars for glyphosate and

glufosinate resistance trait contamination and found that 41 of the 70 seed lot samples were contaminated and that 18 of the 70 samples failed the 99.75% cultivar purity guideline. Three seed lots had glyphosate resistance contamination levels in excess of 2.0%. Unexpected contamination (even at 0.25%) can cause problems for producers that practice direct seeding and depend on glyphosate for non-selective, broad-spectrum weed control (Freisen et al 2002).

There ar also the documented cases of Canola out crossing with wild and weedy relatives. For example, the out-crossing of Canola with *Brassica rapa* (Halfill et al. 2002) and the emergence of multiple resistant *Brassica napus* (Hall et al. 2000)

The contamination of non-GM crops due to hybridization and out-crossing from GM crops can no longer be regarded as a potential problem that ought to be mitigated. Whenever GM crops are grown near non-GM crops, contamination is inevitable. This explains the recognized problem with transgene persistence, emergence of Roundup™-resistant weeds and problems with transgenes contaminating non-GMO varieties ,causing market rejection (for countries that label foods and have a threshold for adventitious presence i.e. EU and Japan). There are threats to the co-existence of non-GMO with GMOs and unknown threats to biodiversity (Lutman et al 2003 and 2005). These difficulties of failed co-existence and lack of liability are becoming an increasing problem. Farmers who do not wish to grow GM Canola are initiating lawsuits because of the unwanted contamination of their non-GM Canola seed banks (Bouchie 2002). This problem is well documented in Canada and several farmers who have had their non-GM Canola fields contaminated have received out-of-court settlements. “Several other western Canadian farmers have willingly agreed to similar settlement terms in order to have their individual issues addressed by Monsanto. For example, in 2007, Monsanto Canada assisted 16 farmers with addressing similar unexpected Roundup™ Ready volunteer issues. In 2005 – the same year Mr. Schmeiser experienced unexpected Roundup™ Ready canola volunteers – Monsanto Canada assisted six different farmers with resolving their particular situations and all costs were picked up by Monsanto Canada.” (http://www.monsanto.ca/about/news/2008/03_19_08.asp).

Seed dispersed from the field by animals, wind and water.

The sites are said to be removed from rivers: 30km from Lichtenburg, 300m from Witbank and 100m from Reitz. However, the localities and associated geography, transport routes and neighboring farms cannot be assessed since the data of these details has not been presented. The fact that some of these sites (Reitz and Witbank) are close to rivers raises the strong possibility that RT73 Canola seed will be enter the river system through wind or water run-off or animal dispersal and therefore disperse to even great distances. This will allow the establishment of feral plants far from the site of the field trial.

There is the added uncertainty of containment since these RT73 plants will seed in the field. The small Canola seeds (approx 2mm) have a high probability of being dispersed outside the defined cultivation areas because of dispersal by rodent, bird, insect, goat, sheep, cattle as well as water. Birds will feed on Canola seed and may disperse the seed over great distances (10 km or more). There is evidence that Canola seed does pass through the digestive tract of birds and can germinate. “Although the passage of viable diaspores was rare, the large number of birds likely to be feeding on canola seed in agricultural fields or at spillages on roadsides and loading zones suggests there is the potential for birds to disperse

viable Canola seed, including viable seed from genetically modified Canola crops." (Twigg et al. 2008).

Similarly, sheep, cattle and deer may enter the field and ingest RT73 Canola and some of this seed will pass through the digestive tract where it can germinate. Studies with sheep fed on Canola have shown that when Canola seed was removed from the diet, the majority of seed was passed during the first 2 days, but seed was excreted in feces for up to 5 days. Seed germination was reduced after 1 day and further still after 2 days, but did not significantly decrease after this (Stanton et al. 2003). A similar effect is predicted with other mammals that may ingest the Canola such as goats, deer and cattle. Other smaller animals such as rodents (mice, rabbits) may also feed on and disperse Canola seed. In addition to the ingestion of seed, evidence suggests that some seed will be dispersed by adhering to the fur of animals.

There is no indication that there will be adequate fencing-off and netting of the cultivated areas (Attachment 2 describing the sites was absent from the application). Fencing and netting to prevent the entry of above mentioned species is required to mitigate these risks of seed dispersal. Additionally, the harvesting methods are not described, nor are the cleaning of harvesting machinery. This is important since evidence does show that seeds can be carried long distances on farm machinery, and by other means that make the containment of transgenes virtually impossible (Briggs et al. 2003). It is also argued by the applicant that feral plants, volunteers or hybrids that do emerge can be controlled with current management practices such as crop rotations and the application herbicides. However, these feral RT73 plants are unlikely to be recognized as distinct from conventional Canola (they appear the same). In fact RT73 is more likely to spread due to the use of glyphosate as a universal herbicide in agriculture and municipalities (RT73 being Roundup™ resistant has a selective advantage) and it will therefore persist and continue to cross-fertilize and spread in the environment. Without an active monitoring programme (as is the case proposed by the applicant), these contaminations will only be will only be detected in transboundary shipments to member states that require GMO testing.

Additionally, published data indicates that these agricultural practices are ineffective in controlling feral populations or hybrids (if recognised as such) in the field. Even after 10 years of planting transgenic Canola, the transgenic volunteers persisted in the agricultural setting despite the compliance to conditions set by the company to limit the emergence of these volunteers (D'Hertefeldt, et al. 2008). This highlights the lack of monitoring in place by the applicant since a commitment of at least 10 years is required whereby the farms that take place in the field trials cannot plant any further Canola (or Brassica spp.) and any emergent volunteers discovered on the farm site (and surrounding area) need to be tested for the presence of the RT73 transgenes (a validated test for RT73 event is available). The applicant has not addressed these risks except to say that they will observe for volunteer emergence in the year following planting and rely on reports from farmers; which we find to be totally unacceptable.

Horizontal gene transfer to other plants, animals, fungi and microorganisms

The nature of the transgenic cassette also raises questions of stability since the cassettes have viral promoters and synthetic elements (i.e. 35S FMV promoter) that is new to nature

and have not been tested for biosafety in terms of stability and horizontal gene transfer. There is existing concern over the use of viral promoters due to increased rearrangements/ deletions affecting cassette integrity and genome stability. These biosafety risks include:

- Increased recombination (rearrangements, deletions, insertions). There is evidence from the laboratory (Koholi *et al.* 1999) and field studies (Quist and Chapela 2001, Collonier *et al.,* Ho et al. 2000) that viral promoters are recombination 'hotspots'.
- The*se* viral promoters result in very high expression levels that may result in unintended (pleiotropic) effects from the expressed transgenes.
- Viral promoter will have and increased recombination potential with other viral elements and the creation of new viruses (Wintermantel *et al.* 1996, Vaden and Melcher 1990, Greene *et al*. 1994).

The effects on genome stability, global gene expression and HGT have not been addressed by the applicant. A comparative assessment has not been made (by comparative genome hybridisation or repPCR/RAPD, RNA microarrays and/or proteomics) to establish if there are any other unintended genome changes (Bao et al. 1993, Pinkel and Albertson 2005). The effects on soil microorganisms have not been addressed. Current evidence indicates that HGT to bacteria does occur at a high frequency when sequence homology is present (de Vries J, Wackernagel W 1998). The *gox* and *epsps* transgenes have homologs in soil bacteria found and therefore there is an increased risk for HGT to occur to soil bacteria. Similarly, HGT could take place between ingested Canola meal to intestinal bacteria (Netherwood 1990). There may be several consequences of gene escape and hybridisation including the spread of herbicide resistance, non-target effects on other plants, animals (Cui and Xia 1999, Hillbeck 1999) and soil microorganisms important for soil function such as nutrient availability and cycling (Benbrook 1999 and Kowalchuk 2003). After almost three decades of world-wide use, there is confirmed herbicide resistance to glyphosate exists in *Lolium rigidum* (annual ryegrass) in Australia and South Africa; *Lolium multiflorum* (Italian ryegrass) in Chile, *Eleusine indi*ca (goosegrass) in Malaysia; and *Conyza canadensis* (marestail) in the eastern parts of the United States of America. http://www.cropscience.org.au/icsc2004/symposia/2/5/2166_killmer.htm

Lack of monitoring and compliance with regulations

Given the likelihood that RT73 transgenes will escape into the environment and that RT73 has an appearance indistinguishable to that of other Canola varieties, an appropriate monitoring program must be put in place. Farmers and local resident cannot be relied on to identify and test feral Canola plants (which are common-place around farms that plant Canola) as a means of monitoring. An active monitoring programme observes for transgene escape by testing for the presence of RT73 transgene (using the RT73 event-specific quantitative PCR method described by the applicant) is required. This should encompass testing Canola harvested and sold in the vicinity for 10 years to determine if RT73 hybrids and feral populations have occurred and also the testing of related Brassicaceae for plants that have outcrossed with RT73 Canola. This is required under international agreements (Cartagena Biosafety Protocol) as well as local legislation (NEMA and Biodiversity Bill). Other authorities have also expressed these concerns "ACRE still has concerns regarding seed spill and remains of the opinion that the post market monitoring plan should include active monitoring for spillage of GT73 seed during import, transportation and processing and include tests for the establishment of feral populations of GT73 oilseed rape" (ACRE 2003).

There are some other inconsistencies in the application. There is no independent observation to maintain that the procedures for transport, planting and harvest actually will take place in accordance with the proposed guidelines. It is unclear how the seed will be distributed after arriving in South Africa and if the individual RT73 seed packages sent to the three trial sites will be sealed and secured. Additionally, a person monitoring compliance with the said procedures should be separate from the person managing the project and should ideally be independent of Monsanto. Another issue is that the signatory for the "Report Verification Team" (page 2, S. Langrell) seems to have been crossed out. Also, the Affidavit by Michelle Vosges has not been fully completed (stamped correctly, but the details are not filled-in on form in the designated places).

Conclusion

The application of RT73 Canola for field release carries unacceptable risks. The RT73 has not been completely characterised and many of the assertions made by the applicant are unsupported by data. There is evidence that RT73 Canola is not substantially equivalent to non-GM Canola and that RT73 is an inferior animal feed.

There are numerous unacceptable risks of RT73 gene escape that include human error, spillage during harvest and transport, dispersal by water, animals (rodents, birds, chickens, sheep, goat, cattle) and the pollen flow from RT73 Canola to other *Brassica* spp. (including related species in the family Brassicaceae). These risks are poorly addressed by the applicant. Since there are numerous threats to biodiversity a full environmental impact assessment (ERA) is required so that these risks can be more clearly addressed and the local legislation (NEMA and Biodiversity Bill) adhered to. There is also a totally inadequate monitoring system that is proposed to monitor transgene escape- there is no proposal for testing of Canola plants or seeds in subsequent harvests for the RT73 transgenes, nor to monitor and assess the risks to biodiversity. This monitoring is required since South Africa is a signatory to the Cartagena Protocol on Biosafety and the Convention on Biological Diversity.

The application for trial release of RT73 Canola should be rejected.

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