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**Interrogating the Science of
Safety - Unknow Aspects of Bt
Toxin that continue to pose a
threat to the Health of Domestic
Animals in India**

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Introduction

Since 2005, shepherds and farmers from different parts of India- particularly from the states of Andhra Pradesh, Haryana, Karnataka and Maharashtra have reported that their animals (cattle, buffaloes, sheep and goat) that have grazed on genetically modified cotton or have been fed genetically modified cotton seeds or cotton seed cake have fallen sick and in some instances have died. Despite several reports and representations to concerned regulatory and research institutions both at national and state levels, alerting them to the seriousness of the issue, there has been a persistent reluctance amongst the scientific establishment to respond, investigate and research into the problem. On the contrary the reaction of the establishment has been bureaucratic, dismissive of the field and clinical observation of shepherds whose animals suffered, and of non-government veterinary scientists who have been tracking the problem, describing these as being “unscientific”, “exaggerated and blown out of proportion”, and not based on insufficient research and hard facts.

The Indian regulatory authorities such as the Genetic Engineering Approval Committee (GEAC) and top Indian research universities have exhibited incapacity to rigorously investigate the problems experienced and observed by farmers and shepherds, and instead consistently argue that because all safety tests in the “pre-commercialisation”, stage provided beyond doubt proof of safety of the technology, the GM toxin, simply could not be the cause of morbidity and mortality. To-date not one public research institution has undertaken to systematically investigate the problem at the farmers field.

In this paper, we critically highlight the numerous unanswered questions with respect to the effects of the Bt toxin on animals, which continue to be unaddressed. We discuss the absence of scientific rigour by regulatory authorities, in particular their circular arguments of safety, being cited as “evidence” that animal deaths were not caused by Bt toxin. The evidence of safety, would not stand any kind of international scientific scrutiny based as they are on incomplete testing / investigation protocols, compounded by admissions by top Indian research institutions of the absence of facilities to test for the effects of the toxin on animals, and citing company data on toxin-safety levels. The paper concludes arguing for the critical need for comprehensive risk-assessment and biosafety protocols, which are completely absent today from the required portfolios for GMO testing.

The case of Bt cotton and Animals in India: Warangal district, Andhra Pradesh, India

Grazing on harvested crop residues that remain on the fields, is a regular and common practice of livestock owners in villages across the length and breadth of India, including in Warangal district, AP. Cotton cultivation in Warangal district was minimal in the 1960s, and gradually increased in area, peaking in the mid-nineties replacing the dryland food crops which predominated the region. Livestock owners had no option but to graze their animals on harvested cotton fields, which began to dominate the landscape. They continued to do so for nearly 12 years, prior to the entry of Bt cotton. Not once did farmers or shepherds experience morbidity or mortality in their animals due to the effects of their animals grazing on cotton fields. Bt cotton was commercially released in Andhra Pradesh in March 2002, after the Government of India granted permission to Mahyco-Monsanto, to market its Bt cotton

India granted permission to Mahyco-Monsanto, to market its Bt cotton variety in South India. In the Kharif season of 2002 the company released two Bt cotton hybrids MECH Bt 12, and MECH Bt-162. It was sown in approximately 9500 acres in Andhra Pradesh, which stands third in cotton cultivation in the country, with an area of 8,87,000 ha under cotton. In Warangal district of Andhra Pradesh, approximately 1200 farmers planted Bt cotton over 1500 acres in Kharif 2002-03.

The first reports of morbidity / mortality in animals after grazing on Bt Cotton, occurred in **January 2005**, which coincided with the third year (2004-05) of Bt cotton in Warangal district. Between 2005 and 2009 Anthra¹ has been closely investigating reported morbidity and mortality observed in sheep and goat flocks, in select regions in the state, where shepherds specifically contacted the organization and in turn the organization was able to respond through fact finding teams to collect retrospective information, as also close- on site clinical observations. The detailing is not a comprehensive summary of events / reports from the entire state (Table 1, Table 2).

During the first 3 years of investigation, symptoms reported by shepherds, were confounded by the concurrent incidence of other common contagious diseases that affect small ruminants, such as peste-du petits ruminants (PPR) and blue tongue. By 2008-09 the symptoms could be isolated, due to the in-situ presence of Anthra's veterinary scientists who continuously monitored the village flocks, which we ensured were vaccinated / protected against all other possible preventable contagious diseases, and thus we were able to narrow down and be precise about the specific morbidity and mortality patterns exhibited by animals that grazed on harvested Bt cotton. Our clinical findings were:

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Our clinical observations were:

- a) Morbidity *selectively* manifests itself symptomatically in animals by the 3rd of 4th day of consuming Bt cotton foliage/ bolls and seeds as *nasal discharge, cough, respiratory distress, occasional bloody urine and the absence of fever*.
- b) Mortality occurs in some animals, especially if untreated, but not in all.
- c) Mortality and Morbidity are observed to occur in those animals that have had a cumulative exposure to the Bt toxin-in the form of grazing / being fed the cotton-seeds/ cottonseed cake, over a number of years.

In Haryana, there was a strong correlation between feeding Bt cotton seeds and cotton seed cake to milch animals, and drop in milk yield and several reproductive disorders such as prolapse of uterus, premature birth of calves, increase in the incidence of abortions and decrease in conception rate.

¹ Antha is an organization led by women veterinary scientists, works on issues related to livestock, peoples livelihoods and the environment and has been researching the impact of Bt cotton on animals in different parts of India

Table 1: 2005-2008: Shepherds observations on morbidity / mortality in flocks grazed on harvested Bt cotton fields

	2005 Jan	2006 Jan-Mar	2007 Jan-Feb	2008 Jan-Mar	2008-09 Dec- Jan
Location	1 district 3 villages 1 mandal	1 district 4 villages 3 mandals	2 districts Warangal-3 Mandal Adilabad-6 Mandals	2 districts Warangal -2 Man Medak-1 Man	Warangal 1 Mandal 1 Panchayat
History	Bt I	Grazed for 2-4 days on Bt I	Grazed for 3-4 days Bt I	Grazed for 3-4 days in Waran- gal BT 1 & 2 Grazed for 3 wks in Medak (1 st exposure)	Grazed for 3-4 days on Bt 1 and Bt 2
Species	cattle, goats, sheep	sheep goats	sheep, goats- Warangal sheep,goats, cattle-Adil	Sheep- Warangal Sheep- Medak	Sheep,Goat, Cattle
Sympt reported by shep- herds	Imprecise infor- mation	<i>dullness, cough, nasal discharge, reddish and erosive lesions in the mouth bloat, blackish diarrhoea occasional red coloured urine</i>	Sheep/Goats/Cattle <i>difficulty in breathing, nasal discharge, mucopuru- lent / blood tinged red coloured urine, bloat Eye / face swelling difficulty in standing occasional diarrhoea Vomiting, convulsion, salivation</i>	Medak- <i>Anorexia Nasal discharge, slight fever. 1 death PM performed</i> Warangal: <i>As above confounded with PPR symptoms</i>	
Vets Clinical observations		NA	As above in S/G- no fever As above in Cattle	As above in Medak Fever, anorexia, mouth lesions, frothing	<i>nasal discharge, cold, cough, respiratory distress (in some ca- ses) Occ. red urine</i>
Vaccines	No details	Not vaccinated against PPR, HS,	Not vaccinated against PPR , HS	Medak – HS, PPR, ET	HS, PPR, ET,

Table 2: Summary of Morbidity and Mortality in Animals as recorded by Anthra.

Year	District	Population	Morbidity	Mortality
2006 (March)	Warangal retrospective	8869 sheep	No information	1825
2007 (February)	Warangal (clinical ob- servations)	350 sheep	40	-
2007 (February)	Warangal (retrospective)	300 sheep		30
2008 (January)	Medak Vaccinated PPR	1000 sheep	3	1
2008 (January)	Warangal Not vaccinated	1000 sheep	40	
2009 (January)	Warangal Vaccinated PPR, HS, SP	7500 sheep 500 goats	31 (21 sheep, 8 goats, 2 cattle)	2 (1 sheep, 1 goat)

2. Investigating the problem: the response by the Research Institutes and Government Departments and the absence of Standardized Protocols of Testing

In 2006 and 2007, shepherds directly approached the local government veterinary doctors in government veterinary hospitals appealing to them to treat their animals, as also to advise them on what to do. In both years, the government veterinarians treated the sick animals with a combination of drugs that are typically used to treat cases of toxicity (Ramdas, 2009). The local Veterinary doctors after noting the history of feeding reportedly asked the shepherds to stop grazing their sheep on the harvested Bt cotton fields. The sheep were treated with a combination of drugs (Atropine, Dexamethasone, Pregneselone) which are commonly used to treat cases of toxicity. The government veterinarians carried out post mortems on the dead sheep and sent it to different government laboratories. In 2006 the samples were tested in state and national laboratories, and in 2007 samples were tested in state laboratories.

By 2008 and 2009, veterinary scientists from Anthra, were located directly with communities in select villages, and carried out post-mortems of dead sheep and goats that had died after grazing on Bt cotton, and sent tissue samples to top research institutions of the country such as the Indian Veterinary Research Institute (IVRI), with specific request that these be tested for Bt toxin. In 2008, *the IVRI reported their inability to test for Bt toxin (see annexure 1)*. The IVRI in 2008 and 2009 recorded histo-pathological lesions in the kidney (chronic nephrosis), liver (chronic hepatitis) and intestinal tissues(chronic enteritis) of the post-mortem sheep/goat, indicative of some kind of “chronic” factor at play.

In 2006, 2007, 2008 plant and animal tissue samples were sent for testing, and the results do not lend themselves to drawing any conclusions, as the information of the tests differed (table 3). Samples sent to different laboratories for the identical samples of plant, actually yielded completely different and contradictory sets of information². Except for 2008, where Bt toxicity testing was specifically requested and the institution specifically responded stating their inability to test as they lacked facilities. There is no information on whether animal tissues were tested for Bt toxicity in the previous two years.

Table 3: Contradictory Results of Testing

Abstract of Tests done: Bt cotton plant samples and animal tissue of animals that died after grazing on Bt cotton (2006-2009) and their implications				
Prepared by Anthra				
Year	Institution that conducted tests	Plant	Institution that conducted tests	Animal Tissue
2006 State and National	Andhra Pradesh Forensic Science Laboratory, Red Hills Western Regional Disease Diagnostic Laboratory, Pune Veterinary Biologicals Research Institution (VBRI, AHD, AP) Department of Agriculture Biotechnology, ANGRAU, Hyderabad	+ve <i>organophosphates</i> , +ve <i>Nitrates and Nitrites</i> -ve <i>HCN</i> +ve Nitrites + ve HCN <i>traces of pesticides</i> Bt protein in leaf and boll: 5 ppt "tolerable range- 5-10ppt"	Veterinary Biologicals Research Institution (VBRI, AHD, AP)	??? no information Not tested for Bt protein/ Bt antibodies
2007 State	Veterinary Biologicals Research Institution (VBRI) Animal Husbandry Department, AP	+ve HCN (VBRI) no report of Bt protein content	Veterinary Biologicals Research Institution (VBRI)	rumen content and spleen and lung -ve HCN Not tested for Bt protein/ Bt antibodies
2008 National	Indian Veterinary Research Institution (IVRI), Izzatnagar. (Bt Cotton samples grazed by the dead sheep in Medak district, AP)	Pods: +ve: saponin, -ve: nitrates and alkaloids Plant: -ve: saponin, nitrates, Nitrites, alkaloids Leaves: +ve: nitrate / nitrite -ve: saponins and alkaloids no mention of Bt protein levels	i) Indian Veterinary Research Institution (IVRI), Izzatnagar 1 dead sheep (Medak district, Andhra Pradesh) ii) Swab samples collected from 3 sheep in Warangal , AP (VBRI)	All Toxicology tests are negative: -ve : phosphine, nitrate/ nitrite, alkaloid, heavy metals, organochlorine /organophosphate are negative Chronic Hepatitis Lack facilities to test for Bt toxin ii) Tested only for "Blue tongue"- result is negative No other tests conducted

The chronic nature of the tissue lesions should have alerted scientists within these research institutions, as to what could possibly be causing this chronic effect? It matched with the history of mortality in those animals that had been exposed to the Bt toxin over an extended

² Letter sent to the GEAC by the Director, Animal Husbandry Department (AHD), Andhra Pradesh, dated May 2007 ref: No 3531/Epid/2006.dated 9/5/2007.

period of time. The admission of lack of facilities to test, coupled with contradictory information emerging from different laboratories should have alerted the scientific establishment, to the urgency and need to systematically research into the problem observed by the shepherds. It also raises several unanswered questions regarding the testing protocols used:

- Did these laboratories test the post-mortem animal tissue samples?
- What was the entire list of test protocols followed by each of the laboratories for animal tissue and plant tissue alike to arrive at a cause of death?
- If we assume it was the identical plant samples sent to all laboratories what explains the different findings?
- The presence of a mineral (eg nitrate/ nitrite/ organophosphate etc) in the plant sample is completely insufficient evidence to deduce / derive that this mineral was the cause of animal death.
- Were animal tissue samples tested for Bt toxin or Bt immune response. Going by IVRI's response of 2008, these facilities do not exist at these state and national level "disease investigation laboratories.", leave aside district level investigative labs.

One of the test results, which warranted further inquiry, was the report that the Bt toxin found in the plant samples were within "**safe and tolerable levels of Bt toxin**"- "*The Bt protein levels detected in the samples of Bt cotton bolls and leaves sent for analysis was recorded as 5 μ /gm. This level is within the tolerable range which is said to be "5-10 μ /gm.³*

In a subsequent RTI filed to the Department of Agriculture Biotechnology by Anthra, to obtain the source of this "tolerable" range, the department responded that this was Mahyco's data of safety, accessible on the official IGMORIS website⁴. They also mentioned that the Bt cotton had been well tested, including testing of foliage, which is a blatant piece of misinformation, as the protocols of testing were on cotton seeds/ cotton seed cake and not foliage. Hence what we have is the public sector research institutions citing company data as proof of safety, once again exhibiting complete scientific incompetency in conducting their own research and evolving their own protocols. From 2006 onwards, Anthra and other Civil Society organizations, submitted their observations, fact finding reports and concerns regarding the test results to the Genetic Engineering Approval Committee (GEAC), Veterinary Universities and Research Institutions, as also the Department of Animal Husbandry. In response to repeated submissions by civil society organizations, the Department of Animal Husbandry also sent their letters of concern to the GEAC.

3. Response by the Regulatory Authorities and Circular Arguments of Safety provided by them as evidence/ proof of safety.

In response to the reports received from civil society organisations regarding mortality in sheep flocks after grazing on Bt Cotton field at Warangal Andhra Pradesh, the GEAC records in the minutes of its 78th meeting held on 22.6.2007, that the committee deliberated at length and arrived at a *general opinion that the report was highly exaggerated and is based more on hearsay than scientific facts*. The committee cited various feeding studies conducted as evidence and proof of safety. They recommended that the Department of Biotechnology (DBT) sponsor a study to assess the problem at Warangal District with the help of the local

³ letter roc no: 14627/Epid/2006/, dated 20/9/2006.

⁴ ABT/ Let No 427 dated Dec 15th 2009

Veterinary Hospital in the district. *The Committee also agreed that, in future, leaf toxicity studies need to be included as part of the bio-safety studies.* The Committee further decided to refer the matter to the State Department of Agriculture for a factual report on the allegation made by the NGOs and the findings of the post mortem report. Exactly 6 months later on 11/1/2008, the GEAC committee in their 82nd GEAC Committee meeting, reversed their decision to have additional animals feeding studies on new events, as taken in the 78th GEAC meeting citing that they (GEAC) had received foolproof evidence that the death of sheep was due to other causes and not Bt toxin. They state in the minutes that the proof of this was analytical reports that the GEAC received from The IVRI and Department of Animal Husbandry, Andhra Pradesh wherein the latter confirmed that the sheep death could not have been due to Bt cotton. The minutes record that a representative of the State Department of Agriculture in the sub-committee meeting held on 11/1/2008.

Anthra decided to access this evidence of safety. It first filed an RTI dated 5.2. 2008 to the IVRI requesting a copy of their “Submission of Safety” to the GEAC. The IVRI responded on 25th February 2008, stating “*no studies have been done by them and that the Animal Nutrition Department of IVRI has not submitted any reports to the GEAC*”

Anthra then filed an RTI to the GEAC dated 5.3.2008, requesting them for the dossier of safety. Their Dossier of Safety sent to Anthra by GEAC, consisted of 4 letters:

- a) A letter from the Department of Animal Husbandry, AP to the GEAC⁵
- b) A letter from the IVRI to the GEAC
- c) A letter from the Sri Venkateshwara Veterinary University (SVVU), AP
- d) A letter from a Joint Director (AHD) of a district (Ranga Reddy) in Andhra Pradesh, which never reported any sheep deaths.

In the first “proof “, the then Director, Department of Animal Husbandry, Andhra Pradesh stated that Bt cotton samples were sent to 4 different laboratories and (abstracted in Table 3) based on these above reports, concludes that the death of sheep might be due to to high content of Nitrate/ Nitrite/ HCN / Organophosphates. The results from each laboratory contradict the other (Table 3). Bt cotton sample is negative for HCN in results from Western Regional Disease Diagnostic Lab, and positive for HCN in VBRI, Hyderabad. Bt cotton sample is positive for Nitrite in VBRI results, and positive for Organophosphate in the AP Forensic Science Lab, Hyderabad, results. Further, the presence or absence of a mineral deposit in a plant sample does not automatically translate into that being the cause of death in the animal. In addition, *three of the four letters clearly stated the need for further bio-safety studies*

Anthra filed another RTI to IVRI (dated 20th March 2008) requesting IVRI to send us the copy of a “Bt Cotton feeding study on goats”, which they have mentioned in their letter to the GEAC, which GEAC produces as the “evidence of safety”. This resulted in a response from IVRI dated April 26th 2008, sending copies of research protocol and methodology to test for HCN, glyphosate, alkaloids, nitrites and nitrates. They also sent a report of toxicity assessment of feeding Bt cottonseeds to rats; but no studies on goats, which was specifically asked for.

It is critical to note here, that there appears to be direct factual conflict between the first and subsequent response from IVRI. One of these is simply not truthful. Not one of these “proofs” can even remotely be construed as “rigorous testing to research the field problem to arrive at a conclusive result”.

The GEAC consistently referred to the “tolerable” range of Bt protein as evidence that death in animals was due to Nitrate/ Nitrite / organophosphates/other diseases. Apart from the fact

⁵ Letter Roc. No. 3531/Epid/2006 Dated 9/5/2007

that the department was citing Company data, there was absolutely no logic to this value, which was borne out by another set of “safe toxic levels”, cited by the GEAC appointed Expert Committee II’s, report on Bt Brinjal. In this report (point 3.1.5), the expert committee describes the level of Bt protein (Cry1Ac protein) found in different parts of the crop to vary between 5 to 47 ppm in shoots and fruits. *For the sake of argument, if we are to go by the earlier submission of all institutions concerned (Agriculture university, cited by Animal Husbandry Department, cited by GEAC) that the reports of Bt toxin (Cry 1 AC protein) are safe and tolerable if they are between 5-10 ppm then it follows that the levels detected in Bt brinjal reported in the biosafety studies and Expert Committee Report, are not tolerable, as it is way above the supposed tolerable levels, which are cited as being safe for sheep!*

This raises serious questions on supposed “tolerable” and safe levels of Bt toxin in plants. Who has decided on this supposed safe level for Bt toxin? What is the scientific evidence for safety? How can there be a safe level of “toxin” with a food product, when the very definition of a “toxin” indicates a poison, or something that is harmful?

What is of serious concern is that in the name of evidence based decisions of safety, we have instead, clear evidence of deception and fraud on the part of all the regulatory bodies in India, and the complete duplicity of passing of circular arguments of safety as “fool proof evidence of safety”:

- a) The absence of protocols to test for prove/ disprove the role of Bt toxin, results in the non –testing for the toxin/ immune response.
- b) The non-testing results in “non-detection” and a negative result.
- c) The negative result of having not detected Bt toxin” is passed off as proof of safety.

It is scientifically untenable that without performing any tests, its absence is cited as evidence that the toxin is safe. This circular argument of “safety” is the basis on which the GEAC claims that reports of animal deaths are “unsubstantiated”, and reversed its decision to carry out further risk assessment tests on goats, as cited earlier.

Regrettably the above “laboratory reports” are the scientific evidence of “safety of the toxin for animals” which have been included in the letter of Shri Prithvi Raj Chauhan, the then Minister of State (Independent Charge) for Science & Technology and Earth Science to Dr Ramadoss former, the then Minister for Health & Family Welfare, to instill confidence in the technology and to disprove any risks involved⁶.

Subsequent studies on the effects of Bt toxin on Sheep, were carried in 2007 by a Veterinary University in Andhra Pradesh, and in 2008 by the Central Sheep and Wool Research Institute. Whilst neither of the studies were designed keeping in mind the field observations of the how symptoms were observed in sheep which had been repeatedly exposed to Bt toxin, and were one time studies, and the researchers chose conclude that no untoward impacts/ effects were detected, there were some troubling results, which raised several unanswered questions:

The 2007, season-long study by the Sri Venkateshwara Veterinary University found:

- i) The presence of higher toxic heavy metals in Bt plants (842.25 ppm of lead in Bt cotton as compared to 134.62 ppm of lead in non-Bt cotton after 45 days), which is 6.25 times higher after 45 days, as compared to the non-Bt cotton⁷.

⁶www.indiagminfo.org

⁷ Studies on the toxicity of Bt cotton plants incorporated in the feed of small ruminants”. Project Report. Sri Venkateshara Veterinary University, Tirupati page 27, Table 18

- ii) The liver marker AST which is known to increase after hepato-cellular injury, as the author of the experiment indicates, increased in the protocol by 37% in Bt treated sheep in comparison to the untreated group of sheep fed on regular cotton, by the second month⁸

The feeding trial study carried out by CSWRI in 2008 designed for only a 3 month period, with a first time exposure of animals to Bt toxin, detected a higher liver weight, testicular weight and fat deposits in sheep fed on Bt diet. These results only served to re-inforce the urgent need for more systematic, comprehensive long-term studies on the effects of GMOs on animals.

In Conclusion

It is evident from the above that there is much to worry about.

The inconclusive nature of BT toxin in Bt cotton and its impact on animals continue to remain. Is Bt toxin acting as a stress factor that is eliciting in a select fashion, a morbid possible allergenic response in the sheep, goats, and other animals, manifested as cold cough, nasal discharge in animals? Is the intense stress a trigger for *Pasteurella haemolytica*, in some of the animals with resultant death? Is the stress factor Bt toxin? Is it some unknown / new toxin? Is it a new allergenic protein? Is it macro / micro mineral imbalances in the Bt cotton plant, (eg excess or deficiency of Nitrate, Nitrite, Selenium, etc) as a result of the Bt protein, which elicits a response from the animal?

There are obvious lies and a host of contradictions within the “safety” parameters being presented to us citizens by those who are “regulating” the technology. There is clearly total failure and inability of our existing public research institutions and National Regulatory Bodies (GEAC), to investigate/ test/ rigorously examine, prove or disprove these field observations, preferring to dismiss the reports as “unsubstantiated”, “exaggerated, and unscientific”, refusing to conduct a single field-based study and instead placing the onus of “proof” on shepherds, farmers and civil society groups who have reported the problem. The argument that the latest guidelines do not require the suggested new risk assessments tests and hence have been dispensed with, negate and ignore the field realities where “non-target organisms” have been affected by the Bt toxin. On the contrary, these unique field experiences and observations, urgently invite new and additional specific regulatory and risk assessment protocols.

There is a clear need for new risk assessment and bio-safety protocols: chronic and long-term toxicity and allergenic tests and inter-generational studies so as to deepen our understanding on long-term implications for human and animal health to understand the unattended effects which often only come to light after several years of exposure of the organism to the GM technology (in this specific case GM cotton containing Bt toxin). There is a clear need to put into place a reliable and citizen accountable Regulatory and Monitoring mechanism, that will respond to problems when they are experienced, and not ignore / dismiss. There is an urgent need that India sets up independent labs for testing which are fully functional and certified to international standards. These must be capable of conducting all the required tests. Standardized and exhaustive testing protocols. India institutes capability/capacity for independent oversight, outside of the Regulatory mechanism that is free of

⁸ Studies on the toxicity of Bt cotton plants incorporated in the feed of small ruminants”. Project Report. Sri Venkateshara Veterinary University, Tirupati page 20, Table 20

both government and bureaucratic / corporations involvement. Until such time that these can be carried out, it is absolutely necessary that we invoke the precautionary principle and impose a moratorium on all further open field trials of GMO crop, until safety is proved without doubt.

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