Journal of Scientific & Industrial Research
Vol. 62, June 2003, pp 554-572

The Rules For Handling Transgenic Organisms And Biopharmaceuticals Produced Therefrom In India*

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Received : 25 September 2002; accepted: 06 March 2003

The Indian government created and promulgated the rules and procedures for dealing with genetically modified organisms and products thereof in 1989 and these rules were enacted under the Indian Environment (Protection) Act (EPA) 1986. In order to ease the enactment of the rules, the recombinant DNA (rDNA) safety guidelines were made subsequently by the Indian government from the Department of Biotechnology in 1990, which was revised twice incorporating the latest revision up to September 1999. The rules also define administrative structures and procedures for handling all kinds of rDNA products and substances. Within the rules, certain rDNA microorganisms have been authorized for use for the production of therapeutic bioactive proteins. Currently, four rDNA products are being produced locally and 12 numbers are consumed in the market. Indian market is fed by local production as well as by imports. The first transgenic plant, insect resistant cotton, was authorized for commercialization in 2002. The use of GMOs and products thereof require generation of environmental safety as well as food safety data under the Indian EPA. Familiarity with the rules and guidelines enables entrepreneurs to facilitate the market introduction process. The paper describes the information needs, the administrative structures and the step-by-step procedures required to be followed under the rules by entrepreneurs for introducing GMOs and products thereof in India market.

Key words : Transgenic organisms, Biopharmaceuticals, rDNA, GMOs, GMO handling

Introduction

Biotechnology encompasses techniques applied to organisms or parts thereof to produce, identify or design substances, or to modify organisms for specific applications. Cell fusion techniques, hybridomas, recombinant DNA (rDNA) technology for rDNA vaccines and Bioactive proteins, protein engineering and structure based molecular design are considered as modern biotechnology. Emanating from the above, the structural and functional genomics complemented with computer-aided informatics and biochips are making fast inroads into the frontiers of modern biotechnology. Differentiated stem cell proliferation and animal cloning techniques are developing very fast. In addition, several technology platforms that have emanated from increased understanding of signal transduction pathways of cells and tissues, cell based immune rejections, tissue engineering, proteomics, bio-informatics including bio-chips and genomic sequencing of organisms; all these areas hold great promise for providing new biotech products that would have wide applications. Conventional biotechnology includes fermentation or conversion of substrates into desired products by biological processes; downstream processing for recovery of metabolites; use of microbes or enzymes for producing value added products; sera, vaccines and diagnostics produced by conventional methods; reproduction, artificial insemination and embryo transfer technology for animal breeding; methods for fish spawning induction; plant cell or tissue culture; plant breeding for producing better seeds or plants cultivars; bio-fertilizers; bio-pesticides; plant growth stimulants; extraction, and isolation of active principles from plants or animals or parts thereof, etc.

World over, no sector of industrial activity was specifically designated as biotechnology industry *per se* before the early 70s although conventional fermentation based industry was at its peak, especially

* Views expressed in the paper are those of the author and they do not necessarily express the views of the organization to which the author belongs.

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in the production of antibiotics, enzymes, fermented beverages, certain organic chemicals, and bio-chemicals. Viral and bacterial vaccines were also produced by multiplying the target microorganisms (viruses) in specific pathogen free eggs or in certain safe cell lines or in defined biological media. The products were used as such or after inactivation. This scenario fast altered with the rapid advances in genetics, microbiology and immunology at the molecular level. The techniques of splicing and recombination of nucleotide sequences at specific sites, the discovery of vectors for transporting trans-nucleotide sequences to organisms and stably integrating composite cassettes comprising promoters, genes, enhancers, markers, and terminators into the chromosomes or through shuttle vectors, thereby enabling the expression of transgenes into unrelated hosts brought revolution to the understanding of biology of organisms. The Polymerase Chain Reaction and the accompanying knowledge about the methods of magnification of nucleotide sequences enabled the production of large quantities of hereditary materials at ease. The combined effects of these developments resulted in the birth of modern biotechnology industry which started during the mid seventies and spanned up to the late 90s at a hurricane speed by the production of a large number of highly potent bioactive proteins including hormones, cytokines, thrombolytic agents, growth promoters, and regulatory molecules of several vital physiological functions that revolutionized human therapy. In agriculture, transgenic plants emerged that were resistant to certain viral and some other microbial diseases. Plants were produced that were resistant to herbicides. Certain transgenic plants were developed that had improved traits like enhancement in vitamins or precursors, enrichment in certain amino acids or reduction in unwanted metabolites. This situation continues to unfold newer frontiers of knowledge and technology in pharmaceuticals, agriculture, bio-chemicals, foodstuffs, and environment management issues. Presently the advancements in stem cell research, production of biochips to understand genomics and proteomics, advances in bio-informatics, xeno-transplantations and organ/tissue revival methods in pharmaceuticals are bringing about newer hopes of prolonging healthier lives with enhanced longevity in human. Transgenic plants also hold enormous potential for increasing productivity and reducing production costs in agriculture.

The upstream capabilities acquired by human kind in modern biotechnology have brought in newer questions of safety with reference to the environment as well as human and animal health. These have resulted in the emergence of newer regulatory laws and closer scrutiny of the genetically modified organisms/substances on a case-by-case basis. The consequent requirement of testing for approvals from regulatory authorities, marketing methods and production techniques have also undergone a sea change the world over. The developing countries are often not able to keep pace with the advancements and technological changes required to be acquired and established within their territory when compared with the progress in the developed nations. Modern biotechnology industry is thus causing the creation of a wide divergence in the technological skills and consequently economic disparity between the developed and the developing nations. The developing countries can bridge these gaps considerably if they concentrate on generating and updating human skills, install sophisticated infrastructure for biotech research, and deliberately channel efforts in specific need-based economic activities in their territories. In addition, training of personnel abroad and creating high culture of alliance with industries within and outside their territory along with pronouncing policies for promoting local industrial development may help to salvage the growing gap.

Consumption Scenario of Biotech Products in India

India has been practising conventional biotechnology for several decades. Products manufactured by the use of genetic engineering, immunological techniques, cell culture methods and hybridoma technology are increasingly being used during the past 10 yrs and local research in these areas has been intensified. Table -1 gives the current consumption and future demand of biotech products in India[1].

Modern biotechnology is rather new to India. The current consumption of modern biotech products is about Rs 5 billion, of which only about Rs 1.4 billion worth of products are produced locally. In 2001, India was producing three recombinant DNA products from basic stage using its own technology; these were hepatitis B vaccine, erythropoietin and G-CSF (Granulocite Colony Stimulating Factor). In

Table I (Past consumption of biotech products in India and future consumption estimates)
(Rupees in Million)

Particulars of biotech sub-sectors	Actual consumption 2000		Future consumption estimate 2005		Future consumption estimate 2010	
1 Human & animal health care products	32700	(37.5)	35320	(37.6)	93540	(40.0)
2 Agriculture (including traded seeds)	26200	(30.0)	28880	(30.7)	78720	(33.7)
3 Industrial products	27320	(31.3)	28500	(30.3)	53590	(22.9)
4 Other biotech products	1080	(1.2)	1300	(01.4)	7940	(3.4)
Total	87300	(100)	94000	(100)	233790	(100)
In million US dollars	**1850**		**2186**		**4270**	

(Figures in brackets indicate contributions in percentage of the total)

2002, India has introduced another one product, namely interferon alpha and may introduce another one recombinant DNA product in the health care sector namely human insulin. In agriculture, genetically modified insect resistant hybrid cotton seeds were approved and introduced in the country in early 2002. More products would be introduced in near future. There is little doubt that the production and consumption of modern biotech products would increase very fast, as such products hold great potential for providing much better solutions to improve the health of people or the quality of life, improve agricultural productivity significantly along with supplying more nutritious food, produce industrial bio-products at much cheaper prices and improve the quality of environment more effectively on a sustainable basis.

Why Regulations are Necessary for Using GMOs and Products Thereof?

It is expected that the Genetically Modified Organisms (GMOs) and products thereof will play an important role in the economic uplifting of the country in its various facets of applications including human and animal health care system, agriculture, industrial products, and environment management. Concurrently, it is also realized that there could be unintended hazards and risks from the use of GMOs and products thereof, if the new technology was not properly assessed before use[3,13]. A gene construct comprising a host-compatible-promoter, a gene of interest and a terminator sequence or a polyadenylation sequence is integrated in a stable manner into the genome of the organism / cell line for the target gene to be expressed and stably inherited. A GM organism can be safe but this can be unsafe too. This will depend upon the trans-genes, the host organism and the environment where the GMO is being tested. GMOs can be microorganisms, plants, and animals. Products made from GMOs can have contaminants, such as transgenic nucleotides sequences, transgenic proteins and lipids, which may come from the host organisms and may remain embedded within the finished products. Finished products containing unwanted materials may create adverse reactions when used. This is particularly the concern when the products are used as pharmaceuticals or food. Biopharmaceutical products produced by recombinant DNA technology are usually used by injectable route where trans-nucleotides sequences entering into cells could express or get themselves integrated. Consequently the concern of safety becomes more pronounced. Therefore, a case-by-case analysis of the safety of each GMOs and products thereof need to be conducted to assess their safety. Whenever GMOs are released into the open environment they require to be evaluated from environmental safety as well as from safety to human and animals. Keeping these in view the Indian Government had enacted the Environment (Protection) Act[2] in 1986 and thereafter, notified Rules & Procedures (Rules) for handling GMOs and hazardous organisms through a Gazette Notification No. G.S.R. 1037(E) dated 5.12.1989 from the Union Ministry of Environment & Forests[3]. The Rules cover all kinds of GMOs and products thereof, which are

controlled commodities for handling and use in the country under the EPA. Guidelines have thereafter been framed in order to facilitate the enactment of the Rules[4-6]. The Rules and the Guidelines apply concurrently to genetically modified organisms and the products derived or produced there from.

Indian EPA Implementation Structure for GMOs

The Indian EPA and Rules define Competent Authorities and compositions of such Authorities for the handling of all aspects of GMOs and products thereof. The GMOs include microorganisms, plants and animals. The products produced from GMOs are also covered under the Rules. Presently, there are six competent authorities under the Rules. The Rules define the broad responsibilities and authorities of the competent authorities [3]. There are 20 para in the Rules 1989. Different paras deals with different aspects of the Rules. Each para can also be designated by suffixing the number of the para and by prefixing the word Rule for identifying them. These rules need to be familiar to people dealing with GMOs and products thereof. Their applicability as well as implications has been discussed by the author elsewhere, especially in the context of transgenic crops[7-9]. The Rules, Authorities and Administrative structures are depicted in figure 1.

Indian Scenario on Transgenic Research in GMOs and Products Thereof

Microorganisms

Efforts are being made to construct transgenic microorganisms that code for bioactive therapeutic proteins. Transformed E.coli coding for several recombinant proteins, such as interferons, interleukins, human growth hormone, bovine growth hormone, granulocyte colony stimulating factors, human pro-insulin, human epidermal growth factor, streptokinase, and recombinant hepatitis B vaccine are being experimented upon in different laboratories in the country. Transformed *Pichia pastoris* has been used for commercial production of recombinant interferon alpha 2b in the country. Other recombinant yeast species of Saccharomyces cerevisae and Hanseneulla polymorpha have been modified to code for specific therapeutic proteins Hepatitis B surface antigen gene has been coded in these yeast organisms and the antigen has been isolated and formulated into dosages form for use as vaccines to protect human against Hepatitis B. Such vaccines are being commercially made in this country. These products have been tested for safety under the Indian EPA and these have been found to be safe. Genetically modified cholera microorganism is being evaluated for its safety as well as efficacy; a non-virulent strain of *Vibrio cholera* was isolated from natural sources and an immunogenic gene coding for a non toxic protein was incorporated into it to make the strain responsible for eliciting immunogenic response and for producing antibodies to it. Genetically modified fungi belonging to *Aspergillus spp* are being experimented upon for the production of value added enzymes. All such microorganisms are also tested for environmental safety (in case of accidental release in to the open environment) as well as for human safety under the Indian EPA.

There are several recombinant products that are being marketed[10] in India including a few produced locally as stated earlier. Table 2 provides the necessary details.

Plants

The first transgenic plant experiment in the field was started in 1995 when *Brassica juncea* plants containing *Bar* gene regulated with plant specific constitutive promoters and linked with *Barnase and Barstar* genes regulated with floral tissue specific promoters *were* planted at Gurgaon (Haryana), India, under contained conditions. These studies were conducted to assess the extent of pollen escape[8,9]. Indian results of pollen escape studies were consistent with reported earlier studies[11]. Subsequently, several experiments have been started in India in the field in different locations using transgenic mustard, cotton and tomato. Several Indian institutes and organizations have claimed to develop transgenic plants, which are ready for green house/ poly-house evaluation and some are ready for field evaluation as well[7].

Animals

Work on transgenic animals carried out in India is yet at rudimentary stage. Lab experiments have been conducted to produce transgenic mice containing different kinds of genes including growth hormone genes. Certain marker genes have also been utilized. Genes that code for substances that produce luminescence were introduced in silkworms and glowing insects were developed at IISc Bangalore; this work only reinstated that these organisms are

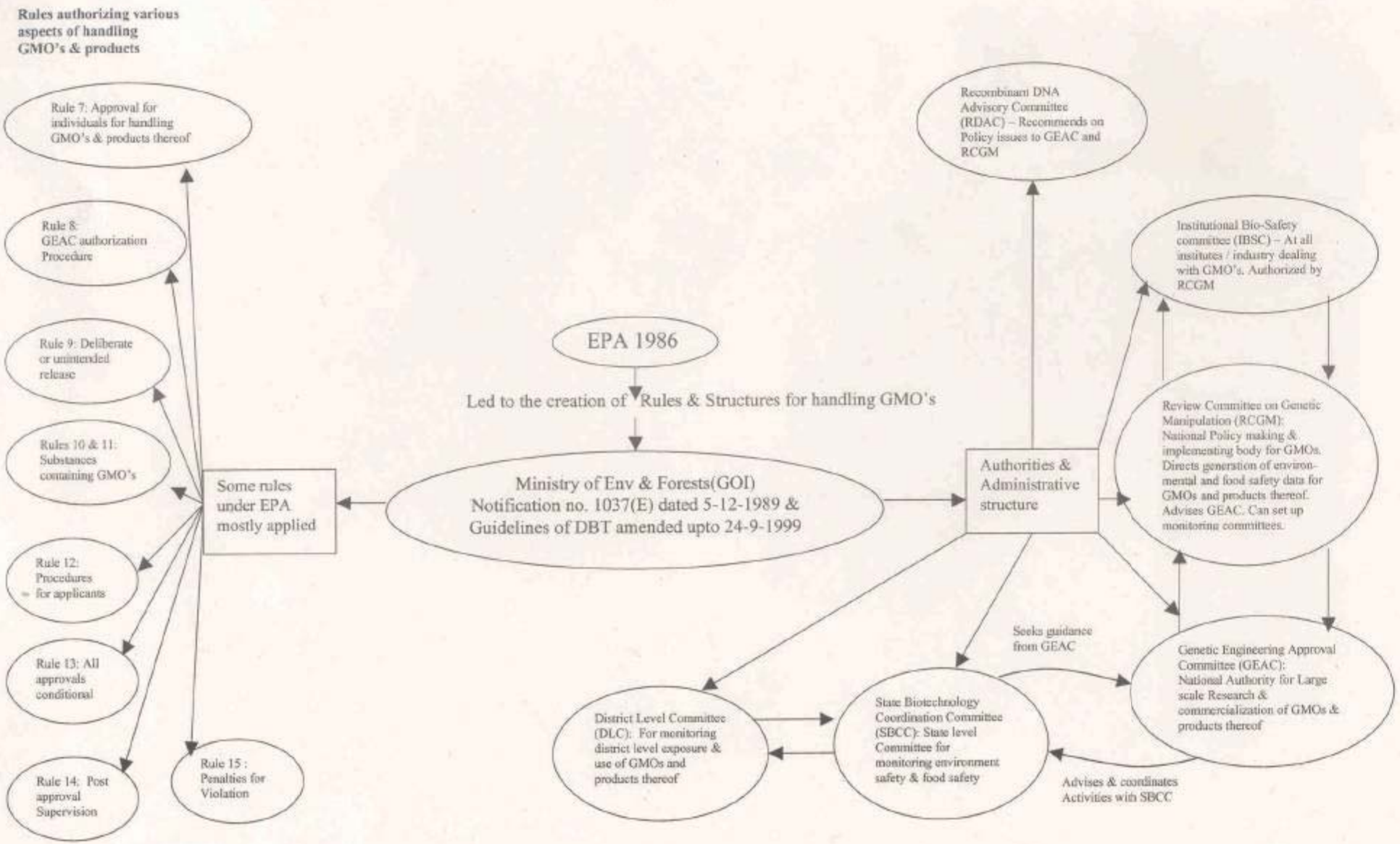


Figure 1 – The rules, and administrative structures for dealing with GMOs and products thereof

Table 2: Recombinant DNA drugs available in Indian market

Sr. No.	Brand	Company	Strength	MRP (in Rs.)
1. Human Insulin				
(i)	Huminsulin-R	Eli Lily & Co.	10 ml, 40 i.u./ml	206.00
			10 ml, 100 i.u./ml	398.00
			5X3ml cartridge, 100 i.u./ml	990.00
(ii)	Huminsulin-N	Eli Lily & Co.	10 ml, 40 i.u./ml	209.00
			5X3ml cartridge, 100 i.u./ml	990.00
(iii)	Huminsulin-L	Eli Lily & Co.	10 ml, 40 i.u./ml	206.00
(iv)	Huminsulin-U	Eli Lily & Co	10 ml, 40 i.u./ml	206.00
(v)	Huminsulin 30:70	Eli Lily & Co	10 ml, 40 i.u./ml	209.00
			10 ml, 100 i.u./ml	398.00
			5X3ml cartridge, 100 i.u./ml	990.00
(vi)	Huminsulin 50:50	Eli Lily & Co	10 ml, 40 i.u./ml	206.00
(vii)	Humalog	Eli Lily & Co	10 ml, 40 i.u./ml	375.00
			5X3ml cartridge, 100 i.u./ml	1920.00
(viii)	Actrapid HM Penfill	Novo Nordisk	3ml cartridge, 100 i.u./ml	993.69
(ix)	Actrapid Novolet	Novo Nordisk	3X5 ml (pack of 5)	124.71
(x)	Human Actrapid	Novo Nordisk	10 ml, 40 i.u./ml	197.56
			10 ml, 100 i.u./ml	508.15
(xi)	Human Insulatard	Novo Nordisk	10 ml, 40 i.u./ml	195.69
(xii)	Insulatard HM Penfill	Novo Nordisk	5X3 ml cartridge	991.50
(xiii)	Human Monotard	Novo Nordisk	10 ml, 40 i.u./ml	197.52
			10 ml, 100 i.u./ml	508.15
(xiv)	Human Mixtard	Novo Nordisk	10 ml, 40 i.u./ml	197.52
			10 ml, 100 i.u./ml	508.15
(xv)	Human Mixtard 30	Novo Nordisk	10 ml, 100 i.u./ml	508.15
(xvi)	Human Mixtard 50	Novo Nordisk	10 ml, 40 i.u./ml	197.62
			10 ml, 100 i.u./ml	517.35
(xvii)	Mixtard 30 HM Penfill	Novo Nordisk	3 ml penfill 100i.u./ml	834.00
(xviii)	Mixtard 50 HM Penfill	Novo Nordisk	3 ml cartridge 100 i.u./ml	993.69
(xix)	Human Fastact	USV	10 ml, 100 i.u./ml	199.24
(xx)	Human Longact	USV	10 ml, 100 i.u./ml	200.94
(xxi)	Huma- Mixact	USV	10 ml, 100 i.u./ml	199.46
(xxii)	Human Rapidica	Sarabhai	10 ml 40 i.u./ml	189.84
(xxiii)	Human Prodica	Sarabhai	10 ml 40 i.u./ml	190.04
(xxiv)	Human Zinulin	Sarabhai	10 ml 40 i.u./ml	189.84
(xxv)	Human Rapimix	Sarabhai	10 ml 40 i.u./ml	190.02
(xxvi)	Insuman Rapid	HMR	10 ml 40 i.u./ml	217.68
(xxvii)	Insuman Basal	HMR	10 ml 40 i.u./ml	217.68
2. Erythropoietin				
(i)	Eprex	Ethnor	1 ml – 2000 iu/ml	890.00
			1 ml – 4000 iu/ml	1690.00
(ii)	Hemax	Hindustan Antibiotic	1 ml – 2000 iu	882.00
			1 ml – 4000 iu	1750.00
(iii)	Epox	WockHardt Merind	0.5 ml 2000 iu/ml	798.00
			1 ml 4000 iu/ml	1550.00
(iv)	Zyrop	Zydus Biogen	2 ml – 2000 K	998.91
			2 ml – 4000 K	1698.00
(v)	Vintor	Emcure	Vial – 2000 iu/ml	550.00
			Vial – 4000 iu/ml	1090.00

- *Contd*

Table 2 - Recombinant DNA drugs available in Indian market – *(Contd)*

Sr.No.	Brand	Company	Strength	MRP (in Rs.)
3. Recombinant hepatitis B vaccine				
(i)	Bevac	Biological E	0.5 ml inj. 10 mcg	100.00
			1 ml inj. 20 mcg	150.00
			10 ml Multidose V	1500.00
(ii)	Biovac B	Wockhardt	0.5 ml inj. 10 mcg	140.00
			1 ml inj. 20 mcg	190.00
			10 ml Multidose V	1750.00
(iii)	Engerix B	SmithBeecham	0.5 ml inj. 10 mcg	272.00
			1 ml inj. 20 mcg	485.00
			10 ml Multidose V	2880.00
(iv)	Enivac HB	Panacea	0.5 ml inj. 10 mcg	100.00
			1 ml inj. 20 mcg	170.00
			10 ml Multidose V	1250.00
(v)	Genevac B	Serum Institute	0.5 ml Vial 10 mcg	90.00
			1 ml Vial 20 mcg	160.00
			5 ml Vial 20 mcg/ml	725.00
			10 ml Vial 20 mcg/ml	1400.00
(vi)	HB Vac	Zydus Vaccine	0.5 ml inj. 10 mcg	195.00
			1 ml inj. 20 mcg	295.00
			10 ml V	2585.00
(vii)	Hepagen Plus	V H B Pharma	0.5 ml Vial 10 mcg	97.00
			1 ml Vial 20 mcg	178.00
			5 ml Vial 100 mcg	834.00
(viii)	Hepashield	Pfizer	0.5 ml inj. 10 mcg	150.00
			1 ml inj. 20 mcg	222.00
			10 ml V 20 mcg/ml	1925.00
(ix)	Hepavax-B	V H B Pharma	0.5 ml inj. 5 mcg	105.00
			1 ml inj. 10 mcg	148.00
			1 ml inj. 20 mcg	198.00
			10 ml V 100 mcg	834.00
(x)	Revac B	Intas	0.5 ml inj.	160.00
			1 ml inj.	230.00
			10 ml V.	2100.00
(xi)	Shanvac B	Shantha Biotech	0.5 ml inj.	140.00
			1 ml inj.	190.00
			10 ml V (Multidose)	1800.00
4. Human Growth Hormone				
(i)	Humatrope	Eli Lilly Ranbaxy		1500.00
				6000.00
				7500.00
				15000.00
(ii)	Norditropin	Novo Nordisk	4 i.u. Vial	1775.00
			12 i.u. Vial	5415.00
(iii)	Saizen	Serum International	4 i.u. Amp	2249.50
			10 i.u. Amp	4750.00
5. Human Interleukin				
(i)	Human interleukin	Ambala Sarabhai	N.A	N.A.
6. Granulocyte Colony Stimulating Factor				
(i)	Neupogen	Piramal Health Care	30 million i.u. Vial	5163.93
2	Grastim	Dr .Reddy's Lab	30 million i.u. Vial	2400.00

-Contd

Table 2 – Recombinant DNA drugs available in Indian Market – (Contd.)

Sr. No.	Brand	Company	Strength	MRP (in Rs.)
7. Granulocyte Macrophagecolony Stimulating Factor				
(i)	Leucomax	Novartis	150 mcg	2650.00
8. Interferon Alpha				
(i)	Alferon	Zydus	3 million i.u. Vial	1190.00
(ii)	Inron Alpha Inj.	Biological E	3 million i.u. inj.	970.00
(iii)	Roferon-A	Piramal Health Care	3 million i.u. Vial	1361.00
(iv)	Shanferon	Shanta Biotech	3 million i.u. Vial	N.A.
9. Interferon Gamma				
(i)	γ-Interferon	Nicholas Piramal	N.A.	N.A.
10. Blood Factor Viii				
(i)	Blood Factor VIII	Hemophila Federation of India	Kogenate	N.A.
11. Follolicle Stimulating Hormone				
(i)	Endogen	Uni Sankyo	Vial 75 i.u.	760.00
(ii)	Endogen	Uni Sankyo	Vial 150 i.u.	1130.00
(iii)	Foliculin	Bharat Serum	Vial 75 i.u.	700.00
(iv)	Foliculin	Bharat Serum	Vial 150 i.u.	1280.00
(v)	Follimon	Biochem	Vial 75 i.u.	850.50
(vi)	Fostine	Zydus Biogen	Vial 75 i.u.	850.00
(vii)	Gonal-F	Serum International	1 Amp 37.5 i.u.	1011.08
(viii)	Gonal-F	Serum International	1 Amp 75 i.u.	2013.50
(ix)	Gonotrop-F	Win-Medicare	1 Amp 37.5 i.u.	850.00
(x)	Gonotrop-F	Win-Medicare	1 Amp 75 i.u.	1445.00
(xi)	Metrodin	Serum International	1 Inj. 75 i.u.	1190.19
(xii)	Ovimen	Life Medicare	Vial 75 i.u.	800.00
(xiii)	Ovimen	Life Medicare	Vial 150 i.u.	1425.00
(xiv)	Ovitrop	Sun Pharma	Vial 75 i.u.	750.00
(xv)	Ovitrop	Sun Pharma	Vial 150 i.u.	1200.00
(xvi)	Puregon	Chemech Labs	1 Amp	735.00
(xvii)	Recagon	Infar	1 Amp 50 i.u. FSH	1462.50
(xviii)	Recagon	Infar	1 Amp 100 i.u. FSH	2925.00
12. Tissue Plasminogen Activator				
(i)	Actilyse	German Remedies	Vial 50 ml	43500.00

amenable to the strategy of genetic manipulation. Efforts have also been made to produce transgenic catfish, tilapia and Indian carps containing growth hormone genes; the objectives were to obtain transformants that mature fast. However, these experiments have not provided any significant success, and none of these works has yet come to the stage of large- scale trials.

Conditions for Trials Using Transgenic Organisms

The RCGM monitors research on transgenic organisms in the laboratory and in the contained open environment/fields. For transgenic plants, experiments are conducted in contained green house to generate several vital safety information before decisions are taken to conduct contained open field experiments. In the field under contained conditions besides designing experiments for collecting data on environmental safety aspects the agronomic advantages of the transgenic plants in small plots are also assessed. The RCGM looks for information on environmental safety including human and animal food safety issues for all kinds of GMOs. Food safety issues are linked with GMOs that may enter into human or animal food chain directly. The information sought from the trials of GMOs is summarized briefly in Table 3.

The genetic materials can be allowed to be imported or transferred within the country by the RCGM for research use only, based on applications submitted through the IBSC.

For conducting experiments with transgenic plants in contained Green House, designs have been worked out for constructing low cost but substantially contained environment where temperature, light and humidity can be controlled to a considerable extent. Nets have been recommended that arrest the entry/exit of insects below 0.6-mm diam. Although similar contained conditions for conducting experiments with transgenic animals have not yet been published, designs are available with the government authorities, and can be sent to the investigators on request.

There are several issues concerning the use of transgenic plants in the country as mentioned above. Entrepreneurs dealing with the introduction of transgenic plants in Indian agriculture would have to follow the above in a step-by-step manner. As the emphasis of this paper is on the methods of introduction of transgenic organisms or products thereof which are to be used as pharmaceuticals and which are to be introduced in India within the frame work of Indian laws, the following paragraphs describes how this can be done.

Step by Step Methods for Introducing Recombinant Bio-pharmaceuticals

There are not many genetically modified organisms that are directly utilized as a drug in therapy. One genetically modified vaccinia virus coding for rabies antigen was introduced a couple of years ago for imparting protection to domestic and wild animals [12]. Several defective viruses and microorganisms are however under testing that are targeted for specific therapy. Several recombinant bio-pharmaceuticals have, however, been introduced the worldover during the last two decades and the number presently exceeds thirty. These products have been produced in a wide range of hosts that include genetically engineered bacteria, yeast and mammalian cells. Plant and insect cell lines have also been utilized; however, products from such hosts have been used mainly for diagnostic purposes. The active substances are usually used as therapeutic or prophylactic peptides and proteins. Concerns of safety from the use of substances produced in transgenic hosts arise from contaminants emanating from the host cells as well as the special processing steps requiring use of toxic chemicals and substances. Countries therefore have relied upon scientifically valid and reproducible purification processes to remove the contaminants, and establish acceptability criteria for each bulk peptide or protein produced by r- DNA technology. The goals that have been set for new molecules produced for the first time in the world are as under:

(a) To evaluate the effects of host organism related contaminants such as DNA, proteins, lipids, nature and extent of post-translational modification.

(b) To set standards of the limits of contaminants into the final bulk material based on scientifically sound evaluation criteria.

(c) To set standards for the presence of toxic processing chemicals used in the purification processes of the bulk and formulated r-DNA bio-pharmaceuticals.

In addition, there has been extensive requirement of documentation indicated in the documentation details (Table 4).

Table 3 - Summary of the biosafety information sought from GMO trials

Particulars	Information sought
Rationale for the development	• Economic, agronomic and other benefits, and rational of development of the GMOs and/or products therefrom
Details of the molecular biology of GMOs and genetically modified products derived therefrom. (Microorganisms, plants and animals, as well as products therefrom)	• Description of the host organisms (micro-organisms, cell lines, plants, animals etc.) • Source and sequence of transgene/s • Sequential block diagram of all trans-nucleic acid stretches inserted • Cloning strategy • Characteristics of expression vectors • Characteristics of inserted genes with details of sequences • Characteristics of promoters • Genetic analysis including copy number of inserts, stability, level of expression of transgenes, biochemistry of expressed gene products etc. • Transformation/cloning methods and propagation strategy
Laboratory, green house trials (for plants) and contained enclosure trials (for animals)	• Back-crossing methods for plants • Seed setting characteristics of plants • Germination rates of seeds • Phenotypic characteristics of transgenics • Organism challenge tests where ever applicable • Effects of chemical herbicides for all herbicide resistant plants • Growth characteristics and general health of animals, measured through specific scientific parameters • Toxicity and allergenicity implications to human if any during handling of GMOs. • Generation of food/feed safety information where applicable through lab tests.
Field trials in open environment	• For GM Plants, comparison of germination rates and phenotypic characteristics, using non-transgenic as controls. • Study of gene flow of plants • Possibility of weed formation for GM plants • Invasiveness studies of plants and animals compared to non-transgenes used as controls • Possibility of transfer of transgenes to near relatives through out crossing/ cross-fertilization • Implications of out crossing/cross-fertilization • Comparative evaluation of susceptibility to diseases and pests for plants and animals • For human food/ animal feed, elaborate determination of composition and assessment of quality of transformed plants/ fruits/seeds as well as animals as the case may be, with appropriate controls. Compositional analysis shall include near equivalence studies of all the major ingredients in GMOs so as to assess substantial equivalence with reference to non-transgenics. Change in the levels of allergens, toxicants if any, beyond acceptable limits is a matter of food safety concern and such substances are unsuitable for commercial release. • Toxicity and allergenicity implications of transformed GMOs. This include microorganisms, plants/fruits/seeds as well as animals; lab animal studies for food /feed safety evaluation is a requisite. • Handling procedures for allergenic substances. • Agronomic evaluation for GM plants • Economic evaluation for GM animals and GM microorganisms

Table 4 - Elements of Documentation for r-DNA bio-pharmaceuticals

1 Characterization of the system of production used
- a) Details of the transgenic expression system used
  - (i) Description of the host cell line
  - (ii) Identification of the genera and the species
  - (iii) The risks involved in handling the cell lines / microorganisms
  - (iv) The classification of the cell line / microorganisms in accordance with any accepted r-DNA cell line / microorganisms usage guideline and their safety status if available.
  - (v) The method(s) of maintenance and growth of the cell line / microorganisms
  - (vi) The nature and hazards of using substrates, inducing agents, etc.
- b) Characteristics of the target transgenic DNA including promoters, genes, enhancers, markers, etc. and the vector used
  - (i) The full description of the source of the target transgenic DNA including promoters, genes, enhancers, markers etc along with documentation of all the trans- DNA sequences
  - (ii) The composition of the vector used indicating the promoter sequence as well as the regulatory mechanisms utilized in the expression cassette
  - (iii) Schematic diagrams of the expression cassette to describe fully the marker genes used
  - (iv) The restriction sites related to specific endo nucleases, and the cell lines used for shuttling and amplification into the host genome
  - (v) The target gene should also contain, along with the nucleotide sequence, the description of the amino acids below the codons
- c) Approaches adopted for expression of the gene
  - (i) The description of the transcripted messenger RNA with its analysis of sequence and identification procedure
  - (ii) The translation information indicating whether the protein product is Chimaric, whether the expression is found as inclusion bodies or as intracellular protein or whether the protein is secreted out
  - (iii) The extent of the target protein produced as percentage of the total cell dry-mass as well as its percentage compared to the total proteins of the cell
  - (iv) The quality of the target protein produced after the cell growth per liter of the fermented broth
  - (v) The full sequence of the recombinant gene along with the promoters, the marker genes and the terminator sequence

2 Description of the production process
- a) Production set up
  - (i) The handling of the stock cell lines
  - (ii) The evaluation and uses of the cell lines in pre-fermentation processes
  - (iii) The preparation of the seed vessel
  - (iv) The main production fermentation conditions need to be described in brief
- b) Growth Kinetics
  - (i) Graphical plots of time versus substrate usage, optical density change, biomass formation, protein products formation, inducing agents used and their effects on the target protein formation
  - (ii) The effect of change of standard parameters like pH, dissolved oxygen, temperature, etc, in the main production fermentation vessel
- c) Fermentation parameters
  - (i) The pH, temperature, aeration, and rpm of the shaft
  - (ii) Volume of seed to the volume of main fermentation vessel
  - (iii) Main substrates used, inducing agents used if any
  - (iv) The fermentation time and the concentration of the target protein in the fermented broth
- d) Approaches for the extraction and purification of the product
  - (i) The methods of handling the cells
  - (ii) The methods of isolating the cell soup containing the target protein. The method of enzyme treatment, if any
  - (iii) The methods of concentration, precipitation (if applied), reconstitution, salt separation (if applicable), absorption and desorption methods, chromatographic methods used if any, and ultra-centrifugation methods if used
  - (iv) Sterilization and final dosage formulation
  - (v) Processes to obtain the product in the finished dosage form

- Contd

Table 4 - Elements of Documentation for r-DNA bio-pharmaceuticals-*(Contd)*

3 In-process control methods
- (i) All the analytical methods used for this in- process control must be described to ensure the regulatory authorities that the chances of entry of unwanted products have been minimized

4 Description of the raw and processing materials used
- (i) Description of all raw materials and processing materials used starting from the stock cells handling to the finished dosage form must be in a tabular form
- (ii) Grades of materials used and their usual sourcing

5 Description of the plant and machinery used
- (i) List of all the equipment along with the indicative capacity
- (ii) Names of the manufacturers
- (iii) List of Main production equipment and the quality control equipment

6 Description of the building and facilities created for the manufacture
- (i) The manufacturing area
- (ii) The flow of the process/ operation starting from the stock culture area to the finishing area,
- (iii) The plot plan of various floors used in the manufacture and processing
- (iv) The building diagram in the plan and / or elevation in the outline indicating the cleanliness maintained in different sections such as class 10,000, calls 1000 and class 100 areas, etc
- (v) Description of the air handling system, which is very important
- (vi) A separate write up indicating the movement of operating personnel in the area

7 Quality Control and Quality Assurance

a) Bulk material:
- (i) Tests relating to the identification of the stock cells
- (ii) The plasmid constructs retention in the cells during cell growth
- (iii) The media used
- (iv) The procedures adopted for testing and the percentage retention of the target plasmid
- (v) Description of the contamination test of the stock culture to assess and monitor the microbial contamination including the media used and the procedures adopted
- (vi) The criteria of acceptance of contamination free culture
- (vii) Characterization of the bio- active molecule produced by monitoring physical and chemical properties of the molecule by the following or more authentic method should be provided
- (viii) Microscopic examination including light, phase contract and electron microscopic studies
- (ix) UV Spectroscopic analysis to show the absorption spectra of the product and comparison of this with authentic material
- (x) Density gradient centrifugation to show single peak
- (xi) Pattern in High performance liquid chromatography to include how many peaks or if only one peak is noticed in the produced and purified bulk
- (xii) Iso- electric focusing analysis to show the pI values
- (xiii) Western Blot and SDS-PAGE to map the peptide/ target protein
- (xiv) Existence of special bonds like disulfide bonds by breaking the protein / peptide with appropriate reagents and subjecting the products to SDS-PAGE mapping
- (xv) Immuno-diffusion Test to find out the absence of contaminants or the extent of the presence of contaminants
- (xvi) Amino acid composition of the purified protein / peptide and its comparison with the authentic material if available (gold standard)
- (xvii N Terminal Amino Acid Sequence analysis and its comparison with the protein / peptide expressed by the transgene
- (xviii) Determination of the biological activity in the appropriate animal model
- (xix) Determination of contaminants per milligram or per any convenient unit of the manufactured bulk purified protein is also to be carried out to indicate the extent of contaminated nucleic acid stretches, proteins, carbohydrates, lipid, detergents, salts and other processing materials used in the purification process, the impacts of the presence of these contaminants are also to be indicated with authentic reference materials if any or by conducting animal toxicity / allergenicity tests in appropriate animal models to ensure the risk associated with their presence in quantities that are to be fixed , are minimal. The limits of contaminants and impurities are to be quantified for each foreign substance, and the acceptability criteria for the bulk peptide / proteins with reference each of the contaminants / impurities need to be quantified

- *Contd*

Table 4 - Elements of Documentation for r-DNA bio-pharmaceuticals – *(Contd)*

(b) Formulated bio-pharmaceutical materials

(i) The ingredients incorporated subsequently in the manufacture of the formulated material using the bulk. The specification of the final product needs to be defined in composition

(ii) The quality control department of the organization would have to certify that the final product has the results within the specifications prescribed and accepted by the regulatory authorities

(iii) The documentation for the formulated material should therefore specify at least the following parameters

- Product presentation
- Physical appearance of the product
- Product inserts, literature and label claim. The label claim must indicate that the product is transgenic. Wherever feasible the name of the host organism / cell line used for the manufacture and the gene utilized for expression should also be mentioned
- Volume/ quantity of the active ingredient per pack / dose
- Potency of the product
- Particulate matter limits for the liquid and the range of the particle size in the finished product
- Preservative usage in percentage
- pH of the solution/ formulated material when reconstituted
- Other extraneous materials used and their extent such as content of DNA, RNA, Carbohydrates, Lipids, processing materials like detergents, salts etc. wherever applicable
- Total protein content and the active protein content
- Sterility status of the formulation
- Toxicity information
- Allergenicity information
- Pyrogen status.
- Any other information relevant to the medical profession

(iv) Status of certification i.e. if the formulated material conforms to any accepted specification.

- Every certificate wherever issued shall be accompanied by other statutory information like the manufacturing batch number like the date of manufacture, date of analysis of the batch, of the date of release of the certificate, authorized signatory to the certificate, etc
- The final formulated product to be marketed / used needs to be certified for acceptance by the manufacturer indicating the date up to which the material can be used for the intended purpose / indication

Recombinant DNA bio-pharmaceuticals that are new molecules are required to be fully investigated for safety before they become eligible for marketing and use. Such molecules produced and evaluated in one country, and allowed for marketing in the country of origin may require a fresh evaluation in another country if the regulatory laws so require. In India, depending upon the quality of evaluation of safety and efficacy as also the sample size used for efficacy studies, the Indian government may require the applicants to conduct de-novo studies or may authorize the conduct of phase III clinical studies in a minimum number of human subjects. Often the phase III trials require a sample size of over 100 persons and the studies should be conducted independently at least in 4 centers under the supervision of qualified medical practitioners. Even during such studies special care is required to be taken to generate data on safety issues to human health that are part of the requirement of the Indian Environment (Protection) Act & Rules. Directly authorization for marketing can be provided only in situations where the recombinant DNA bio-pharmaceuticals have been extensively in use elsewhere and have been evaluated thoroughly over very large number of human subjects, and the products are in use for a couple of years. In such cases also the Indian Environment (Protection) Act & Rules require the generation of post market surveillance data where safety information on issues related to human health are to be addressed and gathered. All protocols used in phase I, II, III and IV require the approval of the government.

Use of all recombinant DNA products requires pre-clinical safety evaluation in relevant animal species. Evaluation carried out in non-relevant animals is of no use. The testing in animals requires, besides the selection of the relevant animal species a statistically significant numbers, their age with sex distribution, the manner of delivery of the dose including the routes of administration and the treatment regimen (Schedule of treatment). The material to be tested must be stable and must be satisfied to be suitable for application / use. All toxicity studies in animals are required to be consistent with a protocol that requires the approval of the government. Further, the use of the protocol as well as the test animals also require the approval of the Animal Ethics Committee of the institute. All studies should follow Good Laboratory Practices. The safety evaluation program should include at least two relevant of animals. Only in special cases, one relevant animal species can be used; however this requires the approval of the regulatory authorities. Toxicity studies should not be undertaken in non-relevant animal species, as the results are misleading and irrelevant. Transgenic animals can be used with the approval of the regulatory authorities provided such animals have the ability of expressing human receptors and that the presence of receptors in the tissues of such animals are comparable to human receptors. Data generated in relevant animal models provide insight in determining pharmacokinetics and pharmacological action besides safety and the usefulness of the product. Some bio-pharmaceuticals produced by recombinant DNA technology can be immunogenic in animals as well as in human (when repeatedly applied). The immunogenic potential of such products requires to be taken a note of and animal studies should document such information at the start and at the end of the animal experiments. Effect of hyper sensitization on the animals also requires to be recorded with effects on vital organs. In this context, the safety of the host cell needs to be taken cognizance to not only from its potential for producing endo-toxins but also its abilities for post translation modification of the translated proteins. Toxic or immunogenic potential of the non-protein components added to the transgenic proteins need also to be evaluated before a decision for approval is taken by the regulatory agencies. For DNA vaccines, it is specifically required to study the fate of the coding sequence in the target tissues / animals and therefore the decay rate of the transgenic sequences need to be studied. Further, data are to be generated on whether the DNA coding sequences get integrated into the tissues of the animal. It is also to be determined whether the vaccine gets continuously expressed and whether such a phenomenon hyper-immunizes the animal and whether such phenomenon of hyper-immunization causes any toxic effect to the blood or to any vital parts of the animals like kidney, liver etc. The protocol has to address generating information on these aspects. The new molecules require generations of pharmaco-kinetic and toxico-kinetic data, evaluation of single doze toxicity as well as repeated doze toxicity, immuno-toxicity, reproductive performance, geno-toxicity and carcinogenicity studies. A well-known molecule does however not require *de-novo* generation of all these data as there may be extensive published information already available on these aspects. However, if the known biopharmaceutical product is produced by a different method where the host cell lines have been modified or changed or where the construct has been newly assembled using new methods or where the product has been purified by applying down stream processing methods that have not been evaluated, then the product will be considered as new. However for such a product only the sub-acute toxicity studies and in some cases chronic toxicity studies in two relevant animals may be adequate. Effects of posttranslational modification of the translated proteins must be kept in sharp watch as indicated above. Post transpationally modified product may behave quite differently as the modification depends upon the translational machinery of the host organism. Carbohydrates, lipids and other residues including Sialic Acid derivative that get incorporated can impart different properties to the resulting protein expressed by a set of well characterical genes as the latter is host dependent while the former i.e. the production of the amino acid sequence of the target protein is not host dependent, but gene-dependent only.

After the conduct of animal studies and after documenting the recombinant DNA biopharma-ceutical product in the manner described above the applicants can seek marketing permission for selling

such products. In India there can be two marketing routes for introducing such biopharmaceuticals. In one route, the biopharmaceutical can be developed in a step-by-step manner in Indian laboratories/facilities and the data generated on safety of the formulated material can be submitted to the GEAC as well as to the DC (G) I. The former authorizes use and marketing under the Indian Environment (Protection) Act while the later authorizes manufacture and marketing under the Drug Act. In the other route, r-DNA biopharmaceuticals can be directly imported and marketed. In such cases also the applicants are requires to provide data as above to the GEAC as well as to the DC (G) I. The only difference is that such data on safety of the products are not generated in India.

The steps required to be followed by applicants/organizations/manufacturers for the introduction of any r-DNA biopharmaceutical product in Indian market based on local manufacture are elaborated in Table 5.

For biopharmaceuticals to be directly imported and marketed, the procedures to be followed by applicants are given in Table 6.

The Kinds of r-DNA Biopharmaceuticals that Require Clearance Under EPA

The information required to be generated, as documented above, applies to all kinds of biopharmaceuticals produced by recombinant DNA technology. The usual products include growth factors, fusion proteins, receptors, hormones, cytokines, thrombolytic agents and plasma factors. They also include monoclonal antibodies produced by the application of phage display methods. Further, they include proteins / peptide DNA vaccines, and genetic materials/ DNA materials used in cellular and gene therapies. They also apply to recombinant primary and secondary metabolites used in therapy such as enzymes, antibiotics, vitamins, blood components, blood proteins, etc. However the rules do not apply to conventional products like bacterial and viral vaccines, antibiotics and other products produced by fermentation using non genetically engineered microorganisms / cell lines. They also do not apply to monoclonal or polyclonal antibodies produced by hybridoma technology or conventional methods respectively.

Conclusions

The Indian Government created the rules and procedures for dealing with GMOs in 1989 under the Indian Environment (Protection) Act 1986. M/s Transgene Vaccines Ltd , Hyderabad started the first semi-commercial scale transgenic experiments with microorganisms in February 1995 using genetically modified *Hanseneula polymorpha* that codes for Hepatitis B surface antigen. The company procured the lab level technology from M/s Rhein Biotech GmbH, Germany. The experiments were conducted at the Institute of Microbial Technology, Chandigarh. The transgenic surface antigen of hepatitis B known as "S" antigen was isolated in bulk and it was proven that the country could handle genetically modified organisms under GMP conditions. However, the company could not subsequently set up its own production facilities. Subsequently, in August 1997, M/s Shantha Biotechnics, Hyderabad introduced a similar product, produced in recombinant *Pichia pastoris,* developed by them. Later on after Shantha, three more companies have come into production of this recombinant vaccine in the country. Subsequently, upto end 2002, three more recombinant products namely erythropoietin, granulocite colony stimulating factor and interferon alpha 2b have been cleared by the Regulatory Authorities for local production. More biopharmaceutical products would be authorized for local production in the coming years. Presently (end-2002), in all twelve biopharmaceuticals produced by recombinant DNA technology have been authorized for marketing in India of which, as stated above, four are authorized for local production. In GM plants, the first transgenic plant experiment in the field was started in 1995. Since then the country has acquired substantial experience in understanding the issues related to the handling of GMOs. In March 2002, transgenic insect resistant Bt cotton was authorized for production in India. The Bt cotton was found to reduce the consumption of chemical pesticides considerably with increased yield of cotton, provided it received the necessary quantities of fertilizers and water, and the plants were sprayed for protection from non-bollworm insects, and where necessary to reduce

Table 5 - Steps to be followed by applicants/institutions/organizations for obtaining the approval for their biotech drugs (recombinant DNA drugs) developed and/or produced in India

The institution/organization makes an application for the approval of its Institutional Bio-Safety Committee (IBSC) to Department of Biotechnology (DBT), Govt. of India.

↓

DBT approves the IBSC and nominates a government's nominee on the IBSC to oversee the activities to ensure that bio-safety guidelines are strictly followed.

↓

IBSC is formed at the institution/organization and DBT takes composition of IBSC on record. Applicants including institution/organization submit applications to IBSC for initiating experiments involving r-DNA products.

↓

IBSC meetings are convened at the institute/organization at least twice a year. Every meeting shall have an agenda. Minutes of all IBSC meetings are sent to Review Committee on Genetic Manipulation (RCGM). IBSC can approve experiments of the Category I and Category II risk experiments. For experiments of Category III and above risks, IBSC sends its recommendations to RCGM for approval. Applicants submit applications in prescribed formats to IBSC/RCGM.

↓

RCGM grants the conduct of experiments of Category III and above risks, and asks for progress reports periodically from the applicants/project-investigators through IBSC.

↓

Experiments concerning drugs produced from Genetically Modified Organisms (GMOs) require generation of animal toxicity data. Before such data are generated, results of at least five trial batches (including each bulk and formulation batches) are evaluated first by the IBSC and then by RCGM to ascertain if a reasonably stable process has been developed. Product acceptability criteria for both bulk and formulated materials are fixed.

↓

Protocol must address risks emanating from host-related contaminants as well as residual toxic chemicals used in processing. RCGM approves the conduct of animal toxicity studies; thereafter, Animal Ethics Committee of the institution/organization has to approve the protocol.

↓

Animal toxicity has to be carried out at least on two relevant species of animals (often, one rodent and one non-rodent species are selected). Species of animal is to be indicated in the protocol.

↓

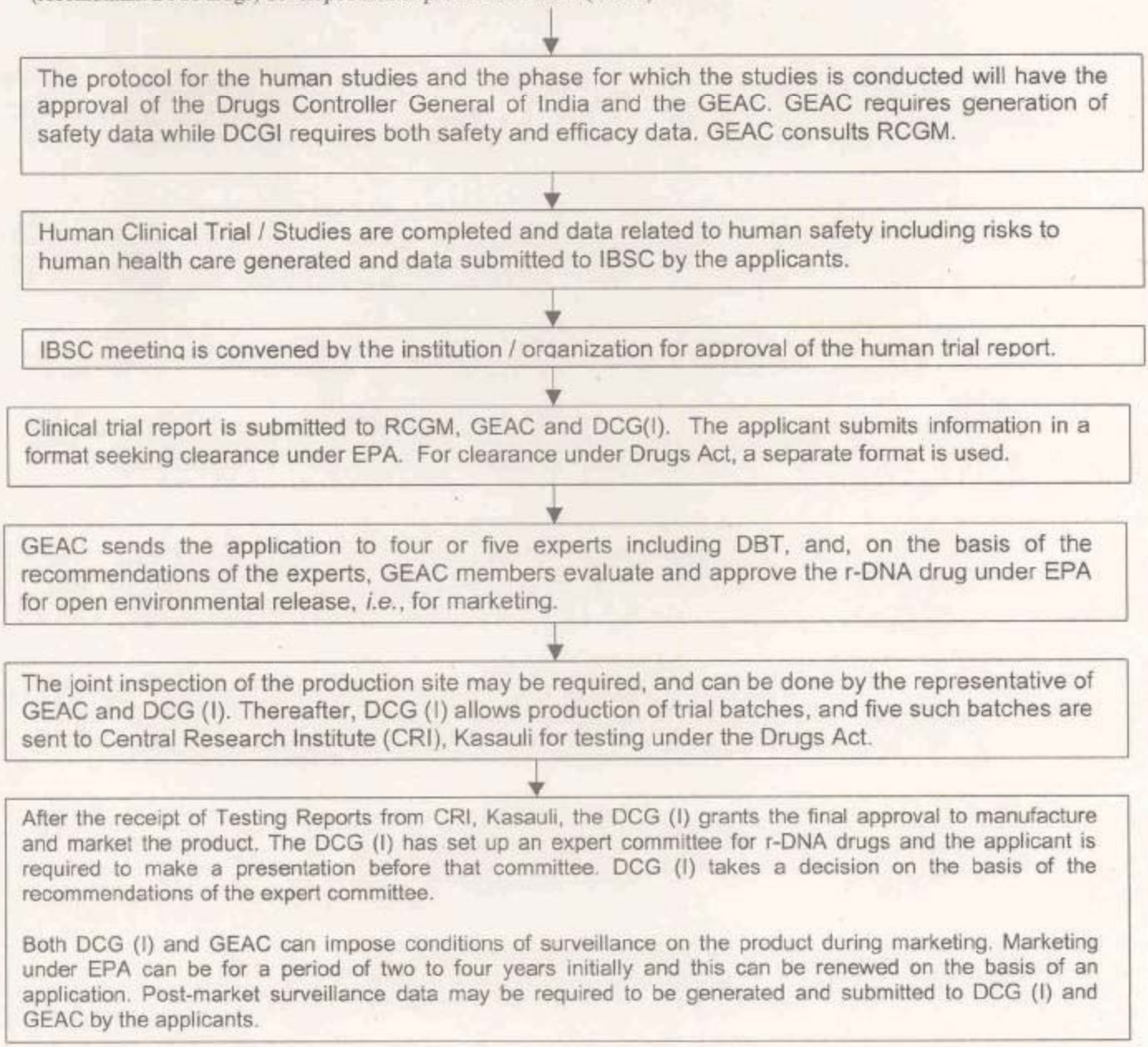
Animal toxicity studies are carried out and IBSC meeting is convened by institution/organization to discuss the report. If satisfactory results are obtained, protocols for Phase I, Phase II and/or Phase III studies are prepared by the institution/organization, and placed before IBSC members for approval. Phase I is needed for a new product/molecule. Phase II is needed when the dose is not known or when the dose finding has to be carried out. Straightaway Phase-III studies can be carried out if the drug is well known and the host is assessed as safe.

↓

Above documents, including protocols for human studies are submitted by the applicants to RCGM and Drug Controller General of India (DCG (I)) for approval. RCGM sends its recommendation to GEAC as it cannot approve human studies.

↓

Table 5 - Steps to be followed by applicants/institutions/organizations for obtaining the approval for their biotech drugs (recombinant DNA drugs) developed and/or produced in India (Contd)



the load of bollworm too. In this context the rich experience gained from hands-on experiments in the field conditions for handling several other GM plants has provided insights about the benefits as well as the risks from the use of such plants[7]. On overall assessment it appears that the benefits from the use of such plants are substantial and they may outweigh the risks, which in many facets are presumptive and not real. There are issues on the flow of trans-genes into the open environment. Transgenic traits would spread into the natural environment over the years, especially in situations where GM plants set seeds by cross pollination and where there are many wild near relatives of such plants as is the case of *Brassica* species. While approving such GM plants, great care has to be taken to assess the potentials of spread of transgenes into the wild relatives through such GM plants, and the consequence of such spread. Obviously, in these cases, a close monitoring would be required for several years by the authorities. Experiments in transgenic animals including fish are yet at developmental stage and the country has to go a long way before such products are developed for commercial applications. A case by case assessment

Table 6 - Steps to be followed by applicants/institutions/organizations for obtaining the approval for their biotech drugs (recombinant DNA drugs) to be imported and marketed in India (Such drugs cannot be considered for import if they are not approved in the country of origin)

The applicant/institution/organization makes an application simultaneously to the Genetic Engineering Approval Committee (GEAC) in a format (in about six copies), and to the Drugs Controller General of India (DCG (I)) in another format in five sets.
↓
GEAC sends the application to four to five experts including the Department of Biotechnology (DBT) seeking their evaluation/comments on the report. DCG (I) also examines the report in-house as well as consults experts.
↓
After receipt of comments of all the experts, the GEAC examines the application along with the comments in its meetings. At this stage, GEAC may ask for 21 sets of application from the applicants. GEAC may decide to direct the applicant to conduct further Clinical Trials if the committee has any doubt about the product in terms of evaluation of safety, lack of information on certain aspects of safety, the inadequacy of the sample size used in the evaluation etc. Phase III clinical trials may be ordered incorporating in the protocol questions that address certain issues of human risks/adversary actions.
↓
If the data are found to be satisfactory by the GEAC, the latter sends its recommendations to the DCG (I) incorporating conditions that require the generation of post-market surveillance information (Phase IV data). If not approved, the GEAC directs the applicant to apply afresh after generating the necessary data under Phase III clinical trial.
↓
After receiving the authorization from the GEAC, if the DCG (I) is also satisfied with the data, it grants its initial approval in accordance with 122A.
↓
Applicants then apply for form 10 along with 122A approval.
↓
Authorization for imports is issued by the DCG (I) incorporating its conditions as well as those of the GEAC, and the applicant imports and markets the drug.

of risks of all kinds of GMOs on a precautionary principle seems to be the wisest path to be followed at this juncture. This will instill public confidence and will enable the country to maximize the utilization of this powerful technology to the benefit of India. Authorities could also publish a list of GMOs and products thereof that are considered environmentally safe and would not require any further scientific scrutiny under the Indian EPA for handling and use, based on published information or documentation or data generated by the government. Also information on the approved GMOs and products thereof as also the brief basis of approval through an internet website is long overdue.

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