

## OPEN BRIEF

Lelystad, 29 juni 2010.

De Minister van VROM

Mevr. J.C. Huizinga

T.a.v. RIVM/SEC/Bureau GGO

Postbus 1

3720 BA Bilthoven

Geachte minister Huizinga,

N.a.v. de beschikking betreffende IM 10-003/00 het volgende;

Het bevreemdt ons, dat u in de beschikking zeer summier reageert op onderstaande tekst, die voorkomt in ons bezwaarschrift dat wij aan u verstuurd hebben en ook te lezen is via

<http://www.gentechvrij.nl/plaatjesgen/med4leidenbezwaarschrifta.pdf>

### **Unregulated Hazards 'Naked' and 'Free' Nucleic Acids**

ISIS Report -Produced for the Third World Network

**Mae-Wan Ho, Angela Ryan Biology Department, Open University, Walton Hall, Milton Keynes MK7 6AA, UK J.Cummins Department of Plant Sciences, University of Western Ontario Ontario, Canada T. Traavik Dept. of Virology, Institute of Medical Biology, MH-Breivika And Norwegian Institute of Gene Ecology, N-9037 Tromso, Norway**

#### **Executive Summary**

A huge variety of naked/free nucleic acids are being produced in the laboratory and released unregulated into the environment. They are used as research tools, in industrial productions and in medical applications such as gene therapy and vaccines. These nucleic acids range from oligonucleotides consisting of less than 20 nucleotides to artificial constructs thousands or millions of basepairs in length, typically containing a heterogeneous collection of genes from pathogenic bacteria, viruses and other genetic parasites belonging to practically every kingdom of living organisms. Most of the nucleic acids and constructs have either never existed in nature, or if they have, not in such large amounts. They are, by definition, xenobiotics – substances foreign to nature - with the potential to cause harm. **Some, such as gene therapy vectors and vaccines, have already been shown to elicit toxic and other harmful reactions in preclinical trials. KNIP**

<http://www.i-sis.org.uk/naked.php>

Geachte heer Bleijs,

Hierbij stuur ik u het aanvraagformulier voor een genterapie studie die binnen het Academisch Ziekenhuis Leiden (handelend onder de naam Leids Universitair Medisch Centrum (LUMC)) zal gaan plaatsvinden. Het project is getiteld: "Intradermal vaccination with naked DNA encoding the fusion protein domain I of tetanus toxin fragment C and MART-1(aa 26-35) for the induction of MART-1-specific T cell immunity in stage IV melanoom patients".

***De aanvraag van 4 maart 2010 van dr. G. van Willigen.***

## ***Het gaat hier toch over naked DNA! En in bovenstaand ISIS rapport wordt toch gewezen op Unregulated Hazards 'Naked' and 'Free' Nucleic Acids?***

Dat u hier niet dieper op ingaat, maar het alleen maar vermeldt, als zijnde een *internet publicatie over mogelijke risico's van nucleïnezuren*, vinden wij *the understatement of the year!*

Het is nota bene een rapport met 72 verwijzingen naar onderzoeken en uitgegeven boeken van gerespecteerde onderzoekers:

### **Conclusion**

The naked/free nucleic acids created by genetic engineering biotechnology are potentially the most dangerous xenobiotics to pollute our environment. Unlike chemical pollutants which dilute out and degrade over time, nucleic acids can be taken up by all cell to multiply, mutate and recombine indefinitely. The need for regulatory oversight at both national and international levels is long overdue. It is irresponsible to continue to exclude naked/free nucleic acids from the scope of the Biosafety Protocol.

1. Traavik, T. (1999a) Too early may be too late: Ecological risks associated with the use of naked DNA as a biological tool for research, production and therapy. pp 29-31. Reported to the Directorate of Nature Management, Norway.
2. See Ho, M.W. (1998, 1999). [Genetic Engineering Dream or Nightmare?](#) The Brave New World of Bad Science and Big Business, Gateway Books, Bath 2nd ed., Gateway, Gill & Macmillan, Dublin.
3. As defined by Traavik, 1999a (note 1)
4. See Ho, 1998, 1999 (note 2); Ho, M.W., Traavik, T., Olsvik, R., Tappeser, B., Howard, V., von Weizsacker, C. and McGavin, G. (1998b). Gene Technology and Gene Ecology of Infectious Diseases. *Microbial Ecology in Health and Disease* 10, 33-59; Traavik, T. (1999b). An orphan in science: Environmental risks of genetically engineered vaccines. Reported to the Directorate of Nature Management, Norway.
5. See Ho et al, 1998 (note 4).
6. See Traavik, 1999b (note 4).
7. See Schindelbauer, D. (1999). Construction of mammalian artificial chromosomes: prospects for defining an optimal centromere. *BioEssays* 21, 76-83.
8. See Helin, V., Gottikh, M., Mishal, Z., Subra, F., Malvy, C. and Lavignon, M. (1999). Cell cycle-dependent distribution and specific inhibitory effect of vectorized antisense oligonucleotides in cell culture. *Biochemical Pharmacology* 58, 95-107; Campagno, D. and Toulme, J.-J. (1999). Antisense effects of ligonucleotides complementary to the hairpin of the *Leishmania* mini-exon RNA. *Nucleosides & Nucleotides* 18, 1701-1704.
9. See Hammann, C. and Tabler, M. (1999). Generation and application of asymmetric hammerhead ribozymes. *Methods: a Companion to Methods in Enzymology* 18, 273-380.
10. Han, Y., Zaks, T.Z., Wang, T.F., Irvine, D.R., Kammula, U.S., Marincola, F.M., Leitner, W.W. and Restifo, N.P. (1999). Cancer therapy using a self-replicating RNA vaccine. *Nature Medicine* 3, 823-827.

11. Beetham, P.R., Kipp, P.B., Sawycky, X.L., Arntzen, C.J. and May, G.D. (1999). A tool for functional plant genomics: Chimeric RNA/DNA oligonucleotides cause in vivo gene-specific mutations. *PNAS* 96, 8774-8778.
12. Reviewed by Lorenz, M.G. and Wackernagel, W. (1994). Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol. Rev.* 58, 563-602; also, Ho, 1998,1999 (note 2); Ho, et al, 1998 (note 4); Traavik, 1999a (note 1).
13. This was said to M.W.H. by a spokesperson of the UK Health and Safety Executive when asked whether there is any recommended treatment for disposal of naked/free DNA.
14. See Lorenz and Wackernagel, 1994 (note 12); also Ho, 1998, 1999 (note 2); Ho, et al, 1998 (note 4) .
15. Mercer, D.K., Scott, K.P., Bruce-Johnson, W.A. Glover, L.A. and Flint, H.J. (1999). Fate of free DNA and transformation of the oral bacterium *Streptococcus gordonii* DL1 by plasmid DNA in human saliva. *Applied and Environmental Microbiology* 65, 6-10.
16. Schubbert, R., Lettmann, C. and Doerfler, W. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Molecular and General Genetics* 242, 495-504; Schubbert, R., Rentz, D., Schmitz, B. and Doerfler, W. (1997). Foreign (M13 DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Nat. Acad. Sci. USA* 94, 961-6.
17. Mercer et al, 1999 (note 15).
18. Brown, P. Naked DNA raises cancer fears for researchers. *New Scientist* 6 October, 17 (1990).
19. Schubbert et al, 1997 (note 16).
20. Doerfler, W. and Schubbert, R. (1998). Uptake of foreign DNA from the environment: the gastrointestinal tract and the placenta as portals of entry, *Wien Klin Wochenschr.* 110, 40-44.
21. Doerfler and Schubbert, 1998, (note 20), p. 40.
22. Yei, S., Mittereder, N., Wert, S., Whitsett, J.A., Wilmott, R.W. and Trapnell, B.C. (1994). In vivo evaluation of the safety of adenovirus-mediated transfer of the human cystic fibrosis transmembrane conductance regulator cDNA to the lung. *Hum. Gene Ther.* 15, 731-744.
23. Noisakran S, Campbell IL, Carr DJ, (1999) Ecotopic expression of DNA encoding IFN-alpha 1 in the cornea protects mice from herpes simplex virustype 1-induced encephalitis. *J Immunol* 162, 4184-90.
24. Yamasoba T, Yagl M, Roessler BJ, Miller JM, Rapheal Y (1999) Inner ear transgene expression after adenoviral vecotr inoculation in the endolymphatic sac. *Hum Gene Ther* 10, 744-69.
25. See Hoffman, R.M. (2000). The hair follicle as a gene therapy target. *Nature Biotechnology* 18, 20-1.
26. Budker, V., Zhang, G., Danko, I., Williams P. and Wolff, J. (1998). The efficient expression of intravascularly delivered DNA in rat muscle. *Gene Therapy* 5, 272-6; Han, et al, 1999 (note 12).
27. Khavari, P.A. Cutaneous gene therapy. *Advances in Clinal Research* 15, 27-35 (1997);

28. During, M.J., Xu, R., Young, D., Kaplitt, M.G., Sherwin, R.S., Leone, P. (1998). Peroral gene therapy of lactose intolerance using an adeno-associated virus vector. *Nat. Med.* 4, 1131-5.
29. Spadafora, C. (1998). Sperm cells and foreign DNA: a controversial relation. *BioEssays* 20, 955-64.
30. Zhang, G. Vargo, D., Budker, V., Armstrong, N., Knechtle, S. and Wolf, J., Expression of naked DNA injected into the afferent and efferent vessels of rodents and dog livers. *Human Gene Ther.* 8,1763-72 (1997).
31. Hengge, U., Chan, E., Foster, R., Walker, P. and Vogel, J., Cytokine gene expression in epidermis with biological effects following injection of naked DNA,. *Nat. Genet* 10,161-6 (1995)
32. Hengge, U., Walker, P. and Vogel, J. Expression of naked DNA in human, pig and mouse skin. *J Clin Invest* 97,2911-6 (1996).
33. Martin, T., Parker, S.E., Hedstrom, R., Le, Thong, Hoffman, S.L., Norman, J., Hobart, P. and Lew, D. (1999). Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection. *Hum. Gene Ther.* 10, 759-68.
34. Zhaqo, T., Robinson, M., Bowers, F. and Kindt, T. Infectivity of chimeric human T-cell leukaemia virus type I molecular clones assessed by naked DNA inoculation. *Proc. Natnl. Acad. Sci. USA* 93,6653-8 (1996).
35. Rekvig, O.P. Fredriksen, K., Brannsether, B., Moens, U., Sundsfjord, A. and Traavik, T., Antibodies to eucaryotic, including autologous, native DNA are produced during BK virus infection, but not after immunization with non-infectious BK DNA. *Scand. J. Immunol.* 36, 487-495 (1992).
36. Brower, V. (1998). Naked DNA vaccines come of age. *Nature Biotechnology* 16, 1304-5;
37. see also Traavik, 1999b (note 4). See Traavik, 1999b (note 4).
38. See Verdier, F. and Descotes, J. (1999). Preclinical safety evaluation of human gene therapy products. *Toxicological Sciences* 47, 9-15; Jane, S.M., Cunningham, J.M. and Vanin, E.F. (1998). Vector development: a major obstacle in human gene therapy. *Annals of Medicine* 30, 413-5.
39. Putnam, L. (1998). Debate grows on safety of gene-therapy vectors. *The Lancet* 351, 808.
40. See Ho, et al, 1998 (note 4).
41. Baba, T.W. Liska, V., Khimani, A.H., Ray, N.B., Dailey, P.J., Penninck, D., Bronson, R., Greene, M.F., McClure, H.M., Martin, L.N. and Ruth M. Ruprecht, R.M. Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infant and adult macaques. *Nature Med.* 5, 194, 203 (1999).
42. See Verdier and Desotes, 1999 (note 38).
43. See Coghlan, A. (1996). Gene shuttle virus could damage the brain. *New Scientist* 11 May, 6.
44. Nelson D & Weiss R. Gene research moves towards secrecy. *Washington Post* Nov 3, 1999
45. Suzuki, K., Mori, A., Ishii, K.J., Singer, D.S., Klinman, D.M., Krause, P.R. and Kohn, L.D. (1999). Activation of target-tissue immune-recognition molecules by double-stranded polynucleotides. *Proc. Natl. Acad. Sci. USA* 96, 2285-90.
46. Fong KM et al (1997) FHIT and FRA3B 3p14.2 allele loss are common in lung cancer and preneoplastic bronchial lesions and are associated with cancer related FHIT cDNA splicing aberrations. *Cancer Res. (CNF)*, 57 (11) ; 2256-67.

47. Asch HL (1996) Comparative expression of the LINE-1 p40 protein in human breast carcinomas and normal breast tissues. *Oncol. Res (BBN)* 8 (6): 239-47.
48. Miki Y. (1992). Disruption of the ARC gene by retrotransposal insertion of L1 sequence in a colon cancer. *Cancer Res (CNF)*, 52 (3):643-5
49. Buendia, M.A. (1992). Mammalian hepatitis B viruses and primary liver cancer. *Semin. Cancer Biol.* 3, 309-20.
50. See Verdier and Descotes, 1999 (note 38); Spadafora, 1998 (note 29).
51. Mao, J.R., Inouye, M. and Inouye, S. (1996). An unusual bacterial reverse transcriptase having LVDD in the YXDD box from *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 227, 489-93.
52. Hoffman, T., Golz, C. & Schieder, O. (1994). Foreign DNA sequences are received by a wild-type strain of *Aspergillus niger* after co-culture with transgenic higher plants. *Current Genetics* 27: 70-6.
53. De Vries, J. and Wackernagel, W. (1998). Detection of nptII (kanamycin resistance) genes in genomes of transgenic plants by marker-rescue transformation. *Mol. Gen. Genet.* 257, 606-13.
54. Schluter, K., Futterer, J. & Potrykus, I. (1995). Horizontal gene-transfer from a transgenic potato line to a bacterial pathogen (*Erwinia-chrysanthem*) occurs, if at all, at an extremely low-frequency. *Bio/Techology* 13: 1094-8.
55. Timms-Wilson, T.M., Lilley, A.K. and Bailey, M.J. (1999). A Review of Gene Transfer from Genetically Modified Micro-organisms. Report to UK Health and Safety Executive.
56. Gebhard, F. and Smalla, K. (1998). Transformation of *Acinetobacter* sp. strain BD413 by transgenic sugar beet DNA. *Appl. Environ. Microbiol.* 64, 1550-4.
57. See Traavik, 1999a (note 1); Timms-Wilson, et al, 1999 (note 24).
58. Pace, N. (1997). A molecular view of microbial diversity and the biosphere. *Science* 276, 734-9.
59. See Ho, 1998, 1999 (note 1); Ho et al, 1998b(note 1); Traavik, 1999a (note 1).
60. See Kohli, A., Griffiths, S., Palacios, N., Twyman, R.M., Vain, P., Laurie, D.A. and Christou, P. (1999). Molecular characterization of transforming plasmid rearrangements in transgenic rice reveals a recombination hotspot in the CaMV 35S promoter and confirms the predominance of microhomology mediated recombination. *The Plant Journal* 17, 591-601; also Ho, M.W., Ryan, A. and Cummins, J. (1999). [The CaMV promoter - A recipe for disaster?](#) *Microbial Ecology in Health and Disease* (in press).
61. See Ho et al, 1998b (note 4) and references therein.
62. Letter from N. Tomlinson, Joint Food Safety and Standards Group, MAFF, to US FDA, 4 December, 1998.
63. See Ho, M.W. , 1998, 1999 (note 2)
64. This was the question asked by Ho et al, 1998 (note 4) who called for an urgent public enquiry; See also Ho, M.W., Traavik, T., Olsvik, O., Midtvedt, T., Tappeser, B., Howard, C.V., von Weizsacker, C. and McGavin, G. (1998). Gene Technology in the Etiology of Drug-resistant Diseases. *TWN Biotechnology & Biosafety Series 2, Third World Network, Penang.*
65. See Ho, 1998, 1999 (note 2)
66. See Jakowitsch, J., Mette, M.G., van der Winden, J., Matzke, M.A. and Matzke, A.J.M. (1999). Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants. *Proc. Nat. Acad. Sci. USA* 96, 13241-6.

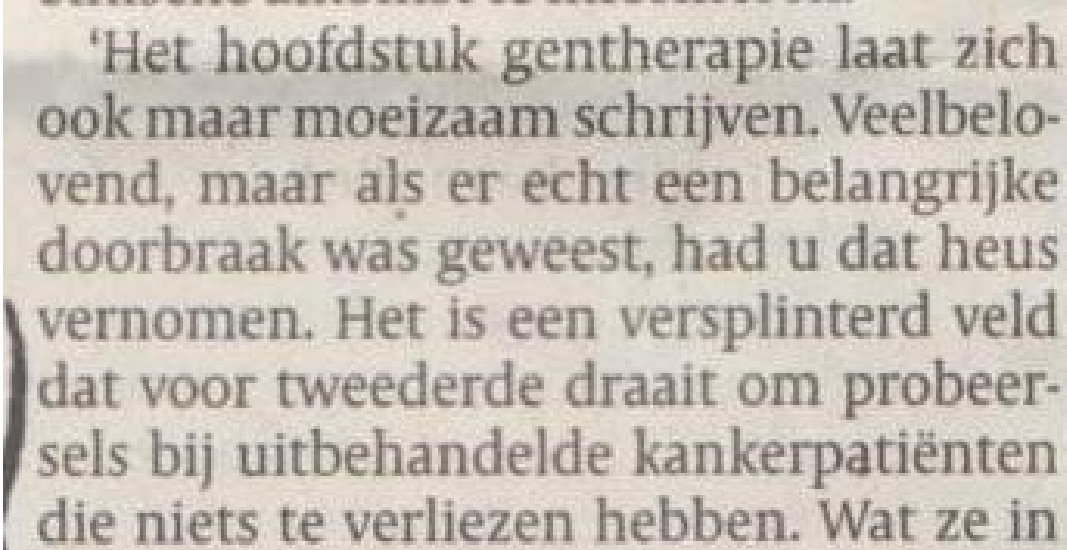
67. Greene, A.E. and Allison, R.F. (1994). Recombination between viral RNA and transgenic plant transcripts. *Science* 263, 1423-5; Wintermantel, W.M. and Schoelz, J.E. (1996). Isolation of recombinant viruses between cauliflower mosaic virus and a viral gene in transgenic plants under conditions of moderate selection pressure. *Virology* 223, 156-64.
68. See Ho, 1998, 1999 (note 2) especially Chapter 13/12.
69. See Ho et al, 1999 (note 30).
70. See Robinson, W.P. and Lalande, M. (1995). Sex-specific meiotic recombination in the Prader-Willi/Angelman syndrome imprinted region. *Hum. Mol. Genet.* 4, 801-6; Wu, T.C. and Lichten, M. (1994). Meiosis-induced double-stranded break sites determined by yeast chromatin structure. *Science* 263, 515-8.
71. See Kohli, et al, 1999 (note 56).
72. Ewen, S.W.B. and Pusztai, A. (1999). Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *The Lancet* 354

<http://www.i-sis.org.uk/naked.php> Met toestemming van dr. Mae-Wan Ho overgenomen.

Hieruit blijkt maar weer de alleenheerschappij van de COGEM, in een land der blinden waar één oog koning is.

Verder maken we van de gelegenheid gebruik te verwijzen naar recente uitspraken van Professor Nico Leschot, klinisch geneticus AMC in de Volkskrant van 26 juni jl.

***Onderstaande woorden van hem schokken ons:***



'Het hoofdstuk gentherapie laat zich ook maar moeizaam schrijven. Veelbelovend, maar als er echt een belangrijke doorbraak was geweest, had u dat heus vernomen. Het is een versplinterd veld dat voor tweederde draait om probeersels bij uitbehandelde kankerpatiënten die niets te verliezen hebben. Wat ze in

"Moet je de patiënt alles vertellen?" Wetenschap, Volkskrant pagina 3, zaterdag 26 juni 2010, fragment.

Tenslotte nog de opmerking over het genoemde gewolmaniseerd hout:

Wij schreven:

*Er zou veel meer preventief te werk moeten worden gegaan: zoals het waarschuwen van mensen tegen gewolmaniseerd hout, dat nog steeds slachtoffers maakt. Bij gewolmaniseerd hout loogt het kankerverwekkende arseen en chroom VI nog na jaren uit, vooral bij regen. Dit is bewezen door een rapport van TNO, gemaakt vanwege het gewolmaniseerd gevelhout, buiten op onze huizen. Ook uit gewolmaniseerde speeltoestellen sijpelt het giftig arseen en Chroom VI naar buiten! En denk eens aan al die schuttingen die van gewolmaniseerd hout zijn gemaakt! Zie ook [www.wolmanzouten.nl](http://www.wolmanzouten.nl)*

Onze bedoeling was, te wijzen op preventief gedrag, zodat er op deze manier geen kanker kan ontstaan. (Zorgen dat je dat hout niet aanraakt, het hout niet verbrandt, er niet op speelt –kinderspeeltoestellen- en dat kinderen er ook niet aan likken!) Maar dan moet deze waarschuwing ook door de media worden ondersteund. Het jarenlang zwijgen van de media over dit onderwerp moet doorbroken worden!

Vriendelijke groet,

Miep Bos, ook namens Wieteke van Dort, Stichting VoMiGEN, en De Gentechvrij Burgers (= the European GMO-free Citizens, waarvan ik woordvoester ben).

Lelystad  
miep@gentechvrij.nl  
[www.gentechvrij.nl](http://www.gentechvrij.nl)

CC

[N.J.Leschot\(at\)amc.uva.nl](mailto:N.J.Leschot(at)amc.uva.nl); [NJ.Leschot\(at\)gmail.com](mailto:NJ.Leschot(at)gmail.com)

Mw. mr. S.Y.M. van der Heijden / Mw. mr. N.M.A. Verkleij, Commissie Medische Ethiek,  
cme(at)lumc.nl

Centrale Commissie Mensgebonden Onderzoek ccmo(at)ccmo.nl

cie. vrom(at)tweedekamer.nl Leden en plaatsvervangende leden van de Vaste Kamer Commissie VROM, te Den Haag.

Leden en plaatsvervangende leden van de Vaste Kamer Commissie VWS, Den Haag

cie.vws(at)tweedekamer.nl

Wetenschappelijk instituut PvdD ,SP, VVD, SGP, D 66. Groenlinks, PvdA, CU.

info(at)greenpeace.nl ;

Diverse media

Bijlage 1: : “Moet je de patiënt alles vertellen?” Wetenschap, Volkskrant pagina 3, zaterdag 26 juni 2010, fragment.