Verbatim Transcript of the Advisory Committee on Releases to the Environment (ACRE) T25 Maize Hearing February 20th 2002.

The Advisory Committee on Releases to the Environment (ACRE) held a public hearing on the criticisms of the risk assessment for T25 GM maize on Wednesday 20th February at Ashdown House, Department for Environment, Food and Rural Affairs, 123 Victoria Street, London.

The aim was for ACRE to explore the scientific basis for the challenges to the risk assessment for the marketing approval of T25 maize.

By questioning a variety of invited witnesses and experts, ACRE probed the scientific evidence and arguments put forward both for and against the marketing of T25 maize to enable the Committee to ensure that it understands the various points of view.

Speakers each gave a short prepared statement and were then questioned by the panel chaired by Professor Alan Gray consisting of ACRE members and a delegation from the Advisory Committee on Animal Feedingstuffs (ACAF). This process of evidence taking and cross questioning was open to the public.

This verbatim transcript records the proceedings of this meeting.

Opening Address

Professor Alan Gray, Chairman

Good morning Ladies & Gentlemen. Welcome to this Open Hearing and thank you for coming. Before I introduce the business for today I want to briefly hand over to ACRE's Secretary, Dr Adrian Butt of DEFRA, who kindly agreed to provide the venue for today.

(*Adrian Butt covers arrangements for the day*)

Let me say something about the background and purpose of today's hearing. I will do this by first of all saying what it is we are here to talk about, then I will say who we are (and introduce you to the people around me). Third I will set out the procedure we intend to follow.
As I am sure you know, we are here to examine the scientific evidence and arguments for and against the marketing of T25 Maize. T25 is the code name given to a genetically modified maize produced by Aventis Crop Science – which has been modified to be tolerant to the herbicide Glufosinate Ammonium by insertion of a gene which codes for the enzyme Phosphinothricin Acetyl Transferase (or PAT). PAT inactives glufosinate inside the plant. T25 maize also contains a promoter, which switches on the

pat gene, from cauliflower mosaic virus but has no functional antibiotic resistance marker gene.

T25 has been bred into a suite of maize lines to produce a number of commercial varieties – in the UK there is just one variety of T25 maize – known as Chardon LL (Liberty Link).

The current situation is that, under the existing European Directive T25 has full marketing consent in Europe for cultivation, feed and food uses. First submitted to the French equivalent of ACRE in 1995, European-wide consent, following a full review by all member states was eventually issued in August 1998. ACRE first saw it in 1996.

I should mention two developments since that date. The first is the Chardon LL Public Hearing, currently adjourned, which was convened to consider an extensive list of objections (about two hundred and fifty written ones) to adding Chardon LL to the UK National Seeds List – that hearing and several of the questions it raised will come up during our meeting today.

The second is the agreement forged between Government and industry (SCIMAC) that no T25 maize, indeed no GM crops, will be cultivated in the UK before the end of the current extensive series of Farm Scale Evaluations, due to finish in 2003 – and indeed that no T25 maize silage would be fed to animals in the UK before the end of these trials, and then only if the Government is entirely satisfied with its safety.

Meanwhile ACRE, and other Government advisors, keep the scientific basis on which we advise Government, and have advised them in the past, under continual review. Our purpose here today is a part of that review process – it is to take evidence from those who have raised scientific concerns, from those who produced T25, and from other scientists with known expertise in the fields of science we hope to cover. We are grateful to you all for coming along today.

The areas we intend to cover – and I will return to these in a minute are the issues of risks from horizontal gene transfer, from the transfer of DNA principally in the soil, but elsewhere. We want to look secondly at the environmental risk assessment, and monitoring. Thirdly we want to look at the issue of composition equivalence, and then finally we are going to look at feed safety assessment in general, and in particular one feeding trial, about which many observers have raised concerns. So that is the programme in general. Now on to who we are. We are of course ACRE, which is the Advisory Committee on Releases to the Environment, or in this case we are a reinforced ACRE, because we have some guests from one of our sister advisory committees, ACAF, that is the Advisory Committee on Animal Feedingstuffs. Clearly much of what we are going to talk about later on in the day is relevant to their scientific expertise, rather than ACRE's as such. ACRE is a statutory advisory committee, which means we work within a strict legal framework, within a body of European and UK law, and we are independent experts, mostly scientists, and I will introduce you to us in a minute. We are charged with advising Ministers on possible risks to human health and the environment from the release of, among other things, genetically modified organisms. I say among other things, because we do advise on introducing non native organisms for biological control, and a whole set of other things. It is important to stress we are a scientific committee. Our approach is precautionary, we are science based, we are evidence based. We do not

even consider possible benefits of these crops, of releasing GMs, and nor do we deal directly with issues such as ethical, cultural, societal aspects of GM. Government take advice from elsewhere on those issues. So being rather peremptory, let me introduce the people round the table so that you know who they are, and what their science background is. Dr John Heritage is one of our guests from ACAF. He is also on ACNFP, which is the Advisory Committee on Novel Foods & Processes. He is from the University of Leeds, a microbiologist, interested in antibiotic resistance genes. Ed Cross is a member of ACRE itself, and Ed is a farmer, who has knowledge of practical farming and agronomy. Professor Ingrid Williams is from the Institute of Arable Crops Research at Rothamstead. Professor Williams is an entomologist, with a particular interest in bees, and pollination biology. Dr Andrew Chesson, from the Rowett Research Institute, another member of ACAF, who is an expert on animal nutrition, and is a member of the European Science Committee on Animal Nutrition. Dr Mark Rees next to him is from Imperial College at Silwood Park. Mark Rees is a plant ecologist, with a particular interest in population of weed biology. Professor Janet Bainbridge who is Chairman of the Advisory Committee on Novel Foods & Processes, is a biochemist, a molecular biologist. Dr Mark Bailey is from the Centre for Ecology & Hydrology in Oxford, and his speciology is microbial ecology, and genetics. You have met Adrian Butt. I am Alan Gray, and I am the Chair of the Committee. My particular expertise is plant ecology and genetics, and my day job is Director of CEH laboratory. Dr Penny Hirsch, next to me is from the Institute of Arable Crops Research at Rothamstead, and her expertise is in microbial ecology and genetics. Professor Chris Pollock is Director of the Institute of Grassland & Environmental Research at Aberystwyth. He is a plant physiologist and biochemist. Professor Phil Mullineaux works at the John Innes Centre, and his interest is in molecular genetics. Dr Chris Spray works with Northumbrian Water, and has a general interest in environmental issues, water quality, and the sciences of biodiversity and wildlife conservation. Dr Susan Hartley is at the University of Sussex. Sue is an ecologist with a particular interest in plant animal interactions. Dr Mike Mayo, who is at the Scottish Crops Research Institute is a virologist, has particular interest in plant biology, and finally Dr Ian Brown, who is Chair of ACAF is at the University of Southampton, and his background is in occupational medicine and toxicology. So now you know what we all do. Right. Let us go through the procedure. Now in order to give everybody concerned an equal and fair chance to present their evidence, and it is about evidence taking, we have decided that something like the Parliamentary Select Committee format suits us best, it is most appropriate. I will ask witnesses to come up and join us here at the front table, where they will be invited to give a short presentation in support of the documentation which they have given us. So we have got something to get our teeth into. I'm going to allow no more than five minutes for each of those, otherwise we will be here for a very long time. Members of the panel will then have the opportunity to ask questions of the witnesses at the table, but most of us will be in listening mode for this. We will also have in the audience one or two scientists, which we will bring up at the end of the session on each particular thing, and ask them for any comments they have on what they have heard, or perhaps they would like to be questioned too. So in order that the whole thing stays on track, and does not

become too discursive, we will deal with each subject area in turn, and I would ask witnesses to confine their evidence to the bit that we are dealing with at the time. OK, and you have done that extremely well thank you, in the submissions. Again, to get most out of the hearing, I am afraid, as in a Select Committee we cannot accept comments and statements from the audience, or come back from the witnesses. Those of you have been involved in this, and I know many of you have, I can see some faces who have been on Select Committees with me, but we do welcome individuals to write to us afterwards, and to say if they believe they have been misrepresented, or their a piece of evidence they think was not properly aired, or they would like to draw attention to further details, and we are going to put a report of this on the web site, so you can write to us care of the Joint Regulatory Authority here, at DEFRA, and finally two things that I feel I must rule out. I have several times stressed the words science and scientific, and it will not be helpful to raise generic issues about GMOs, such as their potential global impact, or what consumers think about them. Indeed, unless the science is relevant, and it impacts on T25, we will not deal , we will deal with other crops, other than T25, so some wider scientific issues may be relevant, 35S or herbicide tolerance for example, but others, general discussions about, I do not know, insect resistance, or other aspects of GM, that is not what we are here for today, and second, I want to emphasise the hearing is to hear scientific evidence, it is to help us give the best possible informed advice to Ministers about the safety of T25 maize. We do not intend to discuss here matters of procedure or regulatory issues. If you would like to tell ACRE what to do, or you believe we have been guilty of sins of permission or omission, then by all means have your say, but we will not explore that. That is a sort of regulatory issue. We will take it on board, but the people to address on things to do with regulation are those who are supporting us in this case in DEFRA, in the Joint Regulatory Authority. So we will not deal in a question and answer way with that, but if you want to raise it do. So, let us begin. The batting order is my fault if you want to blame anybody. It strikes me as logical to go from genetics and environment, and risk assessment, through to food and feed issues, and the sequence too I hope, again you can blame me if you do not like it, gives a fair chance for people with different emphasis to appear alternatively, so that sometimes you are first, and sometimes your are second and so on. So I think if you are happy with that, what I would like first to do is to call Dr Ricarda Steinbrecher, and Dr Mae Wan Ho, and if you would like to come and sit up here, then we can hear in support of your evidence.

Chairman

Let us have Dr Steinbrecher here.

(*Friends of the Earth ask to raise a procedural issue. The Chairman declines*)

(i) Genome stability and horizontal gene transfer

Dr Steinbrecher

Yes. I did not expect to be the first because the order you gave on the paper is different, so now you will allow me please to sort my papers, because I was not prepared for being first . OK. First of all I also wanted to mention that my organisation has not given evidence yet either at the Chardon LL Hearings. I have given evidence on behalf of Friends of the Earth, but so I hope that there will be a next round coming up, where I can give further evidence to another topic. Today I shall focus on horizontal gene transfer, which is the area of my expertise, because I have been working in genetic engineering, horizontal gene transfer, and especially also the plasmids for years. My intention had been to focus on the short briefing, the short statement I gave you, but I also was given by e-mail a paper yesterday, which is the background paper prepared by DEFRA, and it is genetically modified GM T25 maize, which is amended in February 2002, so this actually is looking at a lot of the points which have been raised by different scientists, or members of the public at the hearing, and I find that there are a number of points I am actually in disagreement with, and that might be more helpful to you than me just speaking generally about the topic.

Chairman

That would be very helpful yes.

Dr Steinbrecher

So I do not think I will need to explain what horizontal gene transfer is. Just to briefly let you know the points I have wanted to look at, and where I am happy to sort of elaborate further on is No. 1 on Page 4 of the background paper. You are mentioning, not you, DEFRA, but I think it is probably done in collaboration, that the soil organisms in this country already are carrying the *pat* gene. This is absolute news to me. I have not seen any evidence. I know that Jeremy Sweet from NIAB has looked also at that, and has not found any in his preliminary studies, so I wanted to bring a few points to that, because it is true *Streptomyces* is a species or has like a lot of sub species all across the globe. So they are definitely to be found in soil, but the *pat* gene, which has been used, actually is derived, not only from the Cameroon strain, which is obtained or held in tubing, and is called therefore the strain TU 494, but it is even a sub strain of that which is a mutation, which is actually the ES1 sub strain of that, and that is where *pat* is being found. It is not commonly found in *Streptomyces*, therefore I would like to see your evidence which supports that it is found in soil here, the *pat* gene that is. The other point I wanted to raise is that you are stating that experiments have shown that horizontal gene transfer, from GM plants to soil micro organisms is very unlikely. I do not think experiments have shown that they are very unlikely. We are not able yet to conduct experiments in a way to really find all the things we are looking for, but I would like to look also at the Neilson 2000 publication, not just at the publication in this context. The next point, it was

on Page 6 of the DEFRA statement is that the *pat* gene basically is linked to the cauliflower mosaic virus promoter, and therefore even in the unlikely event, as it is said, that it should transfer to bacteria, bacteria cannot make use of it, because the cauliflower mosaic virus promoter is plant specific, and this has been mentioned in a number of various risk assessments from other committees as well. There is a paper on this, a number of papers around which actually state, and I have it with me as well, that the cauliflower S35 promoter is indeed active also in bacteria, and that is a publication which was already available in 1990, and we have further evidence from 1998 by Fleming *et al* as well. So these are just sort of some points. Another point for example is that you are saying that, and it was also in your introduction, that the impact on biodiversity is being thoroughly studied, in the farm scale evaluation trials, and that these tests will reveal whether there is a problem or not, and depending on that, there is going to be planting or not. For me I have not seen any evidence that you are actually looking at the quantitative, but even more so qualitative component of the micro organisms in soil, soil micro organisms and the effect of glufosinate on them is not being studied in a way that would allow you to come to a conclusion the way you said, so I feel like something is really missing in your evaluation, and I would be happy to give some evidence about shifts which have occurred in the spectrum of bacteria, and of other soil organisms when glufosinate ammonium had been applied. Indeed there are plenty of studies from different groups, and again it is as so often in science, that there seems to be contradictory information, but if you have contradictory information it means even more you have to do further research, rather than going on one side or the other. Most crucially I wanted to come to one point at the very end, which is the horizontal gene transfer, not just to soil, but more so to gut bacteria. You mentioned briefly the Kaatz study from Jena University in Germany, who found according to his own words, and it was in the press, that the *pat* gene transferred as well to bacteria and to yeast in the gut of bees. You are saying in, or DEFRA was saying in a statement, that this has not been published yet, in a scientific paper, which is correct, but you say further, "This work has not yet been published, and so it is difficult for ACRE to give a detailed view, but until then there is no reason to disagree with the press notice from the German researchers who conducted the work, that there is little or no risk to bees or to human health". I find that is not necessarily an application of the precautionary principle, that you are saying, "Until then we are taking the word in the press notice that there is no harm, especially since there was no research behind that", and further concerning gut bacteria, you might be aware, and I hope you are aware of the study, and that is the last point within the transfer to gut I am making, there is a study which AGRO-EVO did, which is called disodium N-acetyl L-glufosinate Toxicology and Metabolism Studies, Summary & Evaluation, which was part of the material given to assess it, and in that study it is actually shown that when rats are being fed with the residue, basically when plants have detoxified glufosinate ammonium you will have this disodium N-acetyl L-glufosinate, which is not harmful any more. When rats are being fed with that actually 1% to 10% of that is being turned back in their gut into the herbicides itself, into glufosinate, which means that gut bacteria somehow are capable to do it, but horizontal gene transfer would enhance that, because the gene is coding for a protein which can work in both directions. It can detoxify the

herbicides, but it can also use the residue as a substrate, and turn it back into the herbicide, because this is part of the pathway of the original bacteria, using that as an antibiotic resistance mechanism .

Chairman

OK. I am sorry, I am going to have to stop you, so that everybody gets a chance too. Have you anything to ask? You have covered a lot of ground there, but.

Advisory Committee Member

It is really two points of just clarity as much as anything else. One is in the survey of *Streptomyces* species that contain the *pat* gene that you draw on, is it really true say that rather than a survey of bialaphos producing strains, rather than those that contain the *pat* gene per se, but with no capacity to produce bialaphos.

Dr Steinbrecher

I am not following which point you are addressing now.

Advisory Committee Member

Well because basically, any form of recovery, and survey of screens will look for a resistance perhaps to , will look for production of bialaphos, which if course is produced by the same strain

Dr Steinbrecher

Of PAT.

Advisory Committee Member

That also had contained the *pat* gene, but my understanding is that *pat* gene, in strains that do not produce bialaphos are much more prevalent. Now I am just wondering on a point of clarity, whether you can make that distinction for us?

Dr Steinbrecher

In the studies which I have read, the studies which they have conducted for example in Germany, they were looking indeed for L-phosphinothrycin in that case, and they could not find any in the agricultural soil, which would mean that PAT is not there, if you do not find it, unless you really can prove it is there. Nobody has done that, and the other one example is Omura *et al*, which is 1984 who tested 2,500 bacterial isolates, and found one producing it. So that is the data I have, and I have not seen anyone producing a study for the UK showing that the *pat* gene is present.

Advisory Committee Member

If one delves a little deeper, for example, looking at the data available from the *Streptomyces* genome sequence, it is quite clear that there are very closely related PAT homologues, so why do you not expect that actually *pat* sequences that could deal with

Dr Steinbrecher

I do not think so.

Advisory Committee Member

This herbicide could be more prevalent than perhaps you are indicating?

Dr Steinbrecher

I do not think so. In the tests which they have originally done, with the tubing strain, it was even that bacteria of the same background, of the same strain, which were not the mutation, they actually died, in the presence of bialaphos. Had they the equipment, genetic equipment to produce the enzymes, they would actually have been able to detoxify it, so therefore that points to , it looks as if we have may be, something which genetically might look similar, but it does not seem to be functional, so I think this needs some investigation.

Advisory Committee Member

Chairman, could I ask a second question?

Chairman

I think so, yes.

Advisory Committee Member

The second one is, to your knowledge, or could you point us to any work, in which the 35S *pat* fusion does actually give resistance to .

Dr Steinbrecher

It was done with NPT.

Advisory Committee Member

Yes I am aware of that. I am just wondering about the *pat* gene.

Dr Steinbrecher

I have not , I mean that is an experiment I would love to see as well, completely, and I would like somebody to carry it, but I think , I have not seen

any substantiation for the claim that it is not working, that this combination is not working in bacteria, there has been no scientific evidence provided, to my knowledge, may be you have it, you know, great, then I would love to hear about it, but .

Advisory Committee Member

Thank you.

Dr Mae Wan Ho

Can you hear me?

Chairman

Yes I can hear you.

Dr Mae Wan Ho

OK. Well, I am actually here to speak against the market approval of T25. Representing the Institute of Science and Society, and my main objection is that there is no evidence that it is a genetically stable line, and to me that is the single most important criterion, even before you talk about risk assessment, environmental or health, because genetic stability itself is also a safety issue, and as you said the public hearing was adjourned, or suspended, because there was evidence that it did not pass the DUS test, and now the new EC Directive on Deliberate Release requires strict molecular evidence, and by which I mean event specific molecular evidence, and something has never really been provided in a satisfactory way, and I repeat that failure to depart from Mendelian ratios, which is very often used, is not acceptable, because that is not a criterion for genetic stability. Now there is a large literature on gene silencing, in which the genes are present, but are not expressed, but what I am really worried about is structural instability, which means that the transgenic DNA can come loose, can rearrange or become lost in part or in whole in subsequent generations, because that would change the transgenic line in subsequent generations, so it is from the safety point of view, it might change as well, and it will also increase the chance for horizontal gene transfer and recombination, and now horizontal gene transfer and recombination is not a theoretical possibility any more. It actually has been shown to take place in the laboratory, and in view of the precautionary principle, then it is not for scientists to say, “Well just because it happens in the laboratory, it does not mean it happens in nature”. What you have to do is to prove that it does not happen in nature, instead of which, when subsequently it has been shown by German scientists that it has occurred, then I believe that there has been a very selective interpretation of the evidence by both ACRE, and the scientists themselves I would have to say, that contradict both precautionary principle and good science, which to me are one and the same thing. Now why do we have to worry about transgenic DNA, and not natural DNA? Transgenic DNA is different from natural DNA. First of all they have never existed in billions of years of evolution, and I would

offer some reasons why I think they are more likely to spread by horizontal gene transfer, and recombination, both by design and otherwise. Now first of all all artificial constructs tend to be unstable, and this is actually a text book topic in Bold & Primrose, which we all grew up on. Transgenic DNA typically, is really in a sense optimised for horizontal gene transfer, because it combines viruses and bacteria, and various gene sequences from many different origins, therefore they have homologies to them, and experiments have shown that homology increases the frequency of horizontal gene transfer, 10 million to 100 million fold. Now transgenic DNA is designed to cross species barriers, and to invade genomes. They are flanked by recombinogenic sequences, such the left and right borders of T-DNA, or the terminal repeats of viral vectors, which enable them to jump into genomes, and very often you find that the enzymes which catalyse jumping in, also catalyse jumping out. Certain receptive hot spots now have been identified in the plant genome and also the human genome, which tend to take up foreign DNA, and the serious question must be raised as to whether these also are recombination hot spots, so that they tend break and join, and therefore let the transgenic DNA out. Now there are well known mechanisms in the cell that actively seek out, and activate and eliminate foreign DNA from the genome. Then there are the cell and embryo culture methods, that are generally used to make transgenic organisms. These are well known to introduce uncontrollable or somaclonal variations which persist in the GMOs afterwards, especially in the plants, and there is now evidence that the transformation process itself of making transgenic plants induces further genetic instability leading to chromosomol rearrangements, genome scrambling in other words. So Monsanto's Roundup Ready soya, commercially grown for years was finally analysed by this kind of event specific analysis. Not only do they find that the gene order of the insert is scrambled up, the host genome at the site of insertion is also scrambled up, and there is a large 534 base pair sequence in there of unknown origin, and unknown safety of course, and all that very different from the original data provided by Monsanto. Now CaMV. There is evidence that it makes the transgenic DNA extra unstable. Not only that, it is actually active across the entire living kingdom, including, I have a paper here, drawing attention to the fact that it is active both in frog eggs and human cells. So, in summary, there is no reason to believe that T25 is stable. Furthermore it has especially hazardous sequences, including the CaMV 35S promoter, and an ampicillin resistance gene, that although it has not got its promoter sequences, if it gets transferred into integrons, and these are very well known now in bacteria, the integron can supply them with a ready made promoter. Finally it also has something called the Origin of Replication, that enables the transgenic DNA to be replicated as a plasmid if transferred to bacteria, thereby greatly increasing the chance of horizontal gene transfer and recombination. Furthermore, the Origin of Replication is itself also a recombination hot spot, and at a recent joint FAOWHO expert consultation on foods derived from biotechnology, there was a strong recommendation that transgenic lines containing this sequence should not be approved on safety grounds. I have to say that the people, the scientists in John Innes Institute who discovered the recombination hot spot in CaMV 35S promoter, have also recommended that this promoter should be withdrawn from use, as well as the Origin of Replication by the way.

Chairman

OK. Sorry to cut you off, but it is , we have a lot of things to do.

Dr Mae Wan Ho

I have finished thank you.

Chairman

Thank you very much. Does anybody want to ask, to clear any evidence? You have covered again a lot of ground for us. You made a lot of salient points, and thank you for that coverage. I would, perhaps to both of you really, you both made reference to German science's studies on horizontal gene transfer to bacteria. You raised the concern that this was evidence that perhaps was misinterpreted. I would like to hear your views on the scientific basis for that judgement that you make.

Dr Mae Wan Ho

Sure, I will give you that. I will actually tell you why I think that is the case, and this is contained by the way in the supplement that I have submitted to ACRE, OK. Well first of all they actually found that the kanamycin resistance began to increase at about one and a half years after the sugar beet was planted, and that is the time at which the debris actually has disintegrated, liberating perhaps the transgenic DNA, so I think I find that suggestive that there might be horizontal gene transfer.

Chairman

This is increasing the incidence of kanamycin in the bacteria.

Dr Mae Wan Ho

May I finish, yes?

Chairman

Well what is the point. I just wanted you to make it clear for me please.

Dr Mae Wan Ho

Yes, and then what they have found is that they have found that when they look at total transgenic DNA, when they added it to non sterile , when they add it to , OK, they actually analysed total samples, and they found evidence that there was transfer by dot blot techniques, which of course is not as specific as PCR, but on the other hand, as you know, if you have a PCR, if the sequence is rearranged, then you will fail to detect a positive, so there are problems with the PCR technology. The reason they say they could not find it is because when they isolate individual colonies, and they only isolated very

few, 4,000, they could not actually pin it down to a species, but the total broth of the bacteria grown up from the soil did actually give positive results.

Chairman

For the gene itself, or for the resistance itself?

Dr Mae Wan Ho

Well they used markers, they used different markers for different parts of the transgenic DNA. In fact this experiment was remarkably well done, and I was very disappointed at the way they interpret it, because you see, you know that only less than 1% of the bacteria can be cultured from the soil, so there is a huge area of unknown, and 4,000 colonies is really quite a small number. Now there are other reasons which, if you want you know, I can take you through them, but I do not think you have this?

Chairman

Yes, I did not have advantage of having it in front of me at this moment in time.

Dr Mae Wan Ho

Sure.

Chairman

I think it is very difficult to ask you direct questions. The detail in all these experiments is extremely important, and to watch what is being assessed.

Dr Mae Wan Ho

But it , sorry. But you see, in the precautionary principle as I said, once you know that it has occurred in the laboratory, then it is for you to prove that it does not occur in nature, not the other way round, and we are perpetually misusing and abusing the precautionary principle, to the extent that it is anti precautionary. When you find evidence suggesting that it has occurred, then you interpret it in such a way to say that "Well, you know, this is not really evidence that it has occurred, whereas you have to prove that it does not occur.

Chairman

You also in your statements as well commented quite correctly, that the consequences of those events are important. Perhaps a couple of words from you on that matter would be helpful.

Dr Steinbrecher

I just wanted to add exactly to that point, that quite often we hear that the likelihood is small for horizontal gene transfer to occur, partly that is because it is as Wan Ho just said, it is very difficult to actually look at all the different bacteria and so far we know that at least forty three different species can develop competence in nature, in natural conditions, but there are many more where we just do not know what is the right background for them to develop competence, so they can take up foreign DNA, so in a way it is enough that horizontal gene transfer occurs once, and then if it has an advantage for it to spread, so therefore, to feel safe with the fact that there is only very little horizontal gene transfer. It is not really a safety assessment, because, with the situation as we know with glufosinate ammonium, and I am sure we will hear from Aventis in a moment about this as well, it has been looked at as a selection marker, because in a way it is an antibiotic, so as a selection pressure, it could actually push for horizontal gene transfer, and we do not know, that could be in soil, but it could also be in gut. As I just mentioned there is now possibility as well, and I find that saying that, glufosinate is not, just simply , I saw earlier in a statement by Aventis that glufosinate is not acting as a selection pressure, has got no research evidence to it, and I think that it really , precautionary principle, we really need to do the experiments, and that is I think what most of us actually want to see is more checking, more experiments, rather than saying, "Well the likelihood is not you know, it is just small, so we can ignore it for the time being. We had better not.

Chairman

OK. Thank you very much to both of you. Were there any more questions? Did you just want to ask something while they are there. Sorry. We must move on, but it is important to try and clarify. If you have notes on it, a new paper, new data, we would be very pleased to see it.

Advisory Committee Member

Within theT25 construct, where would you regard the recombinational or recombination sequences to be present? In the 35S promoter?

Dr Mae Wan Ho

OK. It is actually at the 3' end of the promoter, but there is also the Origin of Replication, and the left and right borders, although I know, well I do not know, because all this commercial confidentiality we do not know, whether the left and right borders are messed up or not. Those are also known to be recombination hot spots.

Advisory Committee Member

OK. Well a follow up question, because in your second stage of your submission, you raise health concerns related to the use of the 35S promoter, and I quote "the CMV 35S promoter if transferred to human or animal cells

could activate cancer associated genes as well as dormant viruses that are there in all genomes". Could you describe a mechanism or where you think that might occur?

Dr Mae Wan Ho

OK. Well we have actually had in two papers preceding the short one that I circulated to you, reviewed a lot of evidence showing that the CMV 35S promoter could actually substitute in part or in whole for the promoters of other viruses. Now if you have a pro viral sequence that is missing its promoter in the genome, then that recombination could actually do that, and there is also the , let me just say that transgenesis itself, you know, the process of causing recombination is associated with destabilising the genome, and part of the reason is because they activate transposons, and transposons and pro viral sequences are very related as you know.

Advisory Committee Member

So would your health concerns therefore extend to the intact cauliflower virus?

Dr Mae Wan Ho

Oh no. Well this is something again that we have argued about with critics, that if you have the entire genome, what happens is that it is not the same, because the promoter and the rest of its genome have been together for millions of years, and they have some stability criteria that we do not know about, just like normal natural DNA has its own stability criteria. However if you chop up the 35S promoter, and link it to something else then it, as I say, becomes promiscuous. It is now active across the entire living world basically.

Chairman

OK. Thank you both very much. Right. Now we need now to call up somebody from Aventis Crop Science. I do not know who. Would you introduce yourselves. I think you are coming up in pairs are you, or singly, or to cover the different areas that we want to discuss with you. You are Dr Rudelsheim?

Dr Patrick Rudelsheim

Indeed.

Chairman

Right. OK. And we have got the statement you have made on this aspect of what we are trying to probe today, so would you like to say something about , in support of that?

Dr Rudelsheim

Thank you Mr Chairman, Members, Ladies & Gentlemen. May be first of all yes, for this topic I will be here on the floor alone. I think for the other topics, other experts will join us as appropriate.

Chairman

OK.

Dr Rudelsheim

And again if during the questioning there are other areas of expertise that are required, I think we have a nice selection here in the room for this important meeting. May I first of all also say that our papers will be available on our own web site so that it is fully transparent to the public, and that we will look forward to the outcome of this hearing, and fact finding exercise. We have heard a lot about genetic stability and horizontal gene transfer, and as you have seen, we have tried to cover those two points in this first presentation. For different reasons, regulative and also commercial, Aventis and its precursor so to speak, have developed a policy to be looking for and essentially trying to come with only one particular event, to bring only one crop traits combination to the market. In the case of the Liberty to Link maize this has indeed been the event T25. In order to bring such a line to the market, it has to fulfil a number of conditions which are indeed selected for, which are checked, and typically such a selection starts from a wide range of different transformants, at least I would say ranging to 100 individual transformants. T25 was chosen, after this kind of selection procedure, as what we call the elite event, essentially for a number of reasons. First of all it contains a single copy insert. It is a short insert, considering this kind of sequences. It did not contain a functional antibiotic marker gene, and it indeed functions in a wide range of climatic conditions, also environmental, but as well genetic conditions, and when I say it functions, it essentially means that on the one hand it performs as expected, it provides the tolerance to certain doses of Liberty. On the other hand it does not show any side effects. I think that is an important parameter that we include in the selection process. The *pat* locus as such, the insert as such is indeed stable, it is stable in the homozygous environment, as in the heterozygous combinations, in hybrids for instance, it has been stable over a large number of generations now. You will recall that the first commercial introduction was in 1996, in North America on a large scale, and since people have been using it in different breeding processes. Yes, partly this is done because of the simple facts of Mendelian segregation, which still is a very rough, but very good indication of overall phenotypic performance, but also later on confirmed by Southern analysis, and I believe you would like me to mention here also, because it was mentioned by one of the previous witnesses. There is a long process of back crossing, integration crosses, but also gradually introgressing it in other material. The parental lines and the different hybrids which were produced have been tested and sprayed again in many environments, the spray, the performance as such is a part of the selection of those varieties, as

well by the breeders, as well as by official instances. We know that it has been questioned that there is some doubt on the distinctness, uniformity and stability, but for instance the most recent results from the French authorities on variety registration have indicated that in addition to Chardon, also another twenty one varieties based on this same maize but genetically different have been accepted and passed at the DUS tests. We have many more varieties. Like I said I think we have already been pointing out on our experience, or experience with our breeders in North America, so that actually we can talk now from really large scale experience, where actually we see both the trait as well as the results from their routine checkings. I would like to believe the stability part is there. Horizontal gene transfer has been a very critical issue. It has been a very difficult one, where also the European Commission has funded already from the early days a lot of very detailed investigations, and as was mentioned before, it is not because we cannot see it, that it will not happen. It just shows that a. it is a very unlikely event, and b. that may be our tools are not capable of finding all of these. Already a while ago the Commission has supported this. Like I mentioned there has been work from Polish, also the work from Smaller. We have heard about other instances of gene transfer with for instance the discussions of the work in Jena with Professor Kaatz. All of these activities have been actually very actively followed by the people who are developing also this material. In many cases this material has been included in those studies. In addition to those which I mentioned there may be smaller ones. We are at this point looking together with a French group also who are looking to a very particular transfer of plastidic sequences to other organisms, and it is clear that it is an area that will continue to cause a lot of attention, not in the least because it is so difficult, and it requires really a very tight set up. Knowing this indeed we cannot say today that it is not happening. The only thing we can conclude is that if it is happening it is indeed a very infrequent event, that you actually need a number of conditions, and actually to make it, to force it to happen, you already need a number of conditions to be met. You can create those, and actually as was said, if you can create them obviously it then would lead you to make some kind of additional recommendations. For us we do not interpret precaution as being the factor issue. Identify something, you now have to prove that it not happening any more. For us it means if you identify something , even with low frequency, you will look at potential impact, you will define whether you have to manage this or not, and based on that you will use your precaution in managing the potential risks. So this is also our argument in the case of looking at something like T25 corn. It is to say while today in fact we believe that all the indications that are available would suggest that it is very unlikely, and we are not even going to attempt to put a figure on it, but very unlikely. Then even in a case that this happens, that we can come back to the point of saying Well on top of that it is very unlikely that such a sequence would be expressed, given the promoter sequences and so on, but even then it will lead to the potential expression of one additional gene that we know very well in that new environment, and based on that we do not believe that this is another concern, or that it has created an additional concern. It had been added that actually glufosinate as such will impact also the soil micro flora, and then again this is also something to consider in view of potential gene transfer. We do have information on the effect of the herbicide

on micro flora in general, in some cases in more detail. This is routine when you are looking at screening for herbicide, and again if there are particular questions we have an expert here available, but we do not believe they are immediately related to the topic of today. So in essence we would like to conclude on the fact that on the one hand we think we have introduced in the case of the potential horizontal gene transfer a reasoning and an analysis, and on the other hand that we have sufficient proof to demonstrate stability, and that in both cases we will continue to monitor what is happening.

Chairman

OK. Thank you. Mark do you want to go first? We are still in this area of gene stability and not only the possibility, but the consequences of gene transfer.

Advisory Committee Member

I think just to pick up on the last point about the impact of Liberty on the soil micro organisms. I mean the half life of the herbicide in the soil is, do you know what that is? Does it degrade rapidly?

Dr Rudelsheim

I think it depends on the soil, but I would be more than happy to ask one of our specialists if you like.

Advisory Committee Member

Would that be OK?

Chairman

Yes sure. Dr Sorvig

Dr Peter Sorvig

My name is Peter Sorvig. I am from Aventis Crop Science Eco Toxicology, and I am responsible for the standard tests for micro flora which we do for every herbicide.

Chairman

The question that Dr Bailey was asking was about half life yes.

Dr Sorvig

The question to half life is that the tests run for twenty eight days. We have not any analytical work done during these tests, but we have done it with natural soil from the field, and we can be sure that the half life is the same as we have it from point, so I am not quite familiar with the values on that, but

they are submitted in the dossiers that are public, and everything refers to that.

Chairman

OK.

Advisory Committee Member

Could I ask the same question I asked Dr Steinbrecher. Do you know of any data that shows that the *35S pat* fusion does not express in bacteria, or does express in bacteria?

Dr Sorvig

I have no indication it would be expressed. Again I think the critical part here is what you mean essentially with expression. It goes back to some of the other discussions on methodology, and what a certain trace amount could be. So I think, you have pointed out earlier also this fact of saying, "Well is it the presence of the gene, or is it the effect as such that you are looking for", and again this may be indeed where some of the differences come from, is that people look at a phenotypic screening, like what you would have to do in cases of screening large amounts of colonies, and then having to check whether one survived.

Chairman

One or two people …. cannot hear you terribly well.

Dr Sorvig

Sorry. The point was that actually it may be a difference also in terms of looking whether one colony in a thousand would have the gene present, or actually whether one colony in a thousand would also have the phenotype that would lead to that.

Advisory Committee Member

I would like to ask if there is anything known about the occurrence of accelerated breakdown of glufosinate in soil after repeat application. Is there any evidence, anything known about that?

Dr Sorvig

The glufosinate? No.

Chairman

Are there any more points. Ed.

Advisory Committee Member

Yes I just wanted to ask what evidence you have for saying that the *pat* gene does occur in soil bacteria in the UK?

Dr Sorvig

As was mentioned earlier we go from the point that it is presently in *Streptomycin*. This is a common soil organism. However I think in the particular case, I was informed by our UK colleagues that there is a study performed at John Innes that would suggest this information.

Advisory Committee Member

But is that specifically the *pat* gene, or is it just something that is similar from *Streptomyces*? Do you know for sure that it is the *pat* gene?

Dr Sorvig

Personally I do not know. We would have to consult on that study then, but it is something to check. But we did not make this statement out of .

Chairman

Any more questions? No. OK. Well thank you very much Dr Rudelsheim. Now we have two people in the audience who have some expertise in this area, and I would be delighted if they could just come up, and perhaps clarify a few things. Professor Peter Young. Peter if you could cover some areas in relation to the soil micro flora and soil biology, and I think Professor Pat Heslop-Harrison is here, that is right yes. Your specialism is genome stability, is that right. Good. Would you like to come up as well, and perhaps we could , members of the Panel could ask you anything that is still unclear to them, or perhaps you would like to say something first? Would you like to say something about what you have heard? Some comments about the business particularly of horizontal gene transfer?

Professor Peter Young

I think I would just like to agree with Dr Steinbrecher and the last speaker that an important aspect is the selection on transferred DNA, not just the conceivable occurrence of transfer, and we should not forget that.

Chairman

OK.

Advisory Committee Member

There is evidence that quite widespread gene transfer has occurred between organisms which are not very closely related, over evolutionary time, but

may be not over the sort of time scales that we are able to monitor if you like in our own life times. Do you have any feeling for the time scales over which some of these very widely different organisms may have exchanged genes? I think that would be quite interesting, because we can make statements that transfer could happen, but we have very little way of knowing the sort of frequencies at which it does happen.

Professor Peter Young

That is right, and it is quite difficult to disentangle the rates of transfer from subsequent selection. I think the examples that we have of transfer among bacteria in our life time so to speak, are those where there is very strong selection, and antibiotic resistance genes are a clear example. There is also a soil bacterial example of *Mesorhizobium* that was introduced in New Zealand, and the genes for colonising the lotus plant, which is why it was introduced, were transferred to other bacteria present in that soil. They were related bacteria, possibly the same species, possibly a closely related species. In both those instances the DNA that was transferred was present in a known mobile genetic element, and in both cases there was strong selection for the transferred or the recipient with the transferred DNA, so that is why we saw it.

Advisory Committee Member

On the last question, I mean do you know of any evidence that demonstrates clearly that there is gene transfer between plants and bacteria in a soil, or an associated environment?

Professor Peter Young

I certainly know that bacteria are not full of plant genes, I mean not of bacteria whose genomes have been sequenced, we are quite clear of that. It is not normal for bacteria to carry around large numbers of genes, even though these bacteria are constantly being exposed to plant DNA, but certainly experiments which have shown that under strong forcing you can detect some apparent transfer, have already been discussed.

Chairman

One more question from here.

Advisory Committee Member

But how stable are those in those forced experiments that you stated? Do you have any idea how stable those recombinants may be, in the absence of selection from what you are saying, I presume, you consider that those genes would not remain?

Professor Peter Young

In the absence of selection there would be no reason for them to increase in frequency, and you would not expect them to form a significant part of the ecological system. If, on the other hand they were strongly selected for, then you might expect there to be selection for more stability in the long run.

Chairman

Shall we go to genome stability. Dr Heslop-Harrison. Will you ?

Professor Heslop-Harrison

Yes, just a couple of points that I would like to state. That many of the gene, I mean stability events of the nature that we are seeing in some of the evidence discussed today, are detected at much lower frequency, in normal material that has not been through any transgenic process, and is a reasonably high frequency. It is detected as well, following any tissue culture. It is a somaclonal variation that Dr Ho referred to earlier, and that does not necessarily just follow from the transgenic part, but from the other part of tissue culture involved.

Advisory Committee Member

Can I ask Professor Heslop-Harrison whether he has a view on whether the conventional breeding of agricultural crops would , how that would deal with any issues of genetic instability that might arise by the sort of effects that he has talked about, so is post hoc breeding technologies , what effect do those have on the consequences of any genetic instability?

Professor Heslop-Harrison

The breeding and selection procedures most certainly will select for stability, whether at the chromosomal level of from the activation of transposable elements or retro transposons, so those will normally be selected out, selected for lines that have very low frequencies of those, and I imagine that is what is being referred to in the first sentence of Aventis's evidence here, that they have policy to bring wherever possible, just one highly stable event. It indicates that there are others which are less stable, so they are already creating that selection for stability at the earliest stages.

Chairman

Thank you. OK. Yes.

Advisory Committee Member

Well I am not an expert on genetics, but if I could ask Professor Young, is it a reasonable general statement to say that if one puts in a gene insert, then is it

inherently unstable because it is an insert in the general case, or is that just not so, because that is the implication that I am given to understand here.

Professor Heslop-Harrison

No I do not think it is generally the case. I mean all genomes are unstable to a certain degree. If the insert carries with it a mechanism for insertion and excisions, then by definition those events may be more frequent, but if it is a piece of DNA which has no sequences of that kind, then there is no particular reason that that will make the genome any more or less stable than it was.

Advisory Committee Member

OK, and in this specific case, the *pat* gene, does it have instability characteristics to it?

Professor Heslop-Harrison

I cannot comment on it in detail not having seen the complete construct. I have heard suggestions that it might be, but I will also counter suggestions that it is not, and I think the Committee has to weigh the evidence.

Advisory Committee Member

OK thank you very much indeed.

Chairman

Yes I am just going to allow you one more, because we have taken an awful long time on this first thing, but it is important to clear up anything that you are still uncertain of.

Advisory Committee Member

I would like your comments on whether cauliflower mosaic virus, the promoter is active in plants and fungi, and how thoroughly that has been looked for.

Professor Heslop-Harrison

It is an extremely active promoter in activating the genes that are put in downstream, and very very effective in a wide range of different plants. I am less aware of the evidence for fungi, but it does work in those, although I am not sure about the efficiency, that is personally rather.

Advisory Committee Member

I meant to say bacteria.

Professor Heslop-Harrison

OK. I am not familiar with the evidence about activity in bacteria.

Chairman

OK. Thanks. I am sorry to curtail this, but thank you very much for coming and helping to clear those points. We are going to have to move I am afraid if we are going to cover the ground, into environmental risk assessment and monitoring. Dr Rudelsheim.

(ii) Environmental risk assessment and monitoring

Dr Rudelsheim

Yes.

Chairman

You are batting first this time I think if . I am going to have to be stricter than I have been so far, so I will tell you when the time is up.

Dr Rudelsheim

Quickly I will introduce Jean-Francois Sarrazin, who is heading our Regulatory Affairs to European group, and thereby very much involved with all the recent changes, and evolutions in ideas on monitoring as well as risk assessment, and Miss Jordan who is at UK level responsible for implementing some of those aspects.

Chairman

You are not going to get 5 minutes each, you are just going to get 5 minutes between you.

Mr Sarrazin

Thank you very much Mr Chairman. In addition I must say that I have been dealing with GM plants since 1990, and in particular in 1995, I submitted the Part C file of T25 to the French authority, so I have some experience of risk assessment under 90/220, but also under the revised Directive 2001/18. As a reminder, at the beginning the T25 Part C file has been submitted to the French authority in 1995, and received the written consent from the same French authority, after the European Review in August 1998. The risk assessment provided by Aventis in this Part C file considers the likelihood of the T25 maize becoming more persistent than non genetically modified maize plants are more invasive in natural habitats. It also considers the impact on wildlife such as beneficial insects, birds or other species that frequent maize fields, and potential impact on agricultural species. In relation to persistent

invasiveness field experiments were performed in USA and Europe from 1992 and 1994 to assess the agronomical characteristics of T25 maize, but the only competitive advantage compared to a non GM counterpart would be that when it is sprayed with glufosinate the genetically modified maize survives the application. During millennia of agronomic improvements to cultivated maize, we have resulted in a species that is unable to exist by itself in the wild, and is completely dependent on man for its survival. As was shown from the field data, the transformation with the *pat* gene did not change its characteristics, therefore the likelihood of T25 variety becoming more persistent than other maize is nil. This point was confirmed recently by a study by a colleague of the Imperial College, which showed that genetically modified herbicide tolerant maize, even in an uncultivated area has died after four years in the ten year study. In relation to wildlife, there was no difference in the type of population of beneficial species present on transgenic and non transgenic maize plants. There were also no differences in susceptibility to a range of pests and diseases. As far as volunteers are concerned these are very, very rare in Europe, and if they do occur field studies have shown that they can be controlled by cultural and conventional herbicides as a non genetically modified variety, then there is no likelihood of crossing to other species in Europe, consequently the weed management in the rotation will not be compromised by the introduction of T25 maize. As you know now, the Directive 90/220 has been revised under the Directive 2001/18, and the environmental risk assessment as it is laid down in the new directive, has been a little bit improved. Specifically these new legislative frameworks transcend the principal environmental risk assessment in the Annex 2, and call for a post release monitoring to be established in connection with its environmental risk assessment that is defined in Annex 7 of the new directive. This new risk assessment emphasises that we have to assess the direct and the indirect effects. The direct effect, this has already been considered for T25, particularly in terms of potential effects on long term genetic organisms, therefore in this respect there is nothing new to consider for T25. Concerning the indirect effect, this section refers to the effect occurring through a causal chain of events, through mechanisms such as interaction with other organisms, transferral of genetic material, or change in use or management. We have addressed the issue of transfer of genetic material in a separate paper, and concluded that this is not a significant issue. Thus the only possible change to the environment that could occur has arisen to changes in farm management, and this is exactly what the farm scale evaluation is designed to assess, and then we go to the third point, that

Chairman

Will you be very brief with that.

Mr Sarrazin

Yes, post release monitoring. Aventis has a policy to develop and implement stewardship for a whole new product that they have marketed, it includes quality control systems, technical training, and information exchange, usage records, after sales monitoring and support. Aventis is an active member of

the industry group SCIMAC. SCIMAC developed a set of guidelines and code of practice which provide an industry standard for product stewardship, which addresses a range of environmental concerns and enables customer choice with identity preservation, specifically the SCIMAC guidelines also addresses the need for post release monitoring. In Section 8 - monitoring and for record keeping, section 9 - monitoring and record keeping of the supply chain. The conclusion I would say that also the T25 risk assessment was originally presented via the Directive 90/220. Due to the voluntary agreement, T25 variety will only be brought to the market after the implementation of the 2001/18. The results of the farm scale evaluation, the SCIMAC Code of Practice and Guidelines, and our own products stewardship package will ensure that T25 will be fully compliant with this new directive.

Chairman

OK. Do you want to say something very ? No. OK. Right.
Professor Pollock.

Advisory Committee Members

I would be grateful if you could provide us with a little more information in detail on post release monitoring. I am particularly interested to know what scale this will be carried out on, ie will it be carried by all people that are growing this material, how this information will be collected and collated, and how it will be analysed.
A supplementary very similar one is how are you actually deciding which elements of the ecosystem should be part of that monitoring, and how long it is going to continue in terms of population and generation times, and what you would therefore say would be a significant change, so those are basically just added to Professor Pollock's .

Mr Sarrazin

Meaning a fairly general remark or a fairly general reaction to this, because it is not only relevant for T25 corn. As it was said it is relevant for all of our products in all of the jurisdictions so to speak, and I want to pick up on the point of saying there are the direct effects, and the indirect effects. There are those which are related immediately to the environmental risk assessment, whereas the environmental risk assessment may highlight certain areas of concern, or certain areas where further information is needed. I think those would be areas where in fact we see a role for our own organisation, following in detail. We have already , when I compare it now with another case, we have already elaborated the practical implementation of such a plan at that scale, with designs including the statistical repetition and so on, which has been forwarded to authorities for their approval. On the other hand there are the indirect effects, where of course in fact you are looking for anything else, where in fact we see that also the expertise is not necessarily within our own organisation, and where we have to build a lot on what others can contribute. The Farm Scale Evaluations actually for us are a good example of that kind of approach where you are looking to a lot of may be indirect in some cases

also, related to the introduction and trying to get as much as possible expert, essentially also external experts, which should be may be more credible, as people tell us, and thereby allowing in fact to some extent a more scientific approach, a more research approach to some of these let us say unknown, still unknown or still less developed areas of concern. So I would see the differences between both, and I think we really intend to have a tailored approach for each of those, when it comes to numbers and so on, I think it depends on either case, well what it would be, and how we would do it.

Chairman

Yes sure. We will come back.

Advisory Committee Member

I would like to come back to you on this, because the paperwork that you put forward on Page two, and rolling over to Page three, does commit you to monitoring and record keeping, for increases in volunteer populations, difficulties in controlling volunteers, and unexpected environmental effects. Now I presume that having committed yourself to that, you must have some idea about the scale, and the density of information that you are going to be accruing, and I would press you to give me a little more detail on it, and tell me what you intend to do with this information.

Mr Sarrazin

Yes, this statement applies to a specific case of UK in SCIMAC , where UK being the country where we already implemented this post marketing plans so, I will ask my colleague.

Ms Jordan

OK. Thank you. Yes you would be aware of the SCIMAC Guidelines, where we actually indicate in Sections 8 and 9, the areas of reporting that will be conducted, and indeed the Farm Scale Evaluations have shown that this has proven to be the case, and growers are able to comply with this. This includes obviously recording where the specific crops are being grown, and during the introduction there will be no change to that . Indeed it details all the agronomic aspects that farmers are currently very used to keeping for other accreditation reasons, as well as normal cropping practice, so that they can follow rotations through their normal farm practice, and the agronomy of those particular sites. So therefore we have records of full rotations, inputs, as well as everything else that will contribute to farmers ensuring that they can operate good agricultural practice, and keep the necessary information that they need. Indeed there is an importance to ensure that volunteers are controlled, and growers are asked to put together a programme to support this, and as the consent holders we ensure that that is the case. Can I add any more? Anything more specific or ?

Advisory Committee Member

Well it was really a question to sort of go beyond the Farm Scale Evaluations. Obviously those are high in your mind, and you have answered in relation to those, but obviously in the marketing consent you are not just talking about Farm Scale Evaluations, and they can go so far, and that is what they are intended to do, and I was wondering how you took on board other angles and other aspects that may occur over generations. It is the scale time one, as well as the scale perhaps aerial and geographically as well.

Ms Jordan

Yes. OK. I will speak from the UK perspective, then perhaps I will pass back to Jean-Francois who is dealing with the European and global situation. During the introduction of these crops, which will be, and I am talking beyond the Farm Scale Evaluations, so those first commercial years. We envisage that none of the recording system will change from that that we are currently doing, so growers will record where these crops are planted, seed rates, inputs etc, and the SCIMAC Guidelines, which were actually ratified as commercial guidelines, not specifically for the farm scales, but they were put together for commercial use, actually details all of that, and also mapping of where volunteers exist, and what control methods are taken to control those. So that document, which can be revised and updated as we go through marketing of these products, is a very comprehensive record of what those growers will do in commercialisation. I think you mentioned a species that will be covered. We will obviously learn from the Farm Scale Evaluations.

Chairman

Right.

Mr Sarrazin

Where we can may be give some additional information on what will happen also in other countries in Europe. I see your point in terms of, to some extent, and that is why we said earlier, depending on the detail we have to go, we can only cover a certain range, and when we are looking at still the detailed research type of activities for the indirect effects, I think we are looking at large scale, but still limited, very well controlled, very well designed set up to really see something or monitor it. The second level is those areas where we are to some extent in control, which are the development, the seed production areas and so on, where actually they are already of large scale, but we do have the immediate contact with those people. A third is really when you go commercial, where obviously you may doubt whether it is in the interests of the farmer that we get all of that information from him, and we have to take that into account too when we develop such a plan. In such a case I think it is up to us to set up a system that can track, and that actually already warns those people for certain things they should look after, that it is a kind of warning system, and that we have then the capacity to intervene when something comes in. So it is really I would say, it is a tiered approach,

depending on the level of detail, and depending on the question that we are asking.

Advisory Committee Member

What are your intentions at this stage for monitoring impacts on soil micro organisms?

Mr Sarrazin

For future there are specific points in the post monitoring marketing we co-operate with external expertise, external university studies, and in particular there in France, there is a study that is named Inter Institute Studies, where in that case it was T25 maize has been grown several years in the same plot since 1996, and there is a plan to make an assessment to compare the population of soil micro flora in the T25 plot in the control, where conventional GM maize has been grown, but that is to say that we will co-operate with institutes, and with specialists from the University or from the public research, so the general idea of our post risk monitoring in this area, to participate, to already existing nature of monitoring, like for example in France is committed to vigilance , and in Germany there is monitoring nature led by the BBA.

Advisory Committee Member

Just a point of clarification in your evidence in Page one. You state that in relation to wildlife there were no differences, in type or population to beneficial species, nor were there any differences in susceptibility to a range of pests and diseases. It is not clear to me whether that is referring to the Crawley study, or to your own data. If it is your own data, could you perhaps elaborate, and tell us which species you have considered, and which pests and diseases you looked at just briefly?

Mr Sarrazin

Yes, the main data that we collected at that moment was data from the USA. They are detailed in the Appendix of the Part C file that we provided in 1995, but we could assess a wide range of pests, mainly of pests. Why USA? Because probably you know that there are more pests and diseases in USA than in Europe in maize.

Chairman

OK I am going to have to cut you off there, and give someone else the floor. Thank you to the three of you. OK, and Dr Sue Mayer from GeneWatch.

Dr Sue Mayer

I have brought a few copies of some other papers. Thanks very much for the opportunity to come and give evidence today. Could I just say before I start, I just wanted to check one thing which was not clear from the Secretariat, that

all the members of ACRE have actually had a chance to read and digest the Proof of Evidence that I gave the Friends of the Earth, as part of their Chardon LL Inquiry. Great. That is lovely. Because there is a lot more detail in there than I was able to do in the two pages, and I have just passed round, I am afraid I have not got copies for everybody, but it is just really to make my additional evidence today a bit easier, to try and help it rely on the facts of the dossier, and supportive evidence to address the questions which I have raised at the Chardon LL Inquiry, and as I expect you have realised, that in my written evidence, I have argued that the risk assessment that was accepted for the marketing of the T25 maize, and the old deliberate release directive, just would not have been acceptable today, under the revised release directive, and for three very obvious reasons. One is that it does not include an assessment of the impacts on the environment of the specific techniques and the management techniques as it would be used, namely with herbicide in this particular instance, but there would need to be a monitoring plan now included with the dossier, and approved, and also there is a requirement now which is different than before, and included in the new guidelines that ACRE has produced, to do with impacts on animal health, and through the food chain. I am not actually going to say very much about monitoring, because I want to really look at the dossier, and the evidence that has been supplied. There is nothing in the dossier about monitoring, that has been considered by ACRE or any of the competent authorities before, and just on those grounds alone, it would not be suitable for being approved legally, and of course it is , we have already heard that , I mean there is no substance that I can detect, scientific substance to the monitoring plans that we have heard about earlier this morning, with SCIMAC. That is not an approved, legally binding, monitoring plan, and you have got your own Sub Committee which is looking at this issue. It is very complex, it is going to need a lot of thought, and the monitoring plans, if they are going to really test the assumptions have got to be rigorous and scientific, verifiable, because we want to pick up any adverse effects if or not they occur, as early as we possibly can, so we can do something about it, and that is one of the main reasons for having a monitoring plan, and that is why it has got to be done properly, and there is nothing in the dossier which addresses monitoring at all, and ACRE has not considered it, as part of looking at T25. There is also this question about animal health, which I am not going to say anything more about now, because there are other people looking at it, but please I would like you to look at the new guidelines, and look at the dossier, and look at the information that was provided then, to see whether they actually match up, because you know, that is the key question in terms of our point around whether the T25 maize has had proper assessment, as we would expect it today for environmental safety. But what I really want to do, and to go through, and I have copied a few documents for us to look at, is to see whether the assessment of the possible impacts on biodiversity in the environment were considered at all in the original dossier submitted by Aventis, and that is something that the Minister has now told us as you know must happen, in the new guidelines, and the Farm Scale Trials have been set up to investigate some of those impacts on biodiversity. You would think that that itself would suggest that the dossier was not adequate, and that the current, approval for marketing, should not be kept in place, but there has

been a fudge round this issue quite honestly. On one hand ACRE have told the AEBC, which I am a member of, that the new principle that has been added to the risk assessment process, that they have suggested to the Welsh Assembly, that in fact there is nothing really very much different, nothing of any substance that should cause anyone any concern, so I think it is only fair though, it is only fair to Aventis to actually look at the dossier, and the way in which it was actually considered by ACRE, and to look at the annexes, the risk assessment annexes, the data which was actually supplied, and that is why I picked out these bits from it to go through with you, to supplement my evidence, and I have got three documents here. The first I have labelled SM1, and those are the papers that are concerned with ACRE's assessment of T25 back in 1996. I am sure you are all really familiar with this. It has got the comments of Advisory Committee Members and the Secretariat's summary. So did the issue of biodiversity and effects impacts on the environment come up at that time? Well this was done by post, and in the postal responses from members there is nothing. There are no questions really around environmental impact that are raised at all, and also in the subsequent minutes of ACRE's meeting there is only a couple of lines, it does not raise any of these questions in any manner at all. The Secretariat, and if you go to page 3, and I have highlighted it for you, do talk about the risk assessment a little bit, and the only things that they bring up as main issues of concern are risk of spread, or risk of transfer of traits, and they dismiss those as being effectively zero, but they do in the first paragraph refer to something called interactions of the GMOs with the environment, and it says they are considered in the dossier under certain pages, 33 to 38, and also on page 39, and I have copied those from the Aventis dossier as SM2, and what I would like to take you to first of all, the first few of those pages 32 to 39 really are to do with agronomic implications, to do with survival, the agronomic characteristics, flowering time, germination rates, which do have relevance to survival in the environment, but what I want to look at in more detail is paragraph 43, on the bottom of page 37, goes on to page 38 over, which is this interactions of GMOs with the environment, and I think Aventis have already referred to this work actually in their submission, so it gives us an opportunity to look at the data in a bit more detail, which is quite useful, and you will see there it says that T25 has been field tested in numerous sites across the US, since 1992, and no toxicity or alteration of population levels have been observed for beneficial insects (*audiotape changed*)..conventional hybrid buffer zones. See termination reports too. So obviously the important thing here, the evidence that has been looked at, substance is in Appendix 13, which I have also copied for you, (I am sure you are already familiar with this), as SM3, which are the monitoring reports. Now I have looked at this, looked through the rest of the dossier, and the only reference that I can find to other species, well to insects I will come back to, to birds or other species that frequent corn fields is repeated several times for different ones, is physical confinement, where it says "There were no problems with birds, livestock, rodents or other wildlife invading the test area". So that seems to be the substance of the no effect on other species other than insects. It does actually rather interestingly note that there were no public responses to the test either positive or negative. There is a little bit more on insects, and that comes back in the later stages, where it looks first of all at insect

susceptibility, talks a little bit about pests that were involved, and there is only one reference, which is on the last but one page, where it actually mentions something that might be considered to be a little bit wider, where it says “Lady beetles were a common beneficial insect observed at test sites, but there were no differences in population levels observed on transgenic or non transgenic corn”, and that is it, there is nothing else that was submitted to the authorities about these impacts, and what I suggest to you is that this, when we look at the FSEs, and we look at the volume of work they involve, and despite the lack of information that was available in that original dossier, it is very difficult to know how you know how the statements to the Welsh Assembly that the new guidance makes little or no practical difference to risk assessment procedures or any practice in the UK can be true. I mean it just does not hold up, and it does suggest that the Welsh Assembly could have been misled on that point, and I am not asking you to second guess the outcome of any evaluations that might need to be done, including all these dimensions properly represented, but ACRE I think does have to admit that there are significant differences, and the dossier that was presented back in 1996, under today’s standards would not be adequate, and I would further suggest that it is not appropriate for an important regulatory process and a committee to look at these issues in a piecemeal way, and that it is not possible to pretend that the FSEs and the lack of monitoring, that that is going to be dealt with under herbicide regulations, that does not hold water, and I just hope at the end of this Inquiry that you will be able to acknowledge that it simply would not be good enough under today’s atmosphere. Thank you.

Chairman

OK. I am not sure whether there are any questions.

Advisory Committee Member

Do you have any specific scientific evidence that would lead you to suggest that there are elements of the risk assessment that have been done to date that need to be readdressed, or is your concern more about the mechanics of how the decision was reached?

Dr Mayer

Well I think if you think about secondary impacts on biodiversity it is quite clear, and we would agree that the Farm Scale Evaluations are addressing some of those, and that has now been recognised as necessary, as a necessary part of the evaluation of GMOs, if they are to be under the new directive, and therefore that is an important piece of information that needs to be collected.

Advisory Committee Member

I am sorry but for the record, I must insist that we distinguish between the farm scale trials as a test of the agronomy of herbicide tolerance from the farm scale trials as a test of GMOs. Farm scale trials have absolutely nothing

to do with the fact that the herbicide tolerance was introduced by transgenesis. They are a test of a novel agronomy, and I think it is very important that that is recorded in the record.

Dr Mayer

Well there are two points on that. One is that you know, ACRE has a statutory responsibility to advise on the deliberate release directive, which concerns genetically modified organisms in the environment, and as part of the revised deliberate release directive, there is now a requirement for the scientific advice on GMOs if they involve a change in practice, to be given, and that is the duty of ACRE to advise on that. Whether you think it has anything to do with the genetic modification or not is immaterial I am afraid in this particular case. However, it is also that point that when you grow GMOs in the wider environment, we may find that there is on a larger scale, that there is some impact that may happen, which we did not detect in smaller field scale trials. That could be a consequence of the genetic modification, rather than it simply being used. For example environmental conditions may vary. Some of the things which have happened with herbicide tolerant cotton in the United States under different environmental conditions, for example and with soybean suggest that you can have different outcomes, as the result of different environmental conditions, and those are things which personally I also think they should be, that should be, both at monitoring and of the Farm Scale Evaluations. So I think there are two sides to this particular point.

Chairman

OK. Thanks. No further questions? Now we have in the audience an expert, but I think we did not ? Are you Dr Ingram?

No, no, no.

Chairman

Oh right, I was looking for Dr Ingram. I think we did not actually during the discussion of this thing, and I think the two parties concerned will agree with me, raise issues in relation to cross pollination and hybridisation, and separation distance and so on, some of those things which we have taken evidence for and accountance of before, so if Dr Ingram does not mind, perhaps we will not ask him to come forward at this stage, because I think we have the benefit of a very extensive report which he did from NIAB, and I would also like to have had Dr Jean Emberlin, who also was working in this area, but unfortunately she could not come either, so I think in fairness we will put that one aside, and review the evidence from literature. OK, now I think we are going to have difficulties getting composition equivalence in before lunch, but I understand you have a problem about .

Dr Rudelsheim

Yes one of my colleagues wants to leave at one o'clock.

Chairman

One of your colleagues has to leave. I expect people desperately need a comfort break. Would there be any problem if we had a slight change of order, if we had Dr Howard first, and then asked Aventis to come on next. If we cut our questions down. Your colleague has to leave at one o'clock?

Dr Rudelsheim

He has to leave at one.

(iii) Compositional equivalence

Chairman

Right. Let us go into this briefly. We may be a bit late for our lunch. If necessary we will break in the middle of it, if that is OK, but let us first of all then ask Dr Howard. So we are now going into the area of compositional equivalence between the T25 maize and its non transformed equivalent.

Dr Howard

Thank you Mr Chairman. I would like to thank you for the opportunity to come and speak here today. I will probably be able to save you some time. What I was asked to do was quite limited, which is to look at some of the documents submitted for the licensing of Chardon LL, and commented on them for the Chardon LL Inquiry, and I am sure the Committee have read those documents. Basically, it was stated in the Aventis documents that T25 maize was not materially different. However some of the measurements that I do not think are disputed showed statistical differences, for instance in the composition of fat and protein of fibre between GM maize silage, and silage from non GM counterparts, as well as statistically significant differences in fat, carbohydrate values of the GM and non GM grain samples, and increases were observed in levels of the amino acids, argenine, histodine and lycine, and the analysis of fatty acid composition found the levels of steric, linolenic and aracidic fatty acids to be statistically significantly different from the non GM counterparts, and the levels of linolenic and aracidic fatty acids had values outside the range recorded in the literature. So they were judged to be not of nutritional importance, and really I think the question I was asking was that there are differences here between the parent strain and the GM modified strain, and that this shows that there may well be differences in phenotypic expression between these two lines, and that then that leads us to hazard identification of possible changes in nutritional value, and the hazard assessment that I looked at was taken purified PAT protein from Canola and feeding it to rats, and one of the main conclusions I came to was that really, if this product is aimed at ruminants as a forage maize feed, then they may be eating well in excess of 50% of their diet, and really a suitable hazard characterisation or assessment would be served by doing a feeding study.

Chairman

Right. Does anyone want to add anything? Thanks for being brief.

Advisory Committee Member

Yes, Dr Howard, just some queries really about the data sets that you have actually looked at to come to those conclusions. In the case of the silage, can you attribute any changes in the pattern of amino acids, and indeed in the fatty acid profiles, directly to the maize itself as opposed to microbe reaction during the silage process itself?

Dr Howard

No I cannot.

Advisory Committee Member

So it could well be that some of the changes are as a result of the actual microbial action, as opposed to changes in the maize per se?

Dr Howard

Yes.

Advisory Committee Member

OK thank you.

Chairman

Dr Chesson.

Advisory Committee Member

OK thanks very much. A very small point if I may?

Chairman

Yes do.

Advisory Committee Member

Thank you. You have spoken about compositional differences, amino acids and fatty acids, but maize is a very heterogeneous substance anyway, and there are lots and lots of, if you like, different values for these, if you look at the whole spectrum of maize. Is this particular T25 Chardon LL outside of all that heterogenicity, or is it within that?

Dr Howard

Well for some of the indices that I have just read out it seems to be outside, with respect to two of the fatty acids, but it really, it seems to me that you can identify a potential hazard there, but the hazard has not been properly assessed, and that is quite easy to do, because what one is interested in is the effect of the whole food on the target species, and that has not been assessed as far as I can tell from what I have been presented.

Advisory Committee Member

OK thank you.

Advisory Committee Member

Dr Howard, you suggest that actually the fact that there are compositional differences, and statistically significant compositional differences introduce a hazard, and you ,

Dr Howard

I did not say that, I said potential hazard.

Advisory Committee Member

A potential hazard, but you describe that hazard in nutritional terms. Is a nutritional hazard or a nutritional difference a safety issue, or is it a market issue?

Dr Howard

Well it could be a toxicological difference. That has not been tested. I mean if you are looking at the whole food in a feeding trial, then you are not just assessing nutrition, but you are assessing toxicological aspects, and we do not know, because for substantial equivalence, relatively simple indices, like the total amount of fat and protein and starch and things have been looked at, but that is a chemical test, and really what we are interested in is biological outcomes.

Chairman

Right. Good. Thank you very much indeed. Right now, Aventis. Right you are Professor Phipps are you?

Professor Phipps

That is right.

Friends of the Earth Representative

Chairman can I ask a very very quick question?

Chairman

Yes sure.

Friends of the Earth Representative

Can you explain what role this witness is to do with T25, because all the other witnesses today have either been involved in Chardon LL or are from Aventis. We know the next witness has worked on BST for Monsanto, but we are not sure what the link is with T25 here.

Chairman

Well let him .

Professor Richard Phipps (University of Reading)

May I introduce myself, which I intended to do before that initial comment. I am a Principal Research Fellow at the University of Reading, working in the Department of Agriculture at the Centre for Dairy Research. I have worked for the last twenty years on the production, conservation and utilisation of maize silage for ruminants, and have published extensively in those and related fields. Much of my focus of current work is now concerned with the use of GM feed ingredients in dairy cow diets. I have worked with T25 in the field, and I have published results of feeding trials with dairy cows, receiving other constructs, not the T25. I am a member of the Expert Working Group formed by the International Life Sciences Institute to consider safety assessments for second generation crops. What I would like to do briefly is to present a number of key points from the paper on compositional equivalence between T25 and the non GM maize. I would like to start really by talking about the concept of substantial equivalence, which was formulated by OECD in 1993, and it is important that that was the starting point, and I think this is a very important issue, the starting point for the safety assessment of GM crops, and has since been developed and expanded by a number of other international organisations. This concept is based on the idea that the existing crops can serve as the basis for comparing properties of a GM crop with an appropriate counterpart, considered safe as shown by history of long safe use. Application of the concept is not a safety assessment per se, but merely helps to identify similarities and differences between conventional and GM crops. Differences are then subjected to further investigation. Three possible scenarios exist. The first is that the crops are substantially equivalent, the second it is substantially equivalent except for the introduced trait, or not equivalent at all. A composition analysis of key components including key nutrients, and anti nutritional factors is together with phenotypic and agronomic characteristics the basis for establishing substantial equivalence which has been endorsed by a wide range of national and international

organisations. To date all forty of the crops that have been approved for use fall into the second category, so once substantial equivalence had been established, the focus moved to the introduced trait. Moving to 2000 in Codex Alemetaris they endorsed the concept of substantial equivalence, and they emphasised again that this was a starting point for a safety assessment, and not the end point, as it identifies the similarities and differences between GM crops and appropriate comparators, which may include its nearest genetic comparator, and other varieties that are already on the market, and I think this is a very key point, because what it does is to place the GM material in relation to the other material that is currently being grown, and that is really of fundamental importance. The possible occurrence of unintended effects in GM crops was examined, and the present approach of detecting such effects focuses on the chemical analysis of known nutrients and toxicants, but might also involve feeding studies with rapidly growing young livestock. So here again we are taking it on to the next step. We are not looking at just one issue. The process looks at a whole series of stages. In the future issues of detecting unintended effects might well be helped by molecular profiling. While the concept of substantial equivalence appears to have wide international support, it has been subjected to the criticism that it is subjective, inconsistent, and may not reveal unexpected differences. The OECD initiative in developing consensus documents to describe and proscribe exactly what is to be analysed for what crop is an absolutely major development, and will help to make it uniform, and uniform across the EU states. In conclusion Mr Chairman, the table presented would support the view that compositional equivalence exists between the grain of T25, and its conventional counterpart, and very importantly, demonstrates its comparability to commercial lines, and shows that it is within the normal and expected range, and I would like to stop at that point.

Chairman

Dr Chesson. Are you going to?

Advisory Committee Member

Professor Phipps, you have produced in the table in your document a set of data which does differ from the data that for instance Dr Howard was able to examine. I wonder if you could say something about first of all the origin of that data?

Professor Phipps

The origin of this data was it was presented by Professor Flachowsky at a meeting in Piacenza in November. It was a summary of work that had been done in Braunschweig, and it has recently been accepted for publication, and I do have paper number, page numbers for that. I am afraid there is a typographical error there. The amino acids should be grammes per hundred grammes of protein and not grammes per kilogram of dry matter. I apologise for that issue. It is grain, and it was the isogenic and a T25 counterpart. As a farmer, because I feed a lot of dairy cows, if I looked at those two maize grain

samples, I would be hard pressed to say I want to feed this one or that one, because the differences between them are very small, and I think it is worth establishing that significant differences can occur between crops, whether it is the whole crop, or whether it is grain, for a number of other reasons, such as environmental factors, and the like.

Advisory Committee Member

Yes, Professor Phipps. Can I pick up on another question relating to the data, and that is simply can you give us some idea of the extent of the sample, or the samples that were collected to provide the range data, ie was it one season, was it one locality, or was it a much wider sample?

Professor Phipps

I would have to refer back to Sidu in the commercial maize lines, but I believe it was really quite an extensive set of data. There is another column in there of historical data, which provides a relatively similar range of values.

Chairman

Could you perhaps provide us with a reprint of that?

Professor Phipps

Absolutely.

Advisory Committee Member

Professor Phipps, can I ask you what role, compositional analysis as opposed to feeding trials would have in the breeding of a conventional variety?

Professor Phipps

Well there are many many thousands of new varieties bred every year, and clearly it is not possible to take all varieties to feeding studies. I think if you look at the literature produced so far on nutritional equivalence of the first generation GM crops, compositional equivalence to all intents and purposes equates to nutritional equivalence, and there is a very large body of evidence to suggest that in the literature, and again information, reprints of those papers could be provided if you wanted.

Advisory Committee Member

Thank you.

Chairman

OK. Thanks. Can we go on then, and we have somebody from Aventis. Be very brief please, because I am proposing that we break after this.

Dr Rudelsheim

We will have to be Mr Chairman, because this person has leave unfortunately. I would like to introduce to you Regina Oberdoerfer, who is working in our nutritional department, and she is specifically specialising on all of the GM questions.

Chairman

Thank you. Welcome.

Ms Oberdoerfer

As Patrick indicated already my name is Regina Oberdoerfer. I am for my scientists background a food chemist. I joined the company in 1998. I am responsible for field trials, compositional studies, and also processing experiments. I want to give you a presentation of the T25 maize, its compositional and nutritional analysis as performed by Aventis Crop Science. Establishing substantial equivalence from the starting point of the assessment for GM crops. This assessment then determines what further studies need to be conducted to determine safety and nutritional value. Within the substantial equivalence assessment, a crop can be defined as either being completely equivalent, equivalent except for the specific modification, or not equivalent at all, as was already described by Professor Phipps. T25 falls into the second category. Analysis has shown that the composition is substantially equivalent to any other non GM maize plant apart from the presence of the *pat* gene and protein. Analyses that specifically look at the gene and protein are covered in a separate paper, that details food and feed safety on all of them. When looking at equivalence between GM and non GM varieties of maize it is important to remember that differences must be looked at holistically, due to the wide range of variation that exists in current non GM varieties. This range is due to three main reasons. Maize varieties are hybrid. The first generation of seeds, so called F1, form a specific cross between a male and female parent line. Breeders intentionally use parental lines with as much genetic distance as possible in order to produce a high level of heterosis. There are a large number of varieties available in any one country that offer differences in morphology, nutritional value, and regional suitability. In addition to the genetic variation, differences arise as a result of their environmental and agronomic conditions in which the plant is grown. Maize is primarily grown around the world as a grain crop. In Europe it is grown as both a grain crop and for whole crop forage. In the UK it is primarily used as whole crop forage, which is ensiled and fed to ruminant animals. Coming to the compositional analysis, a number of studies of the compositional analysis of whole crops forage has been conducted around the world, on T25 since 1994. Some of the results are presented in this paper. It is clear from this study, may be you will take a look at Table 1, which is at the end of this report, that the T25 maize fell well within the usual measured range for the components measured. Phytic acid is an anti nutritional factor that binds to some essential minerals, making them unavailable to monogastric animals. As grain maize is a major constituent of monogastric feed this component is regularly tested in

the US states, as part of the regulatory package. Ruminants are able to breakdown this bound, and so such tests are argued to be irrelevant for whole crop forage, but is shown to be lower than many of the industrial standards. As part of the National Listing process independent data was collected by NIAB in 1999 to assess the forage quality of Chardon compared to other UK grown varieties. This is presented in Table 2. This data collected across four sites shows that Chardon falls within the usual range for all of the parameters of whole crop quality measured. It is also important to know that all varieties vary in the quality of crop produced at different sites. Chardon does not react any differently or unexpectedly from that of other varieties. In addition to the above, and as part of our normal regulatory procedure for continually updating data packages, we have conducted additional studies where more T25 lines, and more truly non GM isogenic lines have been compared in different regions, and under different agronomic conditions. These results are currently being collated for submissions under the new regulations. Coming to the conclusion: compositional analyses have shown that there is no difference between T25 varieties and a range of non GM varieties. Thank you.

Chairman

OK. Right. Yes.

Advisory Committee Member

Just a question that is a bit more detailed about the compositional data that you refer to. First of all, you quite rightly say that there are many, you say a number, of compositional studies that have been published since 1994. From that range, very wide range of studies, how did you actually determine which parameters to present in this paper? Because it seems to me what you have quoted are really sort of macro nutrients as opposed to perhaps more interesting detail relating to things like amino acid and fatty acid composition.

Ms Oberdoerfer

The analytical programme which was designed in the nutritional studies is well, in good shape with some recommendations published by OECD, so at the moment we have a draft paper, a consensus paper from OECD, so the programme which should be tested for grain as the main important commodity for maize, is including fatty acid profile, amino acid profile, minerals, vitamins. For silage, they give the recommendation to stick to the approximates, do additional on fibre, and calcium and phosphorous, on minerals, and that is it, so we are in good shape with our programme.

Advisory Committee Member

OK. Thank you. One more thing. At the end of that page, just before the conclusion, you refer to results that are currently being collated. When do you think they will be available, and what sort of detail is in that data set?

Ms Oberdoerfer

Well it is more, as I already said, we modified our design to be able to submit additional data with the new directive or the update of the directive of the novel food for instance, and we will as soon as this directive is in force, we may be able to update our files accordingly.

Advisory Committee Member

You actually submitted a lot of data derived from comparative NIAB trials of existing maize varieties. I wonder if you can tell me what value you believe this data has in relation to assessing the safety of T25?

Ms Oberdoerfer

Well this data is from the National List of forage to check for forage quality, so I would not recommend to take this list for safety evaluation, it is just that I want to state that you have an idea of the natural variation across different maize variety, and you see that, when you compare in the last column, and just look how our Chardon variety fits into the range, it is in the natural range for these parameters, so it is just to show .

Advisory Committee Member

This is probably just a very quick point, but in your Table 1, the 0.07% for phytic acid, is that a typographical error, or is that real?

Ms Oberdoerfer

No, it is correct. I know it is much lower than the reference range I give which is represented, but you must see that the reference range is for grain, and the commodity we have tested is silage, and we did not find any phytic acid literature values on this silage commodity.

Advisory Committee Member

So what was your organisation R&D reaction to that then?

Ms Oberdoerfer

Well we compare it with our non transgenic value, which, it is not listed here, but the value is 0.06.

Advisory Committee Member

Thank you.

Chairman

Edward.

Advisory Committee Member

In Dr Howard's Submission he said that two of the fatty acids had levels outside the range recorded in the literature from some other research, which I think was probably part of your statement for the competent authority. Could you comment on that? Do you agree that that is the correct assessment, and if so how did it arise, and how does it fit in with what you are saying?

Ms Oberdoerfer

Well, directly from here I would not like to comment. I would just take and see on this original study where the single values are, what is the statistical test performed, and, so in the studies which I perform, I did not make the same observation.

Chairman

OK. Right.

Professor Rudlesheim

Chairman would you allow me one small point?

Chairman

One small point, because I want us to have lunch, yes.

Professor Rudlesheim

I think one of the difficulties I see of the exercise of today, which again I start to value more and more, because it does yield some very interesting suggestions, and probably we should have done this much earlier as always, but one of the difficulties of course is that on the one hand you have the fact finding for things which were in the 1995 Submission, and people have already pointed out on that basis today it would not fit with a new template. Of course it cannot. On the other hand we see that meanwhile there was a point in time, and other things have happened, whether it was on the stability, whether it is on nutrition, and so on. So I think this is an important element to take, and I think will be a challenge also for this Committee, is to see on one hand going back in time, and evaluating whether indeed at that point the right decision was made, which I think indeed you have argued, and we seem to confirm with the additional information we bring. On top of that seeing whether there is any additional evidence that would now , not in view of a directive which is imposing a new format, or is imposing new requirements or feeding studies, would suddenly make it that package is no longer complete, and again I think from our point of view, the value really of these kind of hearings is indeed to have this forum, to look for suggestions, and to see how we can further improve.

Chairman

OK. I mean I did say at the very beginning that we would take very seriously the duty to keep these things under constant review. Indeed we are statutorily obliged to, and as the body of the legislation in the UK and Europe changes, and particularly in relation to 2001/18, which we have to implement by October of this year, the meaning of those things, the actual instructions in the new directive of course is something we keep constantly alive, so we revisit any new evidence that we get, and we are revisiting evidence from a long time ago, and I think Dr Mayer made that point too. Now I am going to declare lunch. I would like to speak to the gentleman who raised the procedural issue if I may personally over lunch, so can we come back here at ten past one, and start again on the next topic. Thank you very much. I am told it is a comfort break rather than lunch, but .

LUNCH

Chairman

Phil Michaels who is a solicitor on behalf of Friends of the Earth, and we spoke during the break, and I am happy to tell you that I have not allowed Phil to make his point here. His point was in relation to the conduct of the meeting, and who was invited, and how it related to the previous inquiry, and I am sure that Mr Michaels will make his point elsewhere, but I did not think it was germane to what we are doing here, and there is clearly of course, and again this is something put to me at the break, a good deal of sensitivity about the origins of people, where they come from, who is paying them, and all the rest of it, and can I remind you that what we are doing here, please credit my Committee with the intelligence to factor these sorts of things into our thinking, and that we are dealing with scientific evidence, which is verifiable and falsifiable. If you read Bob May's advice on what we should do, and I was very much involved in drawing up these guidelines on advisory committees, we do have to try and stick with the information which are trying to glean from you today. I will accede to you it is not necessarily the most, the easiest forum in which to do it, and we are in new territory, but I hope you will bear with us while we try and do this in an open way. OK. Right. Let us start then on the next set of issues related to feed safety assessments, and I think the Aventis, Dr Rudelsheim, if you would like to come up first. OK.

(IV) Feed safety assessment

Dr Rudelsheim

For this topic I can fortunately delegate to one of our experts in the Toxicology Department. Dr Eric Debruyne, who is particularly also specialising with a small group of experts on biotic related questions in toxicology.

Dr Eric Debruyne

Thank you. I just would like to start my paper by discussing briefly how the safety assessment is done in general for GM crops, and what is the intention of the safety assessment as we do it in the dossier that we are submitting for registration. The safety assessment of genetically modified crops is designed to identify whether hazards, nutritional or other safety risk is present, and if present to gather information on its nature, and severity. The safety assessment should include the comparison between the food or feed derived from modern biotechnology, and its conventional counterpart, focusing on determination of similarities and differences. We are talking again about substantial equivalence here, which has been described at length, and I will not talk about this. If the new or altered hazard or nutritional or other safety concern is identified by the safety assessment, the risk associated with it should be characterised to determine its relevance to humans or animal health. For GM foods or feeds which result from crop transformation, this involves the safety assessment of each of the key components in the genetic modification. First the parent crop, existing crops of maize in this case. They have of course been grown and fed for many years without any significant problems. Thus a review of the existing knowledge of the parent crop is essential in order to make a comparison. Secondly the transformation process itself, in order to evaluate the potential impact of the modification on the parent crop. Third, a hazard assessment of the gene product, in order to evaluate the intrinsic properties of the new protein, and fourth a safety assessment of the whole crop, based on its equivalence in terms of phenotypic or agronomic characteristics, compositional equivalence. We talked about it. Safety, wholesomeness, and nutritional equivalence. The concept of substantial equivalence as I said has been discussed by previous speakers. I just would like to emphasise once more that compositional equivalence limited to chemical or analytical equivalence should be considered to be only a starting point to evaluate the safety of the GM crop as compared to the non GM counterpart. As discussed earlier in several presentations, all the comparisons of T25 maize to non GM maize has shown that they are substantially equivalent apart from the presence or not of the PAT protein. Hence further studies were conducted to evaluate the potential impact of this presence on metabolic conversion, nutritional value of the whole crop, and general safety of the PAT protein. The gene product assessments or the evaluation of the potential hazard of the PAT protein is identifying the intrinsic properties of this protein, and we have conducted a fourteen day rat feeding study in order to assess those intrinsic properties. The objective of the study was to assess the potential cumulative toxicity of the purified PAT protein fed by dietary mixture to rats of both sexes over a period of fourteen days, and this study is performed in compliance with the OECD 407 Guidelines for the testing of chemicals. In this study the purified PAT protein was administered by feed and mixture to groups of 5 males and female rats at concentrations of 0, 5 000 or 50 000 parts per million, which are equivalent to 0, 700, and 7 600 milligrams of pure protein per kilo body weight per day for a period of fourteen days. Those dose levels were selected since they represent 100 times and 1,000 times the concentration of the pure protein, the PAT protein that can be found in GM oil seed rape, a meal which is the

highest concentration of the protein that we can find in the products that we are currently working with. The pure protein was produced by bacterial culture, and its physical, chemical and enzymatic properties were confirmed to be identical to the protein present in the GM crops. All treated diets were balanced for their protein content by adding as appropriate to the diet a certain amount of soybean proteins, just to reach the same level of added protein into the normal diet of rats, and during the study several toxicological parameters were measured as recommended. Based on these results it was concluded that the daily consumption of
7 600 milligrams of pure PAT protein per kilo body weight per day, which is equivalent to a human dose of 532 grammes of protein for a 70 kilo human being was totally devoid of any observable toxic effect. Just for comparison to deliver this quantity of PAT protein more than 50 000 tons of grain maize would need to be consumed daily.

Chairman

Can I ask you to be quick.

Dr Debruyne

Right. In addition to this specific study, other toxicity end points were looked at, especially dealing with the *pat* gene and the PAT protein rate, comparing it to toxin allergens in protein sequence databases, and no differences were found, no evidence of homology of the sequence with these proteins were found. In conclusion, the results of the study assessment, studies described before, performed and presented industry that US looked at were consistent with the comparative composition analysis presented before, and the crop behaviour assessment. It is therefore concluded that T25 is equivalent to its non modified counterpart except for the introduced traits, the expression of the PAT protein, and the weight of evidence that we have provided by the dossier, confirms that there is no evidence of a significant risk to humans or livestock following ingestion of the GM crop, and that T25 maize is as safe as its non modified counterpart. Thank you.

Chairman

Thank you very much. Are there questions around the table?

Advisory Committee Member

Could you indicate to us the, if you like, the rationale behind the toxicological testing that you undertake. I mean, what has led to that kind of experimental design being deemed suitable for toxicological studies?

Dr Debruyne

Well, we have applied the design of toxicology evaluations that are performed for pharmaceutical or chemicals in general, so we have applied those guidelines that are recommended for this type of product on the gene product

which is different. It is the only difference that we have been able to detect in the GM crop.

Advisory Committee Member

Thank you.

Advisory Committee Member

Again for clarification please. The fourteen days tox study that you undertook with the PAT protein, was that study you described as relating to OECD guidelines, is there an OECD guideline for a study of fourteen days duration, or is in fact what you are talking about is that the outcome measures you chose to use in the context of this study followed those recommended by the OECD?

Dr Debruyne

The OECD guidelines do not state fourteen days, but they state sub acute chronic toxicity, a difference with acute toxicity which is a single administration, so repeated administration, and it is part of the regulatory process, it can be fourteen days, it can be twenty eight days.

Advisory Committee Member

I mean a chronic study would be considered to be a minimum of twenty eight days usually would it not ?

Dr Debruyne

A sub chronic study yes, but a sub acute one would be a fourteen day. It is a repeated dose. I mean this is just to me jargon, whatever you call it to make it a sub chronic. I mean a sub chronic study would be a twenty eight day or a ninety day.

Advisory Committee Member

Well I mean there are real differences between the two sorts of studies are there not? I mean an acute study would look at mortality as the essential end point, whereas a sub chronic study would look for other histological parameters, pathological parameters.

Dr Debruyne

That is what we did in this study. So we applied the same guidelines, but the duration of the study was fourteen days.

Advisory Committee Member

For clarification, you used a bacterial protein in the studies. Did you check for post translational modifications, and compare them with the protein in the plant?

Dr Debruyne

Well the physical chemical characteristics of the protein were compared, and also their enzymatic activity. I think that post translational modification is essentially glycosylation of the protein, which was only tested at the time with doing electrophoresis and Southern blots. That is what was done at the time.

Advisory Committee Member

Part of Friends of the Earth's Submission includes a statement from the Interdepartmental Group on Novel Feed Developments, and it is a quote that says "Target species testing is a requirement of all new feed additives and ingredients, and therefore the inclusion of this as a mandatory part of GM testing seems entirely justified", and I just wonder how you respond to that, because apparently there have not been target species feeding trials with maize silage.

Dr Debruyne

I think at that time no target study was requested, nor was it part of the guidelines. It is now part of the new recommendation of the 2001/18, and as part of the regular update of our dossier we are conducting a target animal feeding study, but at the time nothing was done. The only feeding study that was done was the chicken feeding study that is part of the next topic.

Advisory Committee Member

Yes. When will the maize, the silage feeding study results be available?

Dr Debruyne

It will be part of the new Submission, so the new regulations you know will be in force in October, so from that time, but the study is almost finished now.

Chairman

I think that is . Nobody else want to ask? OK. Thank you very much indeed. Professor Robert Orskov, could you ? So that we get these things right could you declare your interest. I am going to ask everybody to say where they are from, and what , except the Friends of the Earth. I know what your interest is.

Professor Robert Orskov

I work at the Macaulay Institute in Aberdeen, but I am not speaking on behalf of them.

Chairman

No. Right. Good.

Professor Orskov

I am supposed to be an expert in ruminant nutrition. I have written a few books on the subject, and many papers, but I have to admit that the more I learn the less I feel I am an expert, so I mean you might have asked the wrong person!

Chairman

We all share that sentiment I think.

Professor Orskov

OK. Anyway I think this has already been referred to a couple of times, even now in the last question here, but if I was a consumer of milk, and had to be convinced by drinking this milk by the evidence presented so far, I think it is almost laughable. For instance chemical analysis, what does that mean for the final consumer of milk? Absolutely nothing. Nor does chemical analysis of the grain. I should think Professor Phipps would agree with this, the chemical analysis of the grain does not really help us to evaluate the forage as we are going to feed it to dairy cows, and get milk from it. It does not help us at all. Nor does a two week feeding trial to rats or a two week feeding trial of grain to chickens. It is laughable. It has no meaning, and I think what we have to recognise, is that when we are dealing with cattle we probably have to be even more careful, and I think it has not really been discussed sufficiently, although I am sure all of you are aware of it, that when we are feeding ruminant animals we are feeding bacteria. In fact we can almost in the first instance forget about the animal. We are feeding microbes. There has been some talk about soil microbes, and the effect of that, but hardly anything about the first stage of feeding the forage, which is to ruminant microbes. Maybe there will be in the next Submission in October, some evidence of that, but there is nothing so far and it is really quite remarkable that this has been completely ignored. It is such an easy thing to do, and we have many good microbial laboratories which have not been challenged to do this. There are many good chemical analyses which can be used, mass spec. or anything like it to see whether there are any strange peaks involved with any of these things, and examine what these peaks are, if indeed there are any. So, I have just sort of said before, I may be able to be convinced to drink the milk, yes, may be with the present evidence yes, but if I was to convince the human population, which after all is the final arbiter, and he has got some suspicion about animal products already, so we must not make it worse. Because I

think what we have to recognise is that we have got time on our hands. We are in no hurry. Why be in such a hurry to get it out. We have got to get the foundation right. We cannot afford to go around like this, because it is not all that expensive. So I think at least before we do anything else, we should do microbial trials, to use these samples to feed *in vitro* . We do not even need to go in and feed it to the animals. Let us at least see what happens to feeding the microbes first, rather than feeding a chicken on grain from it. I mean that is a lot more relevant. So, let us then examine carefully the microbial metabolism and microbial growth, and the end product of degradation in comparison with non GM forages. It is not a difficult thing to do, and it is not all that expensive either. We can do it in the lab. We do not really need to go out and do large scale feeding trials. We will come to that point later. When we are satisfied with this, then we can take a small group of cattle, and feed them and see what the milk looks like, whether there are any problems and if we are happy about the composition of the milk. I suspect we will be, but I think we have got to be sure. We have got to be sure so that at the end of the day. We then do it on a larger scale, but I do not think we have come to that point yet, to a large scale feeding trial. We have got a lot of background work to be done before we get to that stage. It does not take an awful long time, a year or two can do it, but we do not need to be in such a hurry, because I think that, when we go through this we really have to be sure that at the end of the day, we as scientists with confidence, can at least assure the final arbiter that as far as we are concerned we are happy with it.

Chairman

OK thanks very much. Yes. I am sorry to look at you, but we are .

Professor Orskov

My friend and former colleague!

Chairman

Well provided it is not a former friend!

Advisory Committee Member

As usual you raise a number issues, and I do not think we have got time to deal with them all. I am a little horrified at your lack of confidence in chemical analysis, given the fact that most crops are actually introduced on to the market on the basis of their chemical analysis, and as far as I recall, we commonly use, and we question experiment equations based on chemical analysis, to predict items like milk production. But that aside, I take your point entirely about the value of working with ruminant animals for materials designed to feed ruminants, but from a safety perspective, I wonder if you could comment on how large an effect you would expect to detect, given the natural variation we find between ruminant animals, and the relatively low numbers most people can afford to use in those sorts of experimentations.

Professor Orskov

Yes thank you. First of all, when I talk about chemical composition, I talk about chemical composition of the grain, when what we are doing is to feed the forage, at least, OK the chemical composition of the forage can certainly give us some indicators, but not the chemical composition of the grain. I am sure you agree with that. Now when you come to ruminant animals, yes obviously we will have a variation, and there is a variation in the products we produce as well, some produce beef some produce milk, some produce wool, and there is a great deal of variation, but before we get to this stage, let us just go through the first stage of the microbes. Of course, then we can argue about how many tests you do of that, whether you should do it with different populations. When it comes to animals itself, I think we would probably have to look at what sort of spread are we dealing with, and then we have to talk to a statistician as to how large the experiments have to be to reach a a probability. At the end of the day it becomes a question of what is the minimal probability you want. I mean it is all a risk assessment is it not?

Chairman

OK you do not want to come back on that? No. No more questions? OK. Right.

Advisory Committee Member

Are you aware of any occasion in which feeding trials or indeed *in vitro* studies on ruminant microbe digestion have thrown up results which are anomalous when compared to straight chemical composition?

Professor Orskov

Oh very much so.

Advisory Committee Member

Could you give us an example of the sort of systems where those anomalies have come through?

Professor Orskov

Well what would chemical composition tell you about accessibility to cellulose for instance? You can have forages that vary from 5% protein up to 12% or 13% protein, with no change in digestibility. So it really does not follow at all. When we are dealing with microbes, their access to substrate cannot really be determined by chemical composition.

Chairman

Right, thank you very much indeed Professor Orskov. I think people have covered those points. Right Professor David Beever from the University of

Reading. Professor Beever, could I again ask you as it were, to declare where you are coming from?

Professor David Beever (University of Reading)

Thank you Mr Chairman. I hold the Chair of Animal Science at the University of Reading, have done so for 9 years. I am the Director of the Centre for Dairy Research, also at Reading. I was previously, for several years a scientist at the Grassland Research Institute. I am a nutritional physiologist, with an active, almost sole interest in research with respect to ruminant livestock. I have an interest in GM feeds for feeding to dairy cows and beef cattle over the last four years. I am involved in some joint studies with dairy cows with Professor Phipps. I have written several independent and peer reviewed papers on the subject. What I want to address very briefly today is some of the concerns that people have had in respect to introducing genetically modified feeds, feed to farm livestock, and the impact this may have on the health of the animal, on the product composition, and also any adverse health effects that may occur in humans that would perhaps be consuming either that crop or that animal. Well the latter one is not necessarily an area where I can make much comment, but at least I can add something in the first two respects. Over this period of time we have, or I have with one or two colleagues, actively reviewed some areas of the literature, important areas of the literature, and I would draw your attention to an estimate that we made, to put it into context, that a dairy cow consuming about 24 kilos of dry matter, which is a very fair estimate, would consume about 60 grammes of DNA a day. Now if all of the forage perhaps, say 60% of that ration was as maize silage, or may be as maize grain, then we can on that basis, determine that the actual novel DNA would be about 50 micrograms to 60 micrograms per day. Now that is quite a small amount, although obviously if it retains its structural and functional integrity then that still could be quite important. I also refer you to the work that has been done on feed processing, and quite a lot has been done, bearing in mind a lot of ruminant and all non-ruminant feeds are processed, prior to delivery to the animal. I refer particularly to MAFF work, done by Professor Forbes in Leeds, where he showed that grinding and milling had no effect on the disruption of the plant DNA, but with increasing temperatures above 95^{o} C he showed partial and subsequently total disruption of that DNA. He also confirmed that with cooking at high pressure, and obviously a lot of ruminant feeds are processed in mills, and undergo pressure as well. The results of Forbes' studies were confirmed when he took commercially available feedstuffs, and showed that there had been virtually no disruption of DNA in intact soybean and rape seed, but when he looked at extracted soya or extracted or expelled rape seed, which is often what is fed, to farm livestock, there was evidence of considerable disruption. In this process of trying to understand for my own benefit, a little bit more about all of this, I looked back at a wealth of literature that was obtained in the early mid 1980s for a completely different reason, but very sound data from the now defunct National Institute for Research in Dairying at Reading, by M^{c} Allan, Smith and others who were looking at the fate of DNA and RNA, of plant origin, and also microbial origin in ruminants. Reference has been made previously, that ruminants synthesise a lot of

microbial DNA and RNA in the rumen. That then subsequently passes to the small intestine with perhaps only small amounts of intact plant DNA, and thereafter there are extensive processes in which that DNA and RNA is broken down, and appears to be lost prior to the terminal ileum. So there is fairly convincing evidence that the processes in the healthy animal are there, both in ruminants and in non ruminants for breaking down plant DNA and also microbial DNA. The situation is not quite as clear with respect to proteins, for whilst we know a lot about the protein digestion in ruminants and also in monogastrics, there has been much less work focused on specific proteins per se. I can only draw your attention to the study by Wherrman which is one which looked at the digestive fate of PAT protein in *in vitro* studies showing almost complete digestion in five minutes in a media which contained pepsin at a pH of 6.5. There have been now quite a lot of studies throughout the world looking at the possibility of novel DNA or novel proteins, finishing up in animal products. We have not got the time to refer to all of those here. I took first the work of Faust from Iowa State, who failed to detect BT protein in milk of cows, and showed that when adding BT protein per se to normal milk from non GM fed cows a 100% detection of the BT protein. So that was quite reassuring data. Similar work in the States, also in Europe have failed to detect BT protein or BT DNA in muscle spleen eggs, dark and white muscle, and also in liver. We have recently reported no DNA fragments in samples of milk taken from cows fed on GM ingredients – GM maize and GM soya. Neither of them were T25, although we are working on T25 at the moment, and I also refer you to the work of Einspainer and Flachowsky in Germany, who perhaps have led this area, with some excellent work, and again confirming and adding to the data that Faust and her colleagues found in the States. And finally there is a large body of data out there, indicating to date no adverse effect on animal performance and animal health, and just one reference which may be worth referring you to is that by Clark and Ipphaguerre, who reviewed twenty three studies. So in conclusion Mr Chairman, on the basis of the evidence that I have presented with the research that I have done, I feel that there is a lot of data out there relating to current constructs, I have yet to find any evidence which would suggest other than that we conclude at the moment there are no issues of significance with respect to animal health, animal performance, or the composition of the products produced by those animals that may ultimately be eaten by humans.

Chairman

Ok thank you very much. Sorry I keep looking across to you because of your expertise in this area.

Advisory Committee Member

Thank you Professor Beever. In the first part of your paper you discuss the potential for breakdown during harvesting, processing of animal feeds that are fed particularly to ruminants. Do you actually equate evidence of breakdown with evidence of safety?

Professor Beever

I have to be guided by the authors of that work in respect that all they were reporting was the extent of the degradation of the DNA molecule to the point where they believed, it was their conclusion, that there was no longer, at the ultimate extent of degradation functional integrity of that DNA. That is for others to judge on the basis of the results which are extensively reported by Forbes.

Advisory Committee Member

Thank you. It could also be argued that, although there are a number of studies which have attempted to detect fragments of DNA, and in rare cases proteins coming from transgenic events, these remain relatively few in number, possibly ten or so at this point in time. On the basis of the evidence that currently exists, would you advocate routine testing of animal products, eggs, milk in particular for evidence of the presence of fragments of transgenes, or the presence of protein, and what do we know about the science of uptake of DNA into things like the mammary gland or into eggs, that might raise cause for concern?

Professor Beever

I refer my comment to those GM constructs that have been extensively tested, and on that basis I am getting to the point where I feel that more exhaustive testing of milk and meat, and eggs as you suggested, may becoming almost a waste of time. There is certainly no evidence with the current generation of GM crops. That does not mean for one minute that I suggest we would not test when there are other situations, where we are looking at new constructs. As to the possibility of fragments of DNA or novel proteins being incorporated into tissue, which would probably be the easiest, or into milk or eggs, the possible concern I would have, and I think it is an area that I would be interested in perhaps pursuing a bit more by literature search would be what happens in the less healthy gut, and that at the moment is an assumption we are making.

Chairman

Anybody further, no. OK. Well thanks Professor Beever for coming and giving us that evidence. Right. Our next people are Emily Diamand and Adrian Bebb from Friends of the Earth. I guess you do not have to declare your interest, but you are very welcome to do so!

Ms Emily Diamand, Friends of the Earth

Well my name is Emily Diamand. I am the Senior Research Officer at Friends of the Earth, and for the last four years I have been involved in compiling our objection to Chardon LL hearings, the evidence of which is in those files. Friends of the Earth is pleased to have this opportunity to address the Open Hearing. Our concerns go far wider than the matters addressed in

this Submission, as you can see by the evidence in these files, and that is just one set of evidence by one objector to the Chardon LL hearings of which there were over 200. However due to the serious time limitations placed on witnesses giving evidence in this forum, FOE does not propose to repeat or summarise any of the points made by its experts in the context of the Chardon LL hearing, and confines itself here to making four short, but we feel important points concerning the serious concerns expressed by the government's own Interdepartmental Group on Novel Feed Developments, the Committee's responsibilities in line with the Code of Practice for Scientific Advisory Committees, the role of this Hearing in the light of the government's guidelines to departments on scientific advice, and in the light of the Phillips Report on BSE, the importance of taking precautionary measures in the face of uncertain risk. According to documents supplied by the Food Standards Agency to Friends of the Earth in November 2000, three members of the Interdepartmental Group on Novel Feed Developments established to co-ordinate policy on animal feedingstuffs across government were consulted in 1996 about the safety of T25 with respect to animal feed. These officials including a representative from the Central Veterinary Laboratory raised specific concerns about the quality of the scientific evidence put forward by Aventis, in particular criticisms were made about the compositional analyses, the claim that T25 is substantially equivalent, the phytic acid analysis and the PAT digestion experiments, and we have presented these papers to the Committee to have a look at. I do not actually think that we are going to have time to go through the whole paper, but if I direct you to paragraph 2 on the first sheet, you will see that it was stated "I would consider the number of replicates generally to be insufficient to conclude substantial equivalence, and would expect to see the effects of location, only one site used, and season, dated from only one year 1994 to be compared before substantial equivalence could be claimed. Furthermore the data presented, even from limited replication, demonstrates significant differences in some of the main chemical constituents namely NDF, ADF and carbohydrate content. It is becoming increasingly evident that these chemical analyses themselves do not entirely describe the nutritive value of a feed, and if substantial equivalence is to be claimed for nutritive value, then it is essential that animal nutritional studies are undertaken". In a separate letter which is included on the next page, another member of this group stated, and I direct you here to paragraph three, "The comparative testing of modified and unmodified product in target species should be used to demonstrate that nutritional value and toxic thresholds have not sufficiently changed. This type of test is non specific, but has the advantage over the current approach of assessing the overall response of the target species. Although no such tests would positively prove total absence of risk, it would be a useful additional test of product safety, and substantial equivalence." And then in paragraph 4 it states "Target species testing is a requirement of all new feed additives and ingredients. Therefore the inclusion of this as a mandatory part of GMO testing seems entirely justified. The current concerns over BSE mean that MAFF must take the precautionary approach towards the introduction of feed ingredients into the food chain. The concerns raised by these scientists appear to have been accepted, or we feel that they have been accepted by ACAF and DEFRA in its review of T25, and in a letter from the ACAF

Secretary dated 5 September 2001, it was stated by the ACAF GM Sub Group that it considered that its, and it refers to T25's substantial equivalence should be confirmed before it may be concluded with confidence that T25 silage poses a low risk to animals. However when Friends of the Earth questioned the Foods Standards Agency as to why animal feed experts were consulted, but their concerns ignored, we were told that the ACNFP had already considered T25, and had advised that the composition of the grain in silage did not differ from that of conventionally bred maize. On this basis it was concluded that there were insufficient grounds for requiring additional data for feed safety purposes. FOE would like to remind the Committee members that the ACNFP considered T25 maize in February and May of 1996, and this was two months prior to the IDG being consulted. The Food Standards Agency has not provided Friends of the Earth with documentary evidence that the ACNFP members were made aware of the concerns of the officials, and considering that the terms of reference of the Interdepartmental Group on Novel Feed Developments was "To review the implication for human health, and animal health of new developments concerning animal feed materials, and to advise Agriculture and Health Ministers where necessary". We are extremely concerned that their serious concerns, and their clear recommendations that further evidence should be sought on the safety of T25 were not put to the ACRE Committee. In fact MAFF wrote to the ACRE Secretariat in July 1996 stating that "MAFF has not identified a need for any further information in this notification, and noting that we are content for a favourable response to be forwarded to the Commission". It is therefore clear that in 1996, serious concerns were raised by members of a government advisory body about the safety of T25 maize for use in animal feed, and clear recommendations were made that further evidence should be sought. As far as Friends of the Earth is aware the ACRE Committee and possibly the ACNFP were not made aware of these concerns and recommendations. ACRE's decision to recommend that UK Ministers take a favourable position with respect to T25 was therefore as far as Friends of the Earth is aware made on the basis of, at the very least, incomplete information from the Ministry of Agriculture. Since that time Friends of the Earth has asked independent scientists to examine the dossier put forward by Aventis, and these scientists, several of whom have been called today as witnesses, have also raised concern about the quality of science, as well as calling for further evidence. FOE considers that when reaching their conclusions and providing their advice in this matter, the Committee must take into account the uncertainty of the current state of the scientific knowledge. We remind the Committee that the government's guidelines to the departments on scientific advice state that departments should not require experts to come to firm conclusions which cannot be justified by the state of scientific knowledge. On the basis of the currently available evidence Friends of the Earth does not believe that the Committee can or should justify firm conclusions on the environmental, animal feed and human health risks of T25 maize. Finally, the Phillips Report into the handling of the BSE crisis recommended that the importance of precautionary measures should not be played down on the grounds that the risk is unproved. In the light of the experience of BSE, the serious concerns raised about T25, the hotly disputed nature of the scientific evidence put forward in favour of it, and the extremely irregular procedures

used during its initial assessment in the UK, Friends of the Earth strongly recommends to the Committee that they ask Ministers to invoke Article 16 of Directive 92/20, and that they ask Ministers to prohibit maize from sale until such time as there is clear evidence on its safety.

Chairman

OK. Thanks for that. Anybody want to ask something specific on that? And then Mr Bebb go next.

Mr Adrian Bebb, Friends of the Earth

We are together.

Chairman

You are together. Do you want to actually say something as well?

Mr Bebb

No I think I will wait for questions.

Chairman

OK. Right.

Advisory Committee Member

I would just like to make a couple of comments really for the record, because I think it is very important. Today's hearing is not concerned with human food and feed studies, but the fact that, in addition to the ACRE Committee, ACAF, and ACNFP are represented, I believe indicates that within our own very specific roles and remits there is very much a great deal of communication, and if you like a joined up approach to the scientific deliberations, which I think is very very important. In addition, you have referred quite rightly to a decision by ACNFP in 1996, but again for the record, I would like to refer you to our web site where you will find out that both in the fiftieth meeting, which was in July of last year, and our fifty second meeting, which was in November, and indeed in January this year, we revisited issues in the light of more recent knowledge, and again the conclusions of those deliberations will be on the web site. So I think you know, your assertion if you like, that there is not a joined up approach, and that ACRE should consider human feed issues is completely wrong. It is without the remit of the ACRE Committee in today's hearing.

Mr Bebb

Can I come back on that

Chairman

Please do.

Mr Bebb

Or add on to it really. I would certainly hope there is a lot more joined up thinking than what happened in the early stages of T25. I think, you know, we did not have the Advisory Committee on Animal Feedstuffs at that stage, but the government had set up their own group to look at the safety, and the testing done on T25, and what these minutes that we have circulated indicate is that they had serious concerns, and they were not echoed, from what we understand either to ACNFP or to ACRE that they had these concerns, and I think you know, this has got to be addressed, and got to be looked at, so yes, we agree, we certainly hope that procedures are much better now, but when this went through, and when it was given the go ahead for commercial consent, then there were serious question marks over its safety.

Advisory Committee Member

Thank you. A rather more specific point. You, and I think one other speaker made mention of the additives directive, and the routine use of animal feeding studies required at least under the EU Additives Directive. I think it is worth making the point that actually, the Additives Directive requires a tolerance study, using a minimum of a tenfold concentration of the additive, and the purpose of that is both to assess the possible margin of safety or to give information about a margin of safety, and to consider the consequences of accidental overdosing. Now the trouble of course is that that is not applicable to many feedstuffs which are incorporated at much higher concentrations in feeds, and so you cannot apply the same rationale to animal testing for additives, and for feed ingredients, and I wonder as a consequence of that, what you think the purpose is of a feeding study in relation to a safety assessment.

Ms Emily Diamand

In the context of the GM crop you mean?

Chairman

In the context of T25 if you like.

Ms Emily Diamand

Well I think that it is not really our place to make that kind of comment, because you have just had a witness who has been through that. I mean obviously we are very concerned that there should be an adequate number of safety tests done, and we would consider a feeding study to be part of that. It does appear that it is widely being accepted at the moment that this would be part of the procedure, and that has been stated several times as well, and

therefore we feel that that should have been applied to T25. The comment about the Additive Directive was not actually made by us. It was made by a member of the Government's Advisory Committee at the time.

Advisory Committee Member

Thank you for your Submission. Many of your criticisms were in relation, not so much to a problem with T25, but a problem that it has not properly been looked at, and many of the studies were insufficient, not long, wrong species, but is there anything you would like to draw the Committee's attention to in relation to a problem that has arisen with T25, even if it is a small one, that you are very unhappy about, that the present studies have thrown up, and I am happy for you to say, "Well the studies were so inadequate that is impossible to say" but there have been a number of studies done on it, and is there anything at all you would want to draw the Committee'' attention to on T25 that has so far occurred, that you are very unhappy about?

Ms Emily Diamand

Yes, we are generally unhappy about the quality of the evidence supporting it. I would not like to make that comment, because there is another witness to come, and I think it would be inappropriate for me to actually make comments about the science, when there are other people yet to speak about it. I think we are particularly concerned that some of the science is so inadequate you cannot really draw any conclusions about its safety, and that is what we are very concerned about.

Advisory Committee Member

Can you draw any conclusions about perhaps a hazard that has occurred within the evidence we have so far, or has it been so bad that you cannot draw any conclusions at all?

Ms Emily Diamand

I think there are hazards that we would be concerned about. There are hazards, well there are lots of potential hazards. There is a potential hazard from the impact of horizontal gene transfer, there are potential hazards that could arise from the change in the composition of the maize, which because of the way it has been examined, it would be difficult to find out whether that had been done or not. The problem that we have is that we cannot say to you "Oh here is a study that shows potential hazards", because we do not feel that many of the studies have even addressed that kind of issue, so you know, we are in a position where we are basically saying what you have just said, that we think there are lots of potential hazards, but we cannot tell from the evidence whether they have actually been addressed or not.

Advisory Committee Member

OK thank you very much.

Mr Bebb

Well I would just like to reiterate our point that you know, we have produced ring binders of evidence to Chardon LL hearings, and we have only had to submit two sides of A4 to this particular hearing. I just want to reiterate that point, and our concerns are much wider than just how the animal feed assessment was given, but also it is quite clear from today that there are disputes about some of the scientific findings. There are acknowledged gaps in the evidence, the environmental risk assessment seems to go walkies. There are a lot of concerns out there, and you have told us that you are going to take a precautionary approach, and we kind of question well, how many more excuses do you need, before you actually implement, or advise Ministers to implement some sort of safeguard clause, and say "Come on, enough is enough here, and we need to look at this more thoroughly to look at the long term safety of it?"

Chairman

I think that covers our general coverage of equivalence and feed testing, and the sort of studies that need to be done in relation to assessing feed and food safety, and there is finally some discussion about a particular study which has raised concern from some observers. Now who is going ? Dr Toby Knowles, is Toby Knowles here. You had a colleague who could not come sadly, but .

Dr Toby Knowles

I am afraid so yes ….

Chairman

Again would you declare your interest Dr Knowles, and tell us where you come from, and what you have been working on.

Dr Toby Knowles

Certainly. I am Toby Knowles. I am from the University of Bristol. I am a professional member of the Royal Statistical Society, and also a Fellow of the Royal Statistical Society, and my professional status means that I am bound by certain guidelines so, I am meant to give you an honest and objective opinion.

Chairman

Good.

Dr Knowles

Yes, Dr Kestin sends his apologies. Really I would like to just read through our statement. I think we have got everything down here, and if I was to do it must off the top of my head I might .

Chairman

It should not take more than five minutes.

Dr Knowles

We were asked by Friends of the Earth to analyse the methods, results and conclusions of the report, the effect of glufosinate resistant corn on growth of male broiler chickens, and we examined the report as though we were looking at a scientific paper that had been sent to us to be refereed, and in the document that we have sent we have summarised our main concerns, and we are very concerned that science of this standard should actually still be discussed at this level. I mean it should have been picked up a long time beforehand that it was sub standard. The chicken study. The report documents a small trial, where two groups of broiler chicken were fed different diets, one based on normal maize, and the other based on maize genetically modified to be resistant to the herbicide glufosinate ammonium. The growth and performance of the broilers for forty two days was measured, and this is a standard production cycle for broilers. The study reported that no significant effects were found. Now a study of this nature is essentially looking for a difference when there is not expected to be a difference to be present, so it is an equivalence study. It is impossible to actually scientifically prove that two things are not different. You just have to go towards an acceptable level of no difference. So it is essentially a very intricate and difficult thing to show. The study overall reported that no significant effects were found. There was a trend for the variance in live weight of the GM fed birds, and the variation in most other variables to be much higher than for the birds fed the non GM maize and it should not be considered as evidence of anything. Our first comment is that there is inadequate replication in the study to identify any meaningful differences in growth or mortality. In the study, four pens of treated birds were compared with four pens of control birds. The number of birds in each pen is irrelevant, and there were over two hundred birds in the whole study, but individuals birds cannot be considered independent experimental units, because there will be some correlation of the results of birds within pens, and to take a very conservative approach, you would use the pen as the experimental unit, so in essence you have only got eight experimental units in this whole trial. You are looking for a difference in a small number of experimental units, you are very unlikely to pick up anything but a major major difference. Individual birds cannot be considered to provide independent measurements of treatment effects. This is because all birds in one pen can be affected by a common growth depressor, for example a malfunctioning drinker, or growth promoting, for example a better climate effect. It is an elementary fundamental error in experimental design which invalidates conclusions drawn from the study. Because there are only four measures of each diet, the power of the study, which detects differences, is very limited. It would only identify very large differences in the effects of the two feeds. Given the variations in body weight at forty two days, and the small number of experimental units used, I back calculated, and the study using the normal accepted parameters for a study of this sort would require a difference of 5.48% in live weight at forty two days, before you could say that

there was a difference between the live weights of the birds in the two groups, and I would suggest that is much too large a difference to look for. To put that in context, if you were a broiler producer you would be interested in a difference of 1%. It would actually affect your profits quite considerably. Comment number two. The methods, this is in the actual report, are inadequately described, to allow this study to be repeated, and this is a fundamental omission, and necessary for any scientific report, and the example we give, the genotype of the birds was not identified. Comment 3. A further major flaw in the design of the study is that there is no positive control. A third treatment should have been included that was known to generate a difference on growth, and this would allow you to identify that the study was actually working. Now this is critical for these equivalence trials, and in the clinical trials, which are much, much tighter, more closely regulated, it is a requirement in these equivalence trials. Comment 4. The report on the statistical analysis is inadequate. It is not clear how the data were dealt with. No degrees of freedom, nor t-values are shown for the tests that have been carried out. It is not even stated whether the errors associated with the means were standard errors or standard deviation or standard error of the mean, and the results are inadequately presented. Comment number five. Feeding grain maize to broilers is only appropriate for assessing the effects of grain on the animals consuming it, not an appropriate model for assessing the possible effects of feeding maize silage, which includes the whole plant to cattle, and Bob Orskov has really covered this point adequately I think. The example we have put in our statement is that for example, a similar study where potatoes are fed to pigs, to assess the likely effect of feeding potato hormes on cattle, would not be an appropriate model. In the UK of course we know that Chardon LL is intended as a crop for the whole plant feeding to cattle, and if this paper were sent to me to be refereed for publication in a journal, there are certain levels of rejection, returned with amendments. This would be rejected outright. No second chance, without further study, more analysis. In summary, inadequate study design and inadequate statistical analysis mean that only unexceptionally large differences in live weight between the treatment would have been detected. Similarly because the study is flawed, no conclusions either way can be drawn with regard to the effects of the diet on mortality. Consequently it is not possible from this experiment to conclude that no toxic elements, able to modify the growth, were introduced by the transformation event, nor that the T25 maize was nutritionally equivalent to other maize varieties. As I said at the beginning it is a real concern that a study with such substantial flaws as this should have reached this stage, and we are still talking about it now, as though it has got some scientific credibility. Where scientific studies are used as evidence for the safety of for example veterinary medicines, there are strictly controlled and applied standards for how these studies are carried out and reported. Standards are not terribly onerous, nor difficult to apply, and are internationally agreed, and the chicken study falls far short for these standards. We suggest it would seem reasonable that in the absence of required minimum standards for evidence, all studies submitted as proof of the safety of a substance, compound etc, should at least be assessed by competent independent people, with an expertise in the area, and we suggest

you regularly call on the Royal Society and the Royal Statistical Society to provide experts for these assessments.

Chairman

Thanks very much.

Dr Knowles

Sorry.

Chairman

Sorry. You have got to the end of your .

Dr Knowles

I know. I have got a couple of points I would like to make.

Chairman

Could you do it quickly?

Dr Knowles

Yes. I was sent this report, I think it was produced by ACRE. It is genetically modified GM T25 Maize Review of the Safety Assessment of T25 GM Policy & Regulation Unit.

Chairman

No that is a background paper. That is not ACRE's.

Dr Knowles

I was just about to say that the section after paragraph thirty two states "Toxicity tests with rats and feeding studies with chickens indicate that PAT is not acutely toxic, and the grain is nutritionally equivalent to other maizes". You just cannot say that sort of thing. ….

Chairman

And one more.

Dr Knowles

I down loaded this from the Internet yesterday. It is from the Food & Drug Administration, the States. It is some of the ICH, International Committee on Harmonisation Steering Committee, and it is the final draft of guidelines that are to be adopted by Europe, Japan and USA. They actually have a section

here on trials to show equivalence or non inferiority, and I was just going to read you this little bit. "There are well known difficulties associated with the use of the equivalence or non inferiority trials, that do not incorporate a placebo, (this is the control I was talking about), or do not use multiple doses of a new drug. These related to the implicit lack of any measure of internal validity, thus making external validation necessary". The equivalence trial is not conservative in nature, so that any flaws in the design or conduct of the trial will tend to bias the results towards a conclusion of equivalence. For these reasons the design features of such trials should receive special attention, and their conduct needs special care.

Chairman

Do you want to have a ?

Advisory Committee Member

Thank you Dr Knowles. I would just like to clarify one thing first of all. That is that this study in your opinion, can tell us nothing about any differences between chicken performance on these two diets, neither positive nor negative?

Dr Knowles

If I was looking at the data, a student had brought it to me, and they were asking my statistical advice as often happens, after the trial, rather than before

Advisory Committee Member

Yes of course

Dr Knowles

I would say, forget it all. You know, you have got to start again really. There are some differences here to look at, to perhaps help guide you the next time you do it. More chickens died, greater variability with the GM crop. You have got some measure of a variance, which will help guide the size of your next study.

Advisory Committee Member

Thank you, and just moving on from that, which is what I wanted to address. You have highlighted design flaws in this particular study, and pointed out the difficulty in doing these sorts of experiments, particularly when looking for differences of say 1%. Could I sort of put you on the spot, and pretending to be that student for a moment, would you like to give me any advice as to how the design of the experiment should be, particularly with regarding the effort of replication?

Dr Knowles

Well this data is probably very useful for giving you some idea of the variation that is present within the groups, and between the groups. I cannot do it now, but you could work out exactly how many animals, based on this data, if you assume that this data is going to be representative at the trial the next time round, how many animals you need to be able to spot a difference of a given magnitude.

Advisory Committee Member

Right, I was just wondering, yes, I realise it is difficult to power calculations on the top of your head, but I was wondering if, in terms of the sort of ball park figure regarding what seem to be, at least we are told, standard protocols for these sorts of trials, which usually seem to involve about twenty to twenty four pens.

Dr Knowles

It depends on the variability that is present, so there is no hard and fast rule

Advisory Committee Member

There is no hard and fast rule, OK. Yes, Right I understand thank you.

Advisory Committee Member

Again a point of clarification. Just, can you confirm that what you are actually saying is, that chicken animal feed studies should be carried out in the same way, and according to the rules and the protocols of studies that relate to veterinary medicines?

Dr Knowles

Well I think you are looking for possible tox effects here. So yes.

Advisory Committee Member

Your answer to that question is yes?

Dr Knowles

The standard should be as high if not higher I would have thought.

Advisory Committee Member

That comment is made as a statistician, as opposed to an expert in animal feed, or in testing medicines?

Dr Knowles

That is a bit borderline is not it?

Advisory Committee Member

In your calculation of the power of the test, did you assume that the errors about the means were standard deviations, or standard errors?

Dr Knowles

I think it is basically possible to work out which way round it is by looking at the figures, though it is not actually stating specifically their views, but if common-sense , and I cannot remember which round it was, but I am pretty certain that my calculations are correct.

Advisory Committee Member

I am not a statistician, so this might be a very naïve question. If you were redesigning this experiment, I understand you cannot give details, would you be more likely to increase the number of pens per treatment, the number of individual birds used by treatment overall, or both?

Dr Knowles

If I was to do a study, it really depends on what facilities you have. You are often constrained by the sort of buildings you have to carry out these studies in, but looking at that study, I would reduce the number of animals in each pen, and increase the size of the pen. I think there is a bit of overkill on the number of animals within the pen.

Advisory Committee Member

You said increase the size of the pen, do you mean increase the number of pens?

Dr Knowles

That is what I meant, the number of pens, sorry.

Advisory Committee Member

OK. Thanks.

Advisory Committee Member

Can I just ask a general point? Leaving aside this particular experiment. Do you think there is a value in doing comparative growth studies as part of the safety assessment, usually with a fast growing species like the broiler chick,

and if you think there is value, what is the purpose, what does it bring to a safety assessment?

Dr Knowles

I do not think that is for me to say. I think that is for your other experts to answer. I will keep my thoughts limited to the statistician.

Chairman

Right. Thanks very much. OK. The Aventis team on the chicken study.

Dr Rudelsheim

Are we happy to run on five or ten minutes?

Chairman

The shorter you can be the better. We are happy to run over a little, but clearly we want you to finish soon, and I would like to bring Professor Stenn in front of us just to clear something.

Dr Rudelsheim

Well in order to then, in view of just your comments Mr Chairman, may be first of all I would like to say that I fully agree with the previous speaker in terms of one point at least, saying that this kind of comment should not have reached this stage, and if indeed we see those kind of serious concerns being raised, I think it would have been really helpful to have those earlier. I think that some of the suggestions that were made indeed triggered some additional questions from our side also to the people, the experts as we tend to call them, experts in this case who have done the study, and I think they helped us in finding some additional information that we have submitted. Additionally I want to point out also that, whatever we may now interpret this to be able in terms of decision making, that the overall goal of this particular study was really one of looking to may be additional unexpected, or unidentifiable effects. We had already gone through the characterisation. We had gone through the compositional. I think that has been amply explained in the previous statement, and we now seem to come to this turning point, as being this is the yes or no for this file, and on the basis of all of these flaws, we should now review our yes, whereas actually this was really intended as let us say a final indication, really an indication that would help us on potentially seeing whether there were any other unexpected or unidentified changes that would impact. The broilers were seen as a very sensitive test system, and I wonder if we would have the same discussion if indeed something would have shown up. I wonder if you would have been in a position, I can tell you from regulatory affairs point of view, I would not be in a position to tell you that this was not a relevant study, with no credibility. I would definitely question it, but I would not be able to make that kind of statement to you. So I want to bring that in front of this group in terms of saying, let us see what the relevance is.

There were a number of good suggestions. We have obviously included them. Dr. Eric Debruyne is also here, and he is prepared to make our statement, but I would say in view of the time period, I think most of the argumentation really is presented in the written statement, where our point is that actually, we do believe that there is value. We have included some of the questions that were raised as additional information, to show that we still believe it is a valuable study. May be it is publishable for a Canadian student, I would accept that! We have definitely brought that comment already back to the people who did that, and we will obviously include some of the suggestions.

Chairman

OK. Well I appreciate you cutting that a little short.

Advisory Committee Member

Just to clarify again one point about your protocols, especially in the light of the previous speaker, who suggested that there would be very few hard and fast rules about protocols. So may be that is something to think about, but what you suggest is that your protocol does not differ to any great extent to the recommended protocol, according to what you have presented here, but it does differ as we sort of touched upon in the last set of evidence, in the relative distribution of the replication effort, whereas you have rather more birds per pen, fewer genders, one instead of two, and hence a lot less pens per treatment.

Dr. Rudelsheim

Well that is right. I think in the paper that we have submitted we have highlighted these differences, and we highlighted three major differences. I just want to refer you to the statement on Page 2, the third paragraph, or the second paragraph in fact, we are actually considering recently published guidelines by the French Food Safety Standards Agency. They have presented an appendix to those suggestions in the regulation proposals. They are not talking about replicates, and I am not a statistician, I am not an expert. I think there are experts in the audience. May be we should ask them, but at least there is a clear understanding, a description of what the statistical power is, and I think may be I can read this to the audience. In that document, the French Food Standard Agency says that the number of birds, (they are not talking about replicates), per treatment group, required to detect a 1% change in a parameter like the body weight, in this type of study, with the power of 90% is 94 birds, and you need 119 birds for a power of 95%, and 173 birds for a power of 99%, which means that in this case, if we look at what we have done in our study, we have 140 birds, which means that we are at the 95% confidence rate. That is all I saying, but again I am not an expert to answer that, I am just stating what we have found recently.

Advisory Committee Member

Yes, I am not an expert either, but I think the crucial thing in that assessment of power is how those birds are distributed, and it is the hierarchical nature of the analysis that is crucial, and if you have loads of birds, but they are all in the same place, that power analysis may not be valid.

Dr Rudelsheim

Well that is right. May be the only comment that I would add to this is that at the time the study was done, it was done as Patrick was saying by experts in the field of poultry research, and they were applying the standard rules that they were applying in their institutions. We did not reinvent the wheel, we just applied their standard operating procedure for the research expertise that they had.

Chairman

OK Ed.

Advisory Committee Member

You said that this was an investigation into unexpected effects, and as a non scientist I am just wondering, I realise this was not a statistically significant difference, but when you had twice as many birds die in one trial than the other, I just wonder why that did not trigger in you an unexpected effect signal, and I just wonder what it would take to trigger a further investigation of an unexpected effect.

Dr Rudelsheim

Well, we have a difference between the controlled group and the treated group. We have a difference. That does not mean that this is an adverse effect. For providing this information we look at what is the normal rate of unexpected death in this type of study in the performing laboratory, and the rate of unexpected death that we have in our study is within that normal range. That is one element, so we say that you know, there is nothing that was not expected in the study in terms of mortality. Certainly the study is not intended to look at a mortality effect, it is just one of the parameters that is considered in the overall evaluation of the study, and thirdly, because the model is very sensitive, you have a very fast growing animal, and you would expect to see other changes in that population, like decreasing body weight, health effects, that you would be able to pick up before these animals die, so that was not reported in the study, so we have evidence, a weight of evidence, shows us that you know, those tests are not treatment related.

Chairman

Several people have called for some statistical input to this, and we certainly would welcome it. Is Professor Senn with us in the audience. Thank you.

Perhaps you could tell us, we have your statement. If you could tell us a little bit about your analysis of this subject.

Professor Stephen Senn, University College, London (Statistical Consultant)

First of all I am Stephen Senn, Professor of Pharmaceutical & Health Statistics at University College, London. What I know about genetics could be written on the back of an envelope. What I know about agriculture could be written on the back of a postage stamp. However I have published several referee papers on the subject of equivalence, which seems to be an issue here, and if I can try and make the issue comprehensible in a simple way, I could perhaps give you the following analogy. An individual who has been acquitted by a court of law on a charge of child molesting is not necessarily your first choice as baby sitter. Different standards apply when you try to prove that something cannot happen, than when you try to prove that something has happened, and it is inappropriate in general to use a test of significance or failure to find significance as a justification for equivalence, and this would not be allowed in the context of drug regulation. Otherwise I am very happy to answer any particular questions regarding my statement.

Chairman

I do not know. I mean perhaps as Chairman I could take the prerogative of asking the sort of more or less final question in a sense. Clearly we are dignifying what is statistically not a terribly powerful experiment here for various reasons, but I wondered if it can tell us nothing about differences in mortality, is it also telling us nothing about differences in weight gain between the groups?

Professor Senn

I would be a little bit more cautious about saying that the study is not powerful. The situation is we do not know how powerful it is, because the data are not presented in a way which would enable us to judge how powerful it is, because the between and within variances are not presented. There are two levels to this experiment, and I suspect that the standard errors have been calculated as if there were one level. It might well be the case that had we got the data, we could see that within a particular pen, there is in fact no correlation between the birds. If that were the case, this would then have the power of a study which had two hundred and eighty birds effectively. If there was a very strong correlation, it would have the power of a study which had eight birds only if the correlation was one. We simply do not know where, between the eight and two hundred and eighty, this study lies because the analysis is inappropriate. I would also like to point out that I have a much more cynical opinion of the standard of statistics in the scientific literature. I fear that, quite contrary to what has been said, this study would be capable of being published in the scientific literature.

Chairman

That is perhaps a good point to finish on. Unless any of my colleagues have a burning question. Thank you very much for waiting all day to say what is very important. I am going to wrap it up, but I am going to do so by thanking you all for coming, and for listening patiently, and in silence unless you were called forward. It has been a very interesting and learning experience for us. It is a very atypical ACRE meeting, because if you saw one of our ordinary meetings, it is quite different, and I think we have learned enormously by it. Thank you for coming. Thank you for presenting the information, and as I said this will translate into something on the ACRE web site in due course, and do not forget my request to you, if you feel that things were inadequately covered, or you want to come back on something that you heard rather later in the day, you can do so via the Joint Regulatory Authority, the Secretariat of which is held in DEFRA, in the department here, so you can write to them. Thanks again.