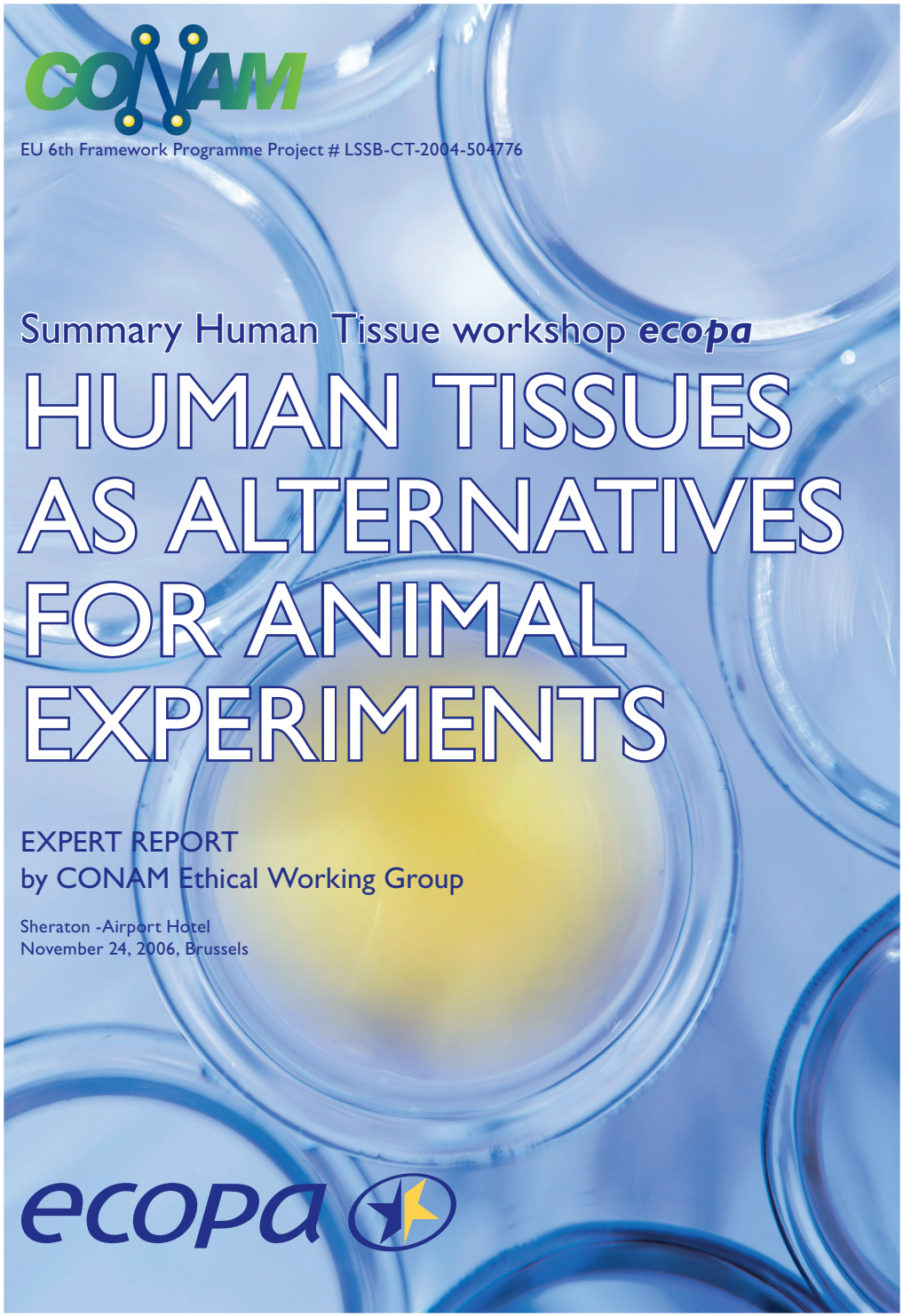

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Summary Human Tissue workshop ***ecopa***

HUMAN TISSUES AS ALTERNATIVES FOR ANIMAL EXPERIMENTS

EXPERT REPORT
by CONAM Ethical Working Group

Sheraton -Airport Hotel
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Executive Summary

Within the ***ecopa***/CONAM initiative an expert workgroup on "Good practices in Human Tissue use for Alternatives" was organized in November 2006. The aim of the meeting was to discuss some of the ongoing practices and to explore options for further joint actions.

Human Tissue is often mentioned as a good option to reduce the number of animals for basic research and to increase the relevance of the data obtained from cell and tissue cultures from animal origin. However, there are not so many successful initiatives. Most initiatives appear to find their own fit in a local national setting. Some have the ambition to extend their services abroad. The potentials seems to be hampered by national legislation regarding the constraints to obtain human tissues, or by the uncertainties in the product-demand chain, or in the scientific qualifications of specific tissues.

During the workshop three themes were considered: scientific state of the art, legal and logistic aspects and recommendations for ***ecopa***.
A summary is given below of the main issues discussed.

MAIN DISCUSSION POINTS

Theme 1: Scientific state of the art (chaired by prof. G.M.N. Groothuis, Groningen University, NL)

Most of the current research with human tissues is performed with liver, skin, brain and tumour tissue. Most of these models relate to pharmaco-dynamic or otherwise basic biomedical research. Only a small proportion of the research is specifically dedicated to the development and use of animal replacement models in biomedical research and in toxicity testing (reconstructed skin models for allergy and corrosivity screening). Tissues can be used as isolated perfused organs, precission cut slices, frozen tissue or in dissociated cell cultures.

The scientific and methodological problems related to the use of human tissues were distinguished in three categories: (i) ***Lack of tissue***, (ii) ***Quality of tissue***, and (iii) ***Limitations of the current in-vitro methods***.

(i) ***Lack of tissue,*** more precisely rest tissue from surgical procedures boils down to problems with concrete availability, the viability/functionality of the cells and inter-individual variance of the donor material. The first two are also related to the logistics that have to be in place, like contacts with surgeons, pathologists, distance and time between operation room and the tissue bank/lab. With respect to availability, practice shows that, in general, patients are willing to donate their rest tissue for research purposes. It appears that it is mainly the procurer who is causing the problem in distributing material. The procurers are not only uncertain about the legal consequences of giving out tissue for research but, in case they do, they generally also ask unrealistic compensation. See also Theme 2. Although in several countries non transplantable donor tissue appears to be a good source for human tissue, it is expected that due to improved procurement and transplantation procedures this source will decrease in the near future.

(ii) ***Quality of tissue.*** Different types of tissue are differently vulnerable to preservation techniques, e.g. corneas of the eye are more easily to preserve than liver cells. It was discussed that standard definitions of 'fresh', 'frozen' and 'cryopreserved' are missing, and that these should be well defined (even per tissue), together with standard protocols that guarantee inter-laboratory or inter-tissue bank quality.

(iii) ***Limitations of in-vitro models.*** Although human tissue cultures seem to be better models than animal tissue cultures in biomedical research, because of easier extrapolation to the human *in vivo* situation, both models seem to suffer from dedifferentiation processes that hamper direct knowledge of the original in-situ activities. Especially tissue with high metabolic activities, like liver cells, need special treatment to keep them from dedifferentiation.

To avoid dedifferentiation problems, another source for human-like functional liver cells could be controlled differentiated human stem cells. However, experimentation with human stem cells is not allowed in all EU countries. A clear distinction must be made for the origin of the stem cells e.g. embryonic and adult stem cells, and their final use e.g. alternative method development, clinical use. The legal aspects and ethics for these subgroups are quite different.

Another aspect in using human tissue cells is the poor standardization (or better formulated, the intrinsic variance) related to the human material. It was discussed to what extent this is actually a weak point or an opportunity. It was generally felt that the standardization sought in animal research thereby reducing the number of animals has a different rational than in the case of human tissue. In animal testing standardization is based on arguments to reduce the contextual variables in order to reach significant results in a small number of animals. Animal models are presumed to be a simplified representation of the human situation. Human tissue is a better representation of the human physiology and at the same time a better representation of the inter-human variance. Standardisation, if necessary for mechanistic research questions, can be achieved by preserving the same tissue line. The degree and relevance of variance between human donors can be investigated in addition for the prediction of pharmacological interventions.

Theme 2: Legal and logistic aspects (chaired by R. Kolar, Animal welfare, D)

EU Directives (Directive 2004/23/EC, 2006/17/EC, Directive 2006/86/EC) on the use of human tissue still leave room for interpretation, and a variety of national legislations exists. The participants mentioned a wide set of 'uncertainties' experienced on the work floor in many hospitals and laboratories that results in reluctance of the key persons to collect, preserve and use human tissue.

This lack of precise knowledge of legislation results in fears and open questions for researchers referring to aspects like:

- amount of necessary paper work
- consent procedures
- the time it will take to procure the tissue
- whether collecting and/or giving out tissue is legal
- who's interest will be damaged (practitioner, patient)
- who owns the material
- to what extent will it be in competition with, and therefore damage, the interest of organ donation programs?

In the absence of Veen, his submitted paper (2006) explains clearly the legal framework. As long as rest materials from surgical actions are 'anonymous', no informed consent procedure is compulsory. However, when material is connected to 'personal identifying data', informed consent for the basic research purpose is mandatory.

As, related to the variance of the material, some donor information (age, gender, race, illness etc.) is important to classify and interpret the metabolic and physiologic profiles of the material, researchers proposed that the material is coded (and therefore not directly linked to a person anymore). However, various coding options imply also various grades of "anonymity', which are interpreted differently in different countries in the EU.

One of the solutions suggested by van Veen and which is applied by Tubafrost (international tissue bank on human cancer tissues), is to fly in tissues from countries that consider coded tissues as anonymous tissue.

As long as the country of origin accepts the simple (but non mandatory) informed consent procedure with respect to the coded material (that is considered anonymously in that country), it is by EU jurisprudence considered legal in the receiving country once it crosses the border, regardless of the interpretation of coded material in the receiving country.

Another issue is the possible interference of human rest material collecting procedures with the transplantation systems currently in practice.

The easiest way is to separate these two tracks technically and socio-politically: 'Rest material' is collected from living patients that give their consent to use waste tissue for basic research, 'material originated from transplantation procedures of organs' is from recently passed patients who subjected to an 'opt in' or 'opt out' regime in their country regarding organ donation. Cancer material and skin can be easily collected as rest material. Liver, lungs and brain tissue can be collected in limited amounts as rest material from tumour surgery or material resected for diagnostic purposes. In some countries the use of non-transplantable donor organs can be used for research purposes. It is crucial whether the national regime is an 'opt out' system. In that case every citizen is considered to be a donor for transplantation and/or basic research unless one declares explicitly that one does not want their organs and tissue to be used. In 'opt in' countries one ought to obtain an explicit consent from the patient by life or the direct relatives (after passing away) to use material for basic research. This needs quite a lot of logistics and paper work.
Intermediate solutions to limit or to efficiently organize the paperwork were discussed (Poland and Germany) by mandating the tissue bank to evaluate the transplantation quality of the organs and to decide on the destination of the organs. In that case the tissue bank is regarded as a trusted distributor to recipient patients and basic researchers.

Special attention was given to the new Directive 2006/17/EC on organ transplantation, which aims to harmonize the procedures regarding organ transplantation. It calls for standard operation procedures (SOP), competence training of transplantation tissue procuring teams, detailed personal data of the donor, traceability of data for 30 years, safety procedures regarding HIV1 and 2 (anti HIV 1-2, Hepatitis B and C), by means of screening of all tissues. Moreover, in the implementation documents it is written that rest material for basic research has to be treated likewise. In absence of any specific EU Directive on rest materials for basic research, in the worst case, this might imply that all rest material can only be used after following consent procedures that are similar to those for organ donation. In addition, all material should have been tested on infectious agents to protect the laboratory staff. Currently, it is common practice that all tissue banks and researchers handle human tissue 'as if' it were infectious (biohazardous) material (without carrying out costly screening tests to confirm this).

The workshop participants stressed the importance to define the difference between procedures related to organ donation and procedures related to rest materials for basic research.

Conclusions and proposals for follow-up actions

(Chair prof. Tj. de Cock Buning, Vrije Universiteit Amsterdam NL)

From the discussion, the following four topics were considered of major importance to facilitate the use of human material in basic and applied research with special attention to the context of the three Rs strategy to reduce, replace and refine animal use in experiments.

(i) *Educating hospital staff* on the legal options to obtain human material and to inform patients regarding the option to donate rest material for basic research.

(ii) Directive on Organ Transplant (2006/17/EC) need to be amended to avoid a legal confusion between transplantation procedures and rest material procedures. It would be effective if forces could be joined, e.g. by means of ***ecopa*** or other associations, to present a proposal for an additional paragraph on rest materials.

(iii) As currently the Directive on animal experimentation (Directive 86/609/EC) is under revision, ***ecopa*** members could join forces to add 'human tissue' as an explicit strategy to reduce and replace animals in basic and applied research.

(iv) Regarding the new EU Framework 7 Program, research related to human tissue (procurement, banking, preserving technologies, trade) needs to be an item from the perspective of 3 Rs but also because of four other trends that highlight the importance of human tissue banks:

   (1) Commercial tissue from USA enters the EU tissue market and the EU does not seem to have a competitive answer to this.

   (2) *Tissue engineering is an option for in-vitro models* for alternative screening tests and depends on tissue banks.

   (3 In the clinical context liver cell therapy seems to become a promising option for liver transplantation and also depends on human tissues banks.

   (4) Newly designed and humanized biologicals cannot be tested in animal models and need screening on human tissue before entering clinical trials. Again human tissues banks are important.

Finally, it was proposed to analyze the Recommendations of the Council of Europe whether their policy proposals are in support of our conclusions.

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About ***ecopa*** and CONAM

ecopa is the European Consensus Platform on 3R-Alternatives. It is an international not-for-profit organisation, officially established in April 2004 according to Belgian law.

ecopa is the only quadripartite umbrella organisation at the European level concerned with the development and implementation of 3R-alternative methods to animal experimentation. The parties involved are Academia, Industry, Animal Welfare and Governmental Institutes.

ecopa is a success thanks to the creation of so-called National Consensus Platforms (NCPs) in the different European Member States. Actually, 13 full member NCPs (Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Hungary, Italy, The Netherlands, Spain, Sweden, Switzerland and the United Kingdom) and 3 associate members (NCPs under formation) exist (Ireland, Norway, and Poland). Recently, Norway and France officially finalised their consensus platform preparations.

As ***ecopa*** believes in international networking, exchange of information and good science as a solid basis for alternatives development, the CONAM (Consensus Networking on Alternative Methods within Europe)-project was initiated under FP6 (Project nr. LSSB-CT-2004-504776), coordinated by ***ecopa***'s chair Prof. Vera Rogiers. Major objectives consist of building new NCPs, creation of a newsletter (***ecopa*** messenger), website expansion, organisation of scientific workshops and meetings and stimulation of international cooperation.

These goals are accomplished by the activities organised within 4 workgroups, including a workgroup on political/scientific developments within REACH. The latter is at the origin of the in-depth analysis with respect to the impact of the REACH chemical legislation in the EU.

November 2006