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INVENTORY OF THE SPANISH INSTITUTIONS AND SCIENTISTS INVOLVED IN ALTERNATIVES TO THE USE OF LABORATORY ANIMALS (REFINEMENT, REDUCTION OR REPLACEMENT)

Repetto G, del Peso A, Salguero M, Repetto M

INVENTARIO DE LAS INSTITUCIONES Y CIENTÍFICOS ESPAÑOLES INTERESADOS EN MÉTODOS ALTERNATIVOS AL USO DE ANIMALES DE EXPERIMENTACIÓN (REDUCCIÓN, REFINAMIENTO O REEMPLAZO)

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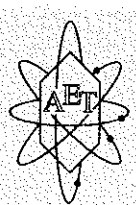
INDEX

INDICE

Incluido en ICyT, IME, EMBASE/*Excerpta Medica* y *Chemical Abstracts*

Indexed in ICyT, IME, EMBASE/Excerpta Medica and Chemical Abstracts



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Inventory of the Spanish Institutions and Scientists Involved in Alternatives to the Use of Laboratory Animals (Refinement, Reduction or Replacement)

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Resumen: Inventario de las Instituciones y Científicos españoles interesados en Métodos Alternativos al uso de animales de experimentación (reducción, refinamiento o reemplazo). Se ha utilizado una aproximación integrada para evaluar el uso de métodos alternativos a la experimentación animal por los investigadores españoles, que incluyó el número de artículos científicos publicados, las ayudas de investigación concedidas, el número de animales utilizados, y entrevistas y encuestas a los investigadores. La mayoria de las comunidades autónomas no han implementado la legislación española que exige que los experimentos con animales sean realizados por personas competentes en centros registrados, y sustituidos siempre que sea posible. Debieran fomentarse los comités éticos y la adecuada preparación de los investigadores y técnicos, y todas las ayudas de investigación debieran requerir el compromiso de los investigadores de cumplir la citada legislación. La producción científica española con métodos experimentales sólo alcanza el 2.13 % del total mundial. Por ello, la inversión en I+D debiera aumentarse desde el 0 85 % del producto interior bruto en 1997, para alcanzar al menos la media europea (1.9 %). Los objetivos prioritarios del Programa Nacional de Salud debieran revisarse, así como estimular a la industria privada para promover ayudas específicas sobre alternativas. Se han organizado diversas actividades para promover los métodos alternativos, incluyendo reuniones y la creación de entidades (GEFTIV, ICLAS/CSIC-WGCM, GTEMA y REMA), con la ayuda de la Red de Distribución Electrónica del GTEMA (3ERRES) a traves de internet (http://tox.umh.es/aet/gtema/). Además es necesaria una buena y efectiva conexión con ECVAM (Centro Europeo para la Validación de Métodos Alternativos) para el apoyo de acciones europeas conjuntas sobre alternativas. Se ha identificando un sustancial número de grupos (98), siendo 75 muy competitivos, con más de 339 científicos implicados, de los cuales se incluyen sus datos concretos

Palabras clave: España, métodos alternativos, experimentación, investigadores, *in vitro*, revisión, inventario.

Abstract: An integrated approach to evaluate the use of alternative methods to laboratory animals among Spanish researchers was used, which included the number of scientific articles, the grants approved, the number of laboratory animals used, and interviews and surveys by the researchers. Most Spanish regions have not been able to implement the Spanish legislation that requires animal experiments only be carried out by competent people on registered establishments, and replaced whenever possible. Ethical committees and the adequate preparation of researchers and technicians should be promoted, and all research grants should require the commitment of researchers to comply with the cited legislation. Spanish scientific production in experimental approaches is only 2.13% of the overall international production. Moreover, the total Spanish investment in R&D should be increased, from the 0.85% of the Gross Domestic Product of 1997, to reach at least the European median (1.9%). The objectives and research tasks included in the National Programme of Health should be revised, and the industry should be asked to fund specific grants for alternatives. Different activities have been organized to promote alternative methods, including the creation of new entities (GEFTIV, ICLAS/CSIC-WGCM, GTEMA and REMA), with the aid of internet network communication (3ERRES-GTEMA, http://tox.umh.es/aet/gtema/). A good and effective connection with ECVAM (European Centre for the Validation of Alternative Methods) is necessary for joint European action pro-alternatives. A substancial number of Spanish groups interested in alternatives were identified (98), 75 very competitive, with more than 339 scientists involved, being their data included in the report

Key words: Spain, alternative methods, experimentation, researchers, *in vitro*, review, inventory

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* A quien dirigir la correspondencia

EUROTOX: European Society of Toxicology, http://www.uta.fi/eurotox/
SETAC: Society of Environmental Toxicology and Chemistry, http://www.setac.org
Instituto Nacional de Toxicología, http:// www.mju.es/toxicologia/intframe.html
CICyT: Comisión Interministerial de Ciencia y Tecnología, http://www.cicyt.es/
CORDIS: Community Research and Development Information Service, http://www.cordis.lu/
V Framework Programme, http://www.cordis.lu/fp5/home.html, http://sost.cicyt.es/programa.htm, http://www.cordis.lu/fifth/src/pr-en-8.htm
European Programmes, http://www.uv.es/cde/GFC/
Ministerio de Educación y Ciencia, http://www.seui.mec.es.

21.4. *Distribution lists*

- [3ERRES] Red Electrónica de Comunicación sobre Alternativas (GTEMA), http://tox.umh.es/aet/gtema/GTEMA2.html
- ESTIV-L, http://www.xs4all.nl/~shorbach/estiv/index.html
- AR- News, http:// arrs.envirolink.org/mailLists/ar-news.html
- COMPMED, Comparative Medicine List, http://www.aalas.org
- WAN-general, http://worldanimal.net
- DISEVEN: Lista de información de eventos, http://www.rediris.es/diseven/)
- FCR-INT: Lista de información de becas y ayudas, http://www.fcr.es/siab/
- FARMACOL: Foro de Farmacología, http: //www.rediris.es/list/info/farmacol.html
- SECAL-L: Lista de distribución electrónica de la SECAL, http://www.secal.es
- TOXICOL: Foro de Toxicología, http://www.rediris.es/list/info/toxicol.html

22. Abbreviations

AET: Asociación Española de Toxicología, Spanish Toxicology Society.
CICyT: Comisión Interministerial de Ciencia y Tecnología, Interministerial Commission of Science and Technology.
CSIC: Consejo Superior de Investigaciones Científicas, Spanish Research Council.
ECVAM: European Centre for the Validation of Alternative Methods.
ERGATT: European Research Group for Alternatives in Toxicity Testing.
ESTIV: European Society of Toxicology *in Vitro*.
ETCS: European Tissue Culture Society
EU: European Union
EUROTOX: European Society of Toxicology.
FELASA: Federation of European Laboratory Animal Science Association
FIS: Fondo de Investigaciones Sanitarias, Spanish Fund for Health Research.
GEFTIV: Grupo Español de Farmacotoxicología *in vitro*, Spanish Group of Pharmacotoxicology *in vitro*
GTEMA: Grupo de Trabajo Especializado en Métodos Alternativos (AET), GTEMA-Spanish Group on Alternative Methods
ICLAS: International Council Laboratory Animal Science.
ICLAS/CSIC-WGCM: ICLAS/CSIC Working Group on Complementary Methods
OECD: Organization for the Economic Cooperation and Development.
REMA: Red Española para el Desarrollo de Métodos Alternativos, Spanish Network for the Development of Alternative Methods.
SECAL: Sociedad Española para las Ciencias del Animal de Laboratorio, Spanish Society for the Science of Laboratory Animals.
SEEA: Sociedad Española de Experimentación Animal, Spanish Society of Animal Research

Inventory of the Spanish Institutions and Scientists Involved in Alternatives to the use of Laboratory Animals (Refinement, Reduction or Replacement)

[Inventario de las Instituciones y Científicos Españoles Interesados en Métodos Alternativos al uso de Animales de Experimentación (Reduccion, Refinamiento o Reemplazo)]

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Resumen.

Se ha utilizado una aproximación integrada para evaluar el uso de métodos alternativos a la experimentación animal por los investigadores españoles, que incluyó el número de artículos científicos publicados, las ayudas de investigación concedidas, el número de animales utilizados, y entrevistas y encuestas a los investigadores. La mayoria de las comunidades autónomas no han implementado la legislación española que exige que los experimentos con animales sean realizados por personas competentes en centros registrados, y sustituidos siempre que sea posible. Debieran fomentarse los comités éticos y la adecuada preparación de los investigadores y técnicos, y todas las ayudas de investigación debieran requerir el compromiso de los investigadores de cumplir la citada legislación. La producción científica española con métodos experimentales sólo alcanza el 2.13 % del total mundial. Por ello, la inversión en I+D debiera aumentarse desde el 0.85 % del producto interior bruto en 1997, para alcanzar al menos la media europea (1.9 %). Los objetivos prioritarios del Programa Nacional de Salud debieran revisarse, así como estimular a la industria privada para promover ayudas específicas sobre alternativas. Se han organizado diversas actividades para promover los métodos alternativos, incluyendo reuniones y la creación de entidades (GEFTIV, ICLAS/CSIC-WGCM, GTEMA y REMA), con la ayuda de la Red de Distribución Electrónica del GTEMA (3ERRES) a traves de internet (http:\\tox.umh.es\aet\gtema). Además es necesaria una buena y efectiva conexión con ECVAM (Centro Europeo para la Validación de Métodos Alternativos) para el apoyo de acciones europeas conjuntas sobre alternativas. Se ha identificando un sustancial número de grupos (98), siendo 75 muy competitivos, con más de 339 científicos implicados, de los cuales se incluyen sus datos concretos.

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INDEX

1 Sumario

El objetivo del presente estudio es evaluar el impacto real que tiene el uso de métodos alternativos entre los investigadores españoles. Se utilizará una aproximación integrada, que incluye la evaluación del número de artículos publicados en revistas científicas, la verificación de las ayudas de investigación aprobadas en relación con metodologías alternativas, el número de animales utilizados, y la realización de entrevistas y encuestas a los investigadores. Como estas aproximaciones son complementarias entre sí, se investigaron en paralelo.

Los métodos utilizados en investigación, enseñanza y ensayo están sometidos a un progreso continuo. Los investigadores están imbricados en la permanente búsqueda de posibles alternativas que mejoren la calidad de su trabajo. Ello es debido, en parte, a la evolución del conocimiento científico y sus aplicaciones prácticas, así como a consideraciones éticas, logísticas, económicas, sociopolíticas y legales.

Los procedimientos experimentales alternativos incluyen aquellos que reemplazan o reducen la necesidad del uso de animales en un ensayo, o que refinan una técnica para reducir el sufrimiento infligido a los animales. El desarrollo de técnicas *in vitro* ha tenido un efecto espectacular tanto en los modelos experimentales como en biomarcadores, en términos de evolución de métodos moleculares y mejoras en el cultivo de células y tejidos.

Para que un procedimiento experimental pueda ser empleado en los ensayos de toxicidad requeridos para la comercialización, transporte y uso de un nuevo compuesto, o con finalidad de control medioambiental, es necesario que haya sido validado científicamente y aceptado por las autoridades reguladoras. Aunque la aceptación reguladora de nuevos métodos toxicológicos ha sido muy lenta, algunos procedimientos han sido aceptados por la industria y los científicos, y son empleados rutinariamente en áreas como la farmacéutica, cosmética, tamizado de plaguicidas, control y monitorización de contaminantes, etc. Es necesario promocionar su validación y aceptación como alternativas reales a los ensayos animales.

El desarrollo del interés por los métodos alternativos surge fundamentalmente del incremento de la importancia que se concede al bienestar animal por los movimientos sociales antivivisseccionistas, la comunidad científica y las industrias interesadas. Gran importancia tienen también consideraciones logísticas y económicas.

Adicionalmente, los requerimientos legales de la Directiva 86/609/EEC fueron incluidos en la legislación española por el RD 223/1988, que proteje a los animales de laboratorio y prohibe estudios innecesarios. La legislación española exige que los experimentos sean sólo realizados por personas competentes o bajo su responsabilidad. Al contrario que en la Directiva europea, no se requiere autorización a éstas personas. Sin embargo, sólo dos comunidades españolas han implementado los requerimientos de esta directiva en sus legislaciones. Por ello, los gobiernos regionales debieran hacerlo lo antes posible, y asegurar su cumplimiento.

El Ministerio de Agricultura, Pesca y Alimentación debería cumplir lo mejor posible con sus obligaciones relacionadas con el control y la información a la Comisión Europea del número de animales utilizados con fines científicos. No debiera permitir la experimentación animal en centros no registrados para ello.

Al menos 558.823 animales fueron empleados en España en 1991, siendo el ratón la especie más usada (56 %), seguido de la rata (24 %) y las aves (12 %). Pocos centros disponen de comités éticos activos, y la preparación de los investigadores y técnicos no es siempre la adecuada.

Se ha observado un incremento importante en la producción científica española en relación con métodos experimentales desde 1966 en comparación con la producción internacional, aunque se alcanza en la actualidad sólo el 2.13 % del total. El incremento en estudios experimentales es proporcionalmente más importante para las publicaciones en las que se emplearon métodos alternativos, aunque con un cierto retraso en España (32 % en vez del 36 %). Las especies más empleadas fueron la rata, el ratón y el hombre. Los investigadores experimentales *in vitro* prefirieren emplear al hombre sobre la rata y el ratón como fuente de material para sus ensayos. El número de estudios fué paralelo *in vivo* e *in vitro*, principalmente en las areas de Fisiología, Genética, Farmacología, Bioquímica, Toxicología, Cirugía y Patología. La importante proporción de estudios genéticos es debida al diseño de modelos transgénicos.

Alrededor de 30.000 españoles trabajan en areas de investigación y desarrollo. En relación con los programas europeos para la promoción de la Ciencia, la Investigación y el Desarrollo, el 6.2 % de los fondos del IV Programa Marco volvieron a España.

La inversión española total en I+D debiera incrementarse lo antes posible desde el 0.85 % en 1997, para alcanzar al menos la media europea, ahora situada en el 1.9 % del producto interior bruto.

En España no existe una inversión fijada en relación con métodos alternativos. De acuerdo con las contestaciones al cuestionario empleado para la preparación del inventario, sólo en casos excepcionales algunos grupos han obtenido ayudas de acuerdo con prioridades relacionadas con métodos alternativos. Existen además grandes diferencias entre los programas regionales, no sólo en la cantidad invertida, sino también en las prioridades.

Los actuales objetivos prioritarios incluidos en el Programa Nacional de Salud no han sido útiles en la promoción de alternativas. La reducción de su ámbito al área de investigación farmacéutica, restringido a nuevos medicamentos, sin opción para las instituciones que trabajan con plaguicidas, cosméticos, aditivos alimentarios, compuestos industriales, etc, debería ampliarse. Para impulsar mejor el desarrollo de estudios alternativos, su redacción debiera priorizar lo más posible el impacto que las alternativas tienen en el mundo real, con objeto de facilitar una evaluación más positiva de los proyectos.

Las ayudas de todas las areas de investigación, sufragadas por entidades públicas o privadas, debieran requerir el compromiso de los investigadores de cumplir la legislación vigente sobre protección animal, incluyendo el registro de los animalarios, la capacitación de los responsables para efectuar experimentación animal, la previsión del número de animales usados, la posibilidad de causar dolor y sufrimiento, los procedimientos para evitarlos, y las razones para no emplear métodos alternativos si estuvieran disponibles.

La inversión privada total española es muy pequeña en relación a la de otros paises, apoximadamente un 15 % menor que la media europea. El inventario ha confirmado que los contratos con diferentes compañías suponen una fuente fundamental de ingresos para las instituciones. La industria ha ido incrementando el empleo de métodos *in vitro*, al menos en

ensayos preliminares para reducir costes y acelerar la producción. Sin embargo, y al contrario que en otros paises, no existe ninguna ayuda privada enfocada directamente hacia métodos alternativos. Debiera estimularse a la industria privada para promover ayudas específicas para alternativas.

Se han organizado diversas actividades para la promoción y el desarrollo de métodos alternativos en España, incluyendo la creación de nuevas entidades como el Grupo Español de Farmacotoxicología in vitro (informal, GEFTIV), el Grupo de Trabajo de ICLAS/CSIC sobre Métodos Complementarios, el Grupo de Trabajo Especializado en Métodos Alternativos (GTEMA) y la Red Española para el Desarrollo de Métodos Alternativos (REMA), con la ayuda de la Red de Distribución Electrónica del GTEMA (3ERRES) a traves de internet, y el patrocinio privado para la organización de cursos, reuniones, etc.

Se ha realizado una encuesta y se ha preparado un inventario de las instituciones y científicos españoles interesados en el area de los métodos alternativos al empleo de animales de laboratorio (reducción, refinamiento y reemplazo). Incluye instituciones, científicos, proyectos en marcha e instituciones financiadoras de proyectos, así como la evaluación global de la situación actual española.

De los 103 cuestionarios recibidos en la encuesta para la preparación del inventario, varios procedían de grupos de otros paises, que fueron incluidos en el inventario, pero no en el análisis de los resultados. El número de grupos interesados en alternativas fue sustancial (98), 75 muy competitivos, con más de 339 científicos interesados, con una media de 4,5 científicos por grupo.

En relación con el tipo de institución, la universidad aportó el mayor número de grupos (46 %), seguida de organismos públicos de investigación (incluyendo al CSIC) (15 %), la industria (13 %) y hospitales y organismos públicos de servicio (ambos 7 %).

La función principal del trabajo fue investigación básica (29 %), seguida por investigación aplicada no regulada (19 %), desarrollo de métodos (16 %), ensayos regulados (14 %), alternativas en la enseñanza y formación (11 %) y validación de métodos (9 %). En relación con el tipo de ensayo, los resultados fueron balanceados, entre ensayos de tamizado o criba (35 %), complementarios (34 %) y sustitutivos (31 %).

Con respecto al área principal de aplicación, la toxicología fue claramente la más frecuente (25 %), seguida por la bioquímica, la biología molecular, la química, y la biología celular (9 %), la monitorización (7%), la biología y la farmacología (6 %), etc. Se evaluan muchos tipos de materiales, incluyendo (14 %), plaguicidas y contaminantes ambientales (11 %), compuestos químicos diversos (10 %), residuos (9 %), cosméticos (7 %), vacunas (6 %), etc.

En cuanto a los modelos empleados, las más utilizadas fueron las técnicas *in vitro* (44 %), seguidas por animales (28 %), modelos en la enseñanza y formación (8 %), embriones (6 %), vegetales y voluntarios humanos (ambos 4 %). Con respecto a los animales, el 63 % de los grupos emplean animales convencionales, mientras que el 22 % utiliza invertebrados y el 15 % transgénicos. Los métodos *in vitro* más empleados son lineas celulares (34 %), seguidas de cultivos primarios (25 %), micro-organismos (19 %) y fracciones celulares (13 %).

En los modelos *in vitro* se emplean una gran variedad de bioindicadores, incluyendo viabilidad celular (19 %), proliferación (14 %), actividad metabólica (14 %), ácidos nucleicos (9 %), citosqueleto / estudios de liberación de enzimas (8 %), sistemas de biotransformación (8 %), morfología (7 %), etc

Aunque sólo una mínima parte de los laboratorios ha participado en estudios previos de validación, la mayoría de ellos está disponible para participar en programas de validación.

A nivel europeo, el 5º Programa Marco de la Comunidad Europea para la Investigación, Desarrollo Tecnológico y Actividades de Demostración (1998/2002) representa una oportunidad para la toxicología *in vitro*, ofreciendo oportunidades en diferentes campos para la promoción y consolidación de actividades.

Es necesaria una buena y efectiva conexión ECVAM para el apoyo de acciones europeas conjuntas sobre alternativas. Deberían organizarse iniciativas como la Reunión de Representantes europeos de entidades responsables de métodos alternativos.

2 Summary

The objective of the present study is to evaluate the real impact that the use of alternative methods has among Spanish researchers. An integrated approach will be used, which includes the evaluation of the number of articles published in scientific journals, the verification of the grants approved in relation to alternative methodology, the number of laboratory animals used, and the preparation of interviews and surveys by the researchers. As all the approaches are complementary, they were investigated in parallel.

The methods used in research, education and testing are subject to continuous progress. Researchers are engaged in a permanent search for possible alternatives in order to improve the quality of their work. This is due in part to the evolution of scientific knowledge and its practical applications, as well as to ethical, logistical, economical, socio-political, and legal considerations.

Alternative experimental methods include procedures that replace or reduce the use or need for animals in a particular test, or that refine a technique in order to reduce the amount of suffering endured by the animals. The development of *in vitro* techniques has had a spectacular effect on both model systems and bioindicators in terms of the evolution of molecular methods and improvements in cell and tissue culture.

Experimental procedures have to be scientifically validated and accepted by regulatory authorities in order to be used in the standard toxicity tests required prior to the commercialization, transportation and use of a new chemical compound, or for environmental control purposes. Although regulatory acceptance of new toxicological methods has been very slow in arriving, some alternative techniques have been accepted by scientists and the industry, and are routinely used in areas such as pharmaceutical, cosmetic or pesticide screening, monitoring and control of pollutants, etc. It is necessary to promote their validation and acceptance as real alternatives to animal tests.

The development of a critical interest in alternative methods arose mainly from the increasing attention to animal welfare shown by animal movements, the scientific community and interested industries. Logistical and economical reasons were also responsible for important input.

In addition, the legal requirements of Directive 86/609/EEC were translated to Spanish regulations in RD 223/1988, which protects laboratory animals and prohibits unnecessary animal studies. The Spanish legislation requires that experiments only be carried out by those competent to do so or people who are directly responsible to them. In contrast to the specifications of the European Directive, authorization is not necessary. However, only 2 Spanish regions have been able to implement the requirements of this directive into their legislations. The rest of the regional governments should be urged to do so as soon as possible, and to make the provisions necessary to assure compliance.

The Spanish Ministry of Agriculture should carry out their obligation to control and provide information to the Commission on animals used for scientific purposes, and assure compliance with legislation. Non-registered establishments should not be allowed to perform experiments with animals.

At least 558,823 animals were used in Spain in 1991, with mice being the species most used (56 %), followed by rats (24 %), and birds (12 %). Few establishments involved in research requiring

the use of laboratory animals maintain active ethical committees and the preparation of researchers and technicians is not always adequate.

An important increase in experimental approaches within Spanish scientific production has been observed since 1966 in relation to overall international production, although, at present, it is only 2.13 % of the whole. The increase in experimental studies is proportionally more important for the publications using alternative methods, but with some delay in Spain (32 % in contrast to 36 %). The species most used *in vivo* were rats, mice and human beings. *In vitro* researchers preferred human beings to rats and mice as sources of material for their assays. The number of studies was parallel *in vivo* and *in vitro*, mainly in the areas of physiology, genetics, pharmacology, biochemistry, toxicology, surgery and pathology. The important proportion of genetic studies is due to the design of transgenic model systems.

About 30,000 Spaniards are working in research and development. In relation to the European Programmes for the promotion of Science, Research and Development, 6.2 % of the funding of the IV Framework Programme was returned to Spain.

The total Spanish investment in R&D in Spain should be increased as soon as possible, from the 0.85 of 1997, to reach at least the European median, now situated at 1.9 % of the Gross Domestic Product.

In Spain there is no fixed amount of investment for alternative methods. From the answers given to the questionnaire used to prepare the inventory, there were only a few exceptions in which Spanish groups were financed by the specific priorities for alternatives. In addition, there are many differences in the regional programmes not only in the amount of investment, but also in priorities.

The present objectives and research tasks included in the National Programme of Health were not very useful in promoting alternatives. The reduced scope of the objective within the pharmaceutical research area, limited to the development of new pharmaceuticals, with no opportunities for institutions working with pesticides, cosmetics, food additives, industrial chemicals, etc, should be much wider. To better stimulate alternative studies, its formulation should prioritize the impact of alternatives on the real world as much as possible, in order to provide more stringent evaluations of the projects.

All research grants in every scientific area, funded by public or private entities, should require the commitment of researchers to comply with present legislation about animal protection, including the registration of animal facilities, the preparation of responsible people for performing animal experiments, the number of animals to be used, the possibility of causing pain or suffering, alleviation procedures, and the reasons for not using alternative methods if they are available.

The total amount of private investment in Spain is low in comparison with other countries, approximately 15 % less than the European median. The contracts of the institutions with different companies have been confirmed in the Inventory as a major source of funding. The industry has been increasingly using *in vitro* methods, at least in preliminary testing, in order to reduce cost and speed up production. However, unlike the situation in many other countries, no private aid or grants directly focused on alternative methods could be identified in Spain. Spanish industry should be asked to fund specific grants for alternatives.

Different activities have been organized for the promotion and the development of alternative

methods in Spain, including the creation of new entities such as the Spanish Group on Pharmaco-Toxicology *in vitro* (GEFTIV), the ICLAS/CSIC Working Group on Complementary Methods, the GTEMA- Spanish Group on Alternative Methods, and the Spanish Network for the Development of Alternative Methods (REMA), with the aid of internet network communication (3ERRES-GTEMA) and private support for the organization of meetings, courses, etc.

A survey was carried out and an inventory of institutions and scientists involved in the area of alternatives to the use of laboratory animals (refinement, reduction and replacement) in Spain was prepared. This included institutions, individual scientists, ongoing projects and project financing institutions as well as an overall evaluation of the state of the art in Spain. The situation in the Spanish universities, industry and the Administration was also evaluated.

Of the 93 questionnaires received in the survey for the preparation of the Inventory, several were from groups from other countries, and were included in the inventory, but not in the analysis of the results. The number of Spanish groups interested in alternatives was substancial (98), 75 very competitive, with more than 339 scientists involved, a media of 4.5 scientists per group .

In relation to the type of institution, the university was the most active in the number of groups (46 %), followed by governmental research facilities (including CSIC) (15 %), industry (13 %), hospitals and governmental service facilities with 7 %.

The main purpose of the work was basic research (29 %), followed by non-regulated applied research (19 %), development of methods (16 %), regulatory testing (14 %), alternatives in education and training (11 %) and method validation (9 %). In relation to the type of testing, the results were balanced, with screening (35 %), complementary or adjunct (34 %) and replacement (31 %) tests.

With regard to the main area of application, the most frequent was clearly toxicology (25 %), followed by biochemistry, molecular biology and cell biology (9 %), monitoring (7%), biology and pharmacology (6 %), etc. Many types of materials were evaluated, including pharmaceuticals (14 %), pesticides and environmental pollutants (11 %), diverse chemical compounds (10 %), wastes (9 %), cosmetics (7 %), vaccines (6 %), etc.

Regarding the model systems employed, the most often used were *in vitro* techniques (44 %), followed by animals (28 %), models in education and training (8 %), embryos (6 %), vegetables and human volunteers (both 4 %). With respect to the animals, 63 % of the groups employed conventional animals, while 22 % used invertebrates and 15 % transgenics. *In vitro* methods largely employed cell lines (34 %), followed by primary cultures (25 %), micro-organisms (19 %) and cell-free systems (13 %).

A wide variety of bioindicators were used in *in vitro* models, including cell viability (19 %), cell proliferation (14 %), metabolic activity (14 %), nucleic acids (9 %), cytoskeleton / enzyme release studies (8 %), biotransformation systems (8 %), morphology (7 %), etc

Although only a minimum part of the laboratories have participated in previous validation studies, most of them are available to participate in validation programmes.

At European level, the 5th Framework Programme of the European Community for Research, Technological Development and Demonstration Activities (1998/2002) represents an outstanding challenge for *in vitro* toxicology, by offering several opportunities on different grounds to promote and consolidate this activity at the European level.

A good and effective connection with ECVAM is necessary for joint European action pro-alternatives. Initiatives such as the Meeting of Representatives of European Entities Responsible for Alternatives should be organized periodically.

3 Introduction

One of the present initiatives of the European Centre for the Validation of Alternative Methods is to identify Laboratories and scientists within Europe which are active and expert in the various areas of alternatives to the use of laboratory animals. However, one of the difficulties in organizing activities to promote alternatives or to prepare international studies for the validation of new methods is to identify the active scientists interested in this area.

To aid in resolving such difficulties, a survey was carried out and an inventory of institutions and scientists involved in the area of alternatives to the use of laboratory animals (refinement, reduction and replacement) in Spain was prepared. This included institutions, individual scientists, ongoing projects and project financing institutions as well as an overall evaluation of the state of the art in Spain. The situation in the Spanish universities, industry and the Administration was also evaluated.

The main aim of this report is to indicate the state of the art in relationship to the use of alternative methods in Spain over the last thirty years, as well as the most recent development trends. In this context the more specific goals are:

- to set up a picture of activities and initiatives in order to ensure better co-operation and to promote concerted action between the various partners for the development, validation and acceptance of *in vitro* methods
- to provide a record of the main reference points
- to identify the main Spanish institutions and scientists involved in alternative methods
- to trace future challenges

The report is organized in four main parts.

- The first part (3 to 8) focuses on an analysis of the activities which have contributed to the most recent developments in this area. The growth of critical interest in alternative methods arose mainly from the increasing attention paid to animal welfare by animal movements, the scientific community and interested industries. The legislation and the number of animals used and Spanish scientific productivity are reviewed.
- The second part of the report (9 to 12) contains information about public and private sources of financing, promoters of alternative methods and related activities.
- The third part of the report (13 to 22) contains the results of data collection obtained through questionnaires addressed to more than 1000 scientists. The inventory of Institutions and Scientists is followed by final comments and proposals. Finally, we present conclusions and recommendations that will give useful information to ECVAM and the European Commission. Bibliographic and legislative references and websites with information about alternatives are also included.

This report offers a view on the state of the art of *in vitro* methods in Spain to ECVAM, to the European Commission, to scientists, to private companies, and to the general public.This report is also an operative tool for those who want to establish contacts and to work in collaboration, to learn more about the subject and to share expertise, and for those who want to gather information on specific areas of interest, methods and approaches. For these reasons, the more specific objectives are:

- to clearly identify as many as possible of the facilities involved in alternative methods in

Spain, giving identical objective information for all of them

- to encourage the development of alternative methods
- to enable young scientists to choose the institution best suited for their training in new developing fields
- to enable these institutions to be consulted and participate in validation and quality assurance programmes
- to establish a network of consulting institutions in alternative methods
- to optimize the outcome of past experience
- to facilitate access to the field
- to address future investments
- to build up a record of the main reference points, such as scientific societies, public institutions and private groups
- to set up a picture of activities and initiatives, in order to ensure better co-operation and promotion of concerted action between various partners for the development, validation and acceptance of *in vitro* methods

The authors intend to update this compendium periodically to take into account any changes in the facilities and to allow institutions not yet included in this edition to be added in any subsequent editions. For this purpose, a copy of the questionnaire is attached. This publication of the compendium must be regarded as a prototype. Therefore, suggestions for improvements in the quality of the information made available will be welcome.

4 Alternative Methods

The methods used in research, education and testing are subject to continuous progress. Researchers are engaged in a permanent exploration of possible alternatives to improve the quality of their work. This is due in part to the evolution of scientific knowledge and its practical applications, as well as to ethical, logistical, economic, socio-political, and legal considerations.

4.1.- The three 'Rs'

Alternative methods include procedures that replace or reduce the use or need for animals in a particular test, or that refine a technique in order to reduce the amount of suffering endured by the animals. The concept of the Three Rs (replacement, reduction, and refinement) was developed by Russell & Burch (1959) to provide a framework for improving the conduct and ethical acceptability of experimental techniques on animals (Festing *et al*., 1998).

In practical terms, non-necessary experiments *in vivo* or *in vitro* should be avoided by making use of integrated strategies, prediction modelling (quantitative structure-activity relationships, but also kinetics prediction), and the availability and interchange of pre-existing information and experimental results.

Given that animals used in research may experience pain, suffering or lasting harm, the first step must be to consider whether less sentient or non-sentient alternatives can be used instead (replacement). Where this is not possible, care needs to be taken to minimize the pain that any individual animal may suffer (refinement), both during the actual experiment and before or after the conduct of experiments. Lastly, the number of animals used in a given project needs to be minimized (reduction), while ensuring that the objectives of the study can still be achieved; typically, this will also reduce the sum total of animal suffering.

However, the most promising alternatives encompass the use of lower organisms with limited sentience and/or non-protected by legislation controlling animal experiments, including bacteria, fungi, algae, plants, and invertebrate animals; vertebrate use at early stages of development --from fish, amphibians, reptiles and birds to mammals; and, particularly, the employment of *in vitro* methods using material from these organisms (Repetto, 1995; Balls, 1998).

Table 1. ALTERNATIVE METHODS

1. Avoidance of non-necessary experiments *in vivo* and *in vitro*:

Protocols and previous studies:
Availability of information, exchange.
Flexibility.

Integrated strategies

2. Mathematical Prediction Modelling:

Environmental fate of chemicals
Pharmaco-toxicokinetics (PB-PK)
Quantitative Structure-Activity relationships (QSAR)

3. Improved design of animal studies:

Reduction: number of animals used
Refinement: minimization of pain and distress; new model systems

4. Use of lower non-protected organisms:

Bacteria, fungi, protozoans, algae, plants, invertebrate animals

5. Vertebrates at early stages of development:

fish, amphibians, reptiles, birds, mammals

6. *In vitro* methods:

Organs: bath, perfusion, culture, slices, reconstituted organs
Explants, cellular reaggregates, micromass, cocultures
Dispersed primary cell cultures
Cell lines / transgenesis
Cell-free systems

7. Others:

Human studies: volunteers, epidemiology, toxicosurveillance.
Models in education and training: mechanical models, audio-visual systems, computer simulations and virtual reality

(Balls, 1998; Repetto *et al*., 1999)

4.2.- Recent Advancements

International harmonization (via the International Conferences on Harmonization) of standards governing the toxicity testing of pharmaceuticals appears to have resulted in nearly a 50% reduction in the number of animals used to test some pharmaceuticals (Lumley and Cauteren, 1997).

The development of *in vitro* techniques has had a spectacular effect on both model systems and bioindicators, in terms of the evolution of molecular methods and improvements in cell and tissue culture. Among the advances produced are new coculture and microaggregate methods, and the use of growth factors, matrices, inserts, chambers, plastics and membranes. Genetically manipulated model systems have increased our understanding of the action mechanisms of chemicals. A number of technological advances have increased the specificity and sensibility of the employed biomarkers, including those with biochemical, morphological and electrophysiological bases. Molecular biology techniques have made us aware of both the way in which different genes are expressed and the relevance of their alterations. In addition, the procedures can be performed with a high degree of robotization and automatization, increasing the productivity and reliability of the assays (Repetto *et al*., 1999).

Table 2. BIOINDICATORS USED *IN VITRO*

1	**Cell and tissue morphology**	**Form, size, differentiation** **Membranes, organelles**
2	**Cellular Viability**	**Dye uptake, adhesion, phagocytosis**
3	**Cellular Proliferation**	**Proteins, DNA, cell cycle**
4	**Metabolic Activity**	**Regulatory Substances** **Energy use, enzymes, pH, Bioluminescence, Calorimetry** **Macromolecular Synthesis** **Selective induction of proteins**
5	**Cytoskeleton / Membranes**	**Composition and stability** **Ionic Permeability** **Transport Systems**
6	**Cell signalling**	**GJIC, cytokines, NO**
7	**Nucleic Acids**	**Gene expression / inhibition** **Mutation / degradation / apoptosis**
8	**Biotransformation Systems**	**MFO, P450**
9	**Defence systems**	**GSH, G6PDH, metallothioneins**
10	**Specific indicators**	**NS, liver, Immune S, Reprod S**

(Repetto *et al*., 1999)

One important piece of information needed in risk assessment is that concerning the concentration range at which a chemical exerts adverse effects on the organisms living in the

aquatic or terrestrial environment. Without this information we can neither make predictions nor establish safety factors. According to the basal cytotoxicity concept, a majority of chemicals cause toxicity by basal cytotoxicity, while a clear minority cause toxicity by interference with either organ-specific cell functions or extracellular bodily functions. According to this reductionistic view, the toxicity of a compound can be broken down into a number of elements, each of which can be identified and quantified in appropriate model systems (Ekwall, 1994). It seems possible that a limited number of cell lines or isolated cells from invertebrates and vertebrates may be sufficient for basal cytotoxicity screening.

4.3.- Validation and acceptance of toxicological methods

The Sixth Amendment (79/831/EEC) to the Classification, Packaging and Labelling of Dangerous Substances Directive (67/548/EEC) imposed a mandatory requirement for manufacturers and importers of new substances to provide a set of toxicological, eco-toxicological and physico-chemical data before placing a new substance into the common market for sale or use.

The scientific community accepts the utility and results obtained in basic research using a variety of *in vivo* and *in vitro* procedures for the investigation of physiological or pharmacological effects, toxicodynamic mechanisms, toxicokinetic processes, etc. However, in order either to be used in the standard toxicity tests required prior to the commercialization, transportation and use of a new chemical compound, or for environmental control purposes, it is necessary for the experimental procedure to be scientifically validated and accepted by regulatory authorities.

Validation is the process by which the reliability and relevance of a procedure are established for a specific purpose. Thus, after its development, the procedure has to successfully pass through prevalidation (previous interlaboratory assessment), followed by validation of its reproducibility and relevance to *in vivo* toxicity (final interlaboratory assessment), independent assessment of the study by a panel of experts and, finally, progression toward regulatory acceptance (Balls *et al*., 1995; Repetto, 1995).

Although regulatory acceptance of new toxicological methods has been very slow in coming, some alternative procedures have been accepted by scientists and the industry, and are routinely used in areas such as the monitoring and control of pollutants. It is necessary to promote their validation and acceptance as real alternatives to vertebrate tests.

Accepted testing protocols are published by national and international regulatory activities. The most widely used as reference are the guidelines of the Organization for Economic Cooperation and Development (OECD), listed on internet at the web site http://www.oecd.org/ehs/test/testlist.htm.

Efforts have been taken to reach international consensus on alternative methods for testing and assessment, including the development of testing strategies, in order to further reduce the number of animals used in safety testing of chemicals and to minimize animal suffering. Governments will accept alternative methods for the testing and assessment of chemicals once these tests are sufficiently validated in accordance with agreed criteria (Walker *et al*., 1998). However, despite much effort, considerable financial expenditure, moral, practical, and scientific argument, plus legal mandates to use acceptable alternatives to animal testing, progress has been distressingly slow (Balls, 1998).

5 Public opinion

The development of a critical interest towards alternative methods arose mainly from the increasing attention to animal welfare shown by animal right movements, the scientific community and interested industries (REMA, 1998). For these reasons, animal research presents many kinds of implications.

5.1- Ethical implications

There are many ethical aspects concerning the scientific use of living organisms, founded on the basic principle of the mutual respect for all life and the avoidance of unnecessary suffering. In practical terms, although the necessity or legal obligation to perform some types of assays exists, it is still necessary to establish the balance between the benefits that may be obtained and possible injury previously. The true relevance and reliability of the procedures used and the existence of alternatives should be known to all.

5.2.- Scientific aspects

From a scientific point of view, animal procedures are not always the most appropriate, since they can often be improved, and in many cases, even replaced. Important advances have been produced in models and bioindicators, which should be applied by scientists. Alternative methods used in pharmacology, toxicology, biochemistry, etc, have advanced tremendously in recent years, not only from the perspective of scientific advance, but also in terms of their social impact.

In vitro research is now a scientific activity with its own personality, giving origin to scientific societies, meetings and journals. This is due not only to a receptive social environment which favours a major reduction in the use of laboratory animals, but also to the pioneering efforts of scientists.

5.3.- Logistic, economic and industrial implications

Economic and logistic aspects sould also be taken into consideration in the real world. *In vitro* methods are often cheaper than animal studies, in addition to providing more rapid results, an important incentive for industry.

5.4.- The social pressure

A social movement has been consolidated since the 50's, leading to the formation of a number of groups concerned about animal rights. Although the groups created in Spain are less powerful than their European partners, they are increasing their social influence. Among the more active groups which have taken a position against animal research are the *Asociación para la Defensa de los Derechos del Animal (ADDA*), the *Asociación para la Defensa y Prevención de la Crueldad con los Animales* (ADPCA) and the *Asociación Nacional para la Defensa de los Animales* (ANDA).

6 A new context in the use of laboratory animals

6.1.- European legislation

The social pressure and scientific advances produced have promoted important political and legislative changes. Among several important decisions for researchers are the following:

- The most important was the European Directive 86/609/EEC, of 24 November 1986, concerning the protection of animals used for scientific purposes. In particular this Directive states, at Art. 23:
  "*The Commission and Member States should encourage research into the development and validation of alternative techniques which could provide the same level of information as that obtained in experiments using animals which involve fewer animals or which entail less painful procedures, and shall take such other steps as they consider appropriate to encourage research in this field. The Commission and Member States shall monitor trends in experimental methods*"

  According to Article 7.1, experiments only will be carried out *by authorized competent people or under their direct responsability.*

  The three Rs are included in Article 7, as follows:
  7.2 An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available.
  7.3 When an experiment has to be performed, the choice of species shall be carefully considered and, where necessary, explained to the authority. In a choice between experiments, those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm and which are most likely to provide satisfactory results shall be selected.
  7.4 All experiments shall be designed to avoid distress and unnecessary pain and suffering to the experimental animals.

- Similar words are used in the Council of Europe Convention for the protection of vertebrate animals used for experimental and other scientific purposes (EEC 1986b), urged to be signed and followed, particularly in relation to education and training, by the European Member countries by the Council Resolutions 86/C331/01 and 86/C331/02.

- In 1993 the Member States of the European Union resolved that the number of vertebrate animals used for scientific purpuses should be reduced by 50 % to the year 2000 (COM (92) 23 final, DOCE C138, 1-98)

- Moreover, art. 4 of Directive 76/768/EEC, as modified by the Sixth Amendment (Council Directive 93/35/EEC of 14 June 1993) established that after January 1, 1998, cosmetic ingredients or combinations of such ingredients tested on animals would not be allowed on the market. A recent directive established the postponement of the date mentioned for not less than two years (30 June 2000) until *in vitro* methods were validated (97/18/EEC).

- There are movements against the *in vivo* production of monoclonal antibodies by the ascites method. The ECVAM Scientific Advisory Commitee have endorsed an statement about the availability of scientifically acceptable *in vitro* methods for all levels of

monoclonal antibody production. The regulation of some countries (The Netherlands, UK, Germany, Switzerland...) prohibit the ascites method, and it is expected that many others will do so in the near future.

- Adherence to regulations and guidelines not primarily designed to promote animal welfare, such as those associated with Good Laboratory Practice and international standards such as ISO 9001 or UNE 45001, might also lead to a reduction in the use of animals, because they ensure that procedures are carried out consistently, to predefined standards which are less likely to be erroneous or inappropriate (de Vrey, 1997).

- Legislation and internal review procedures employing inspectors and/or ethics or animal experimentation committees have been developed, in part, as a response to the demand for the use of humane techniques. However, the law and requirements for review procedures vary between countries, and between institutes or companies within a country (Festing et al., 1998).

6.2.- Spanish legislation

- The Real Decreto 223/1988 on the Protection of animals used for research and other scientific purposes, of 14 March 1988, published 18-12-88, incorporates the Council Directive 86/609/EEC into Spanish legislation. Some points can be outlined:

    - Article 11.1 states that *experiments only could be carried out by competent people (researchers, project responsibles or specialists) or under their direct responsability.* Authorization is not required, in contrast to the specifications of the European Directive 86/609/EEC.
    - *An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available* (Article 11.2).
    - According to article 4, the registration of public establishments correspond to the Ministry of Agriculture, Fishing and Food, while regional authorities are responsible for the rest, and for the communication to the Ministry of the animals used.
    - Requirements for registration of establishments and their obligations are referred in Article 6, including the notification of the number of animals used, and the prevision of use in 3 months time.

- Thanks to a Ministerial Order of 13 October 1989 (E, 1989), that develop the Real Decreto 223/1988, the rules for the registration of producer, and animal user establisments and for the authorization for the employement of animals are established. The order is focussed on public and not in private establisments, as public centres are under the direct competence of the Ministry of Agriculture.

- The Instrument of Ratification of European Convention for the protection of vertebrate animals used for experimental and other scientific purposes was signed and published in the 25-10-90 (E, 1990).

- The Real Decreto 822/1993, of 28 May, established the Principles of Good Laboratory Practices and their application to non-clinical studies on substances and chemicals, have influence in toxicity testing studies.

6.3.- Regional legislations

As the Spanish organization is nearly federal in structure, many of the responsibilities in relation to animal experimentation are transferred to the regional governments. They are particularly responsible for the registration of non-public establishments, and for the communication to the Ministry Agriculture, Fishing and Food of the animals used within their regions. However, up to now, only two of the Spanish regions have established their own legislation:

1 Catalonia: The Law 5/95 of 21 June, published the 10-7-95 (DOGC), on the Protection of animals used for research and other scientific purposes, was completed with the Decreto 214/1997 of 30 July, and the Decreto 164/1998 of 8 July, that modify some aspects of the decreto 214/1997. Among the improvements introduced to the national legislation, ethical commitees are required for every centre, and a Commission on Animal Research was created.

2 Navarra has a Foral Order of 5 August 1991 to assure the fulfillment of the RD 223/1988, with no further specification.

3 Other regions (Galicia, Cantabria, Canarias, Madrid, Murcia, Baleares, Pais Vasco...) have prepared legislation more focused on domestic animals.

7 Animals used

It has been reported that the total number of research animals used in the world is falling; about 35 % in the US from 1985 to 1995; from 5.6 to 2.9 million in the UK from 1974 to 1992; and in the Netherlands from 1.6 million in 1978 to only 673,000 animals in 1974 (Roush, 1997)

The first report from the European Commission to the Council and the European Parliament of the statistics on the number of animals used for experimental and other scientific purposes in the Member States of the European Union was published in 1994 (EU, 1994).

The purpose of reporting to the Council and Parliament is to implement Articles 13 and 26 of Directive 86/608/EEC. Under Article 13, the responsible authority in each Member State shall "*on the basis of requests for authorisation and notifications received, and on the basis of reports made,...collect...statistical information on the use of animals in experiments...*"

Article 26 requires an appropiate summary of the statistical information collected under Article 13 to be provided to the Commission "*at regular intervals not exceeding three years, and for the first time five years following notification of this Directive*". This is an important report in terms of indicating the manner and the extent to which individual Member States currently comply with their obligations under these Directive articles.

According to the Spanish official data included in the first European report (EU, 1994), 558,823 animals were used in 1991, mice being the species most used (56 %), followed by rats (24 %), and birds (12 %) (Table 3). The figures for other countries were very different: from 3,645,708, France; 3,181,768, the United Kingdom; 2,402,710, Germany; 876,058, The Netherlands; 683,293, Italy; 304,370, Denmark; 87,117, Portugal; to 25,300, Greece and Ireland.

Some criticism has arisen about the overall data, as there is no reference to any shortfalls in data (Straughan, 1994). The report also reveals a very unsatisfactory state of affairs with respect to data collection. It notes that the Commission "found it impossible to produce fully consistent, transparent, Community-wide figures", and that "the national figures do not give the full picture, since many laboratories were unable to complete the tables or failed to return them to the authorities"

A survey about the use of animals in Spain, prepared by Martín Zúñiga in 1997, was answered by 12% (n=21) of the centres using animals within the country. Of the 127 centres registered by the Ministry of Agriculture, the author estimated there were at least another 20-25 % which were not officially registered, particularly in the regions of Andalucia, Extremadura, Baleares, Valencia and Madrid. Only 15 of the 38 universities using animals were registered, but curiously enough, except for one (UAB), they were all registered as users, when they are also producers of animals. Only 7 hospitals from the National Health Service were registered, while at least 15 hospitals have experimental units. Only 4 centres from the National Research Council (CSIC) were registered, although more than 14 of them are using animals. Fortunately, the industries (6) and pharmaceutical laboratories seem to be registered (52), and they use the most animals.

Table 3. Total number of animals used for scientific purposes in Spain in 1991

SPECIES	NUMBER
Mice	**313,250**
Rats	**135,659**
Guinea pigs	**17,464**
Other rodents	**1,201**
Rabbits	**17,307**
Apes	**0**
Other simians	**7**
Prosimians	**18**
Dogs	**1,479**
Cats	**96**
Other carnivores	**70**
Horses, donkeys and cross-breds	**5**
Pigs	**1,109**
Goats and sheep	**728**
Cattle	**74**
Other mammals	**0**
Birds	**69,018**
Reptiles	**0**
Amphibians	**390**
Fish	**621**
TOTAL	**558,823**

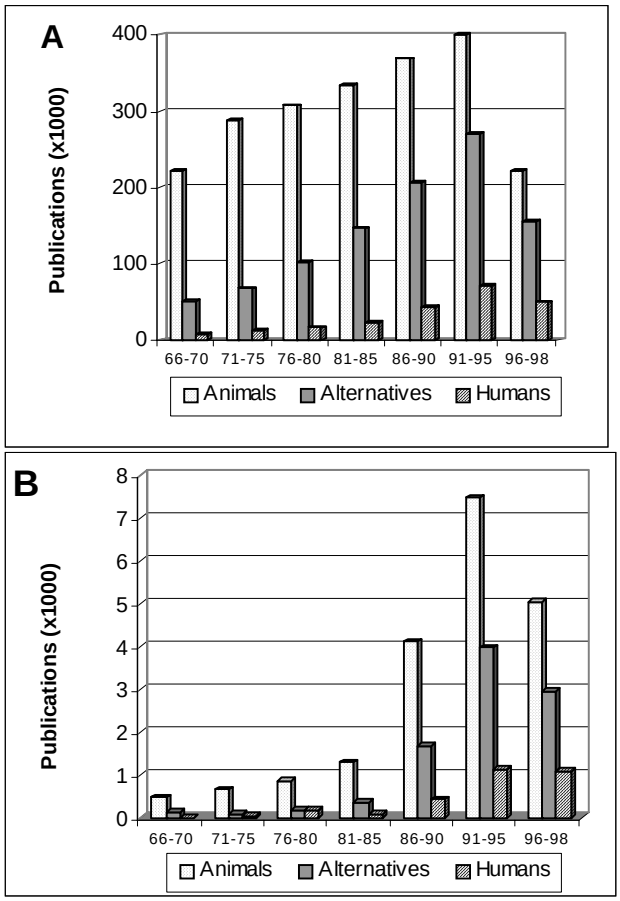
The same author (Martín Zúñiga, 1997), employing data from Catalonia, which used 325,590 animals in 1996, estimated that the actual number of animals used in Spain may be about 700,000. In addition, Martin Zúñiga detected inadequate preparation of technicians and researchers, a low level of animal welfare, and few centres with active ethical commitees.

Appropiate preparation of researchers and technicians should be encouraged (de la Peña *et al.,* 1995). ICLAS and FELASA recommendations on the education and training of persons working with laboratory animals should be followed (FELASA, 1995; Martín Zúñiga *et al*., 1997) and the creation of active ethical committees should be promoted.

8 Scientific productivity and alternative methods

The best way to explore the scientific productivity of a country is to compare the number of scientific articles published in relevant journals or the number of patents presented (Pestaña, 1996). Spain contributes 2.2 % of world scientific productivity according to the 1997 Science Citation Index. The figures are 2.65 % of world scientific productivity according to the Institute for Scientific Information (ISI, US), while about 0.6 % of the patents presented were by Spanish teams.

Figure 1. Increase in the publications corresponding to experimental methods in total (A) and for Spanish groups (B) included on Medline database from 1966



To evaluate Spanish scientific productivity related to *in vivo* and *in vitro* experimental procedures, the number of articles of relevant scientific publications has been recently identified by searching the bibliographic database Medline (Repetto *et al*., 1998). The areas where experimental animals and alternatives are habitually used --education, biomedical and environmental basic research and toxicity testing-- have been searched from 1966 to 1998. A variety of descriptors were used for the partial searches to assure their sensitivity, but with a high degree of selectivity.

The sample used in the search contained about 7 million bibliographic references from the last 32 years. The descriptor '*animal testing alternatives*' , that should identify most alternative studies, is not useful, because it only describes less than 600 articles published since 1966. Moreover, it is not possible to differentiate conventional animal experimentation studies from

those with innovations that should be considered as alternatives. This is in agreement with a recent publication by Langley *et al*. (1999), that concluded that the relevant search terms used by existing bibliographic databases are insufficient. For this reason, the group of alternative methods is underestimated in our results, because it has not only been reduced to *in vitro* studies, but also, to avoid association to other non-experimental studies (i.e., tests with bacteria or cell fractions), other studies have not been included.

Figure 2. Relative increase in the number of Spanish experimental publications included on Medline database in relation to world-wide publications

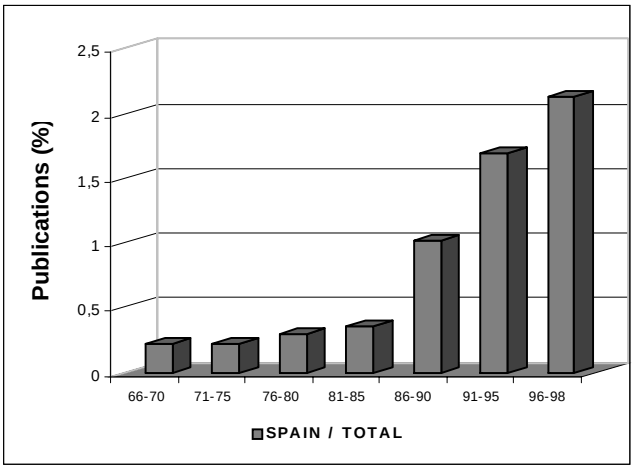
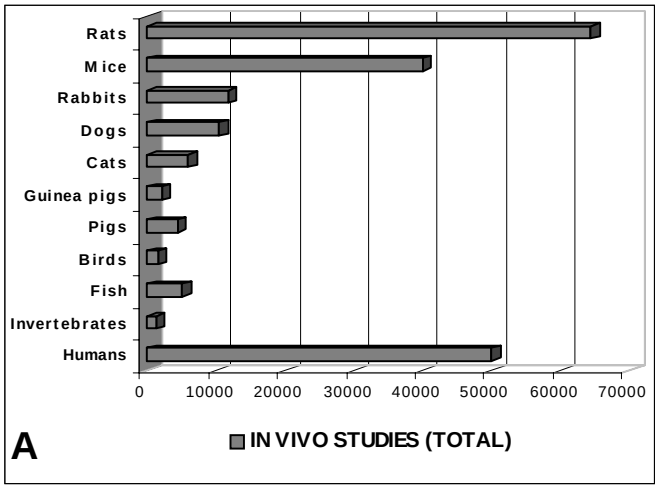
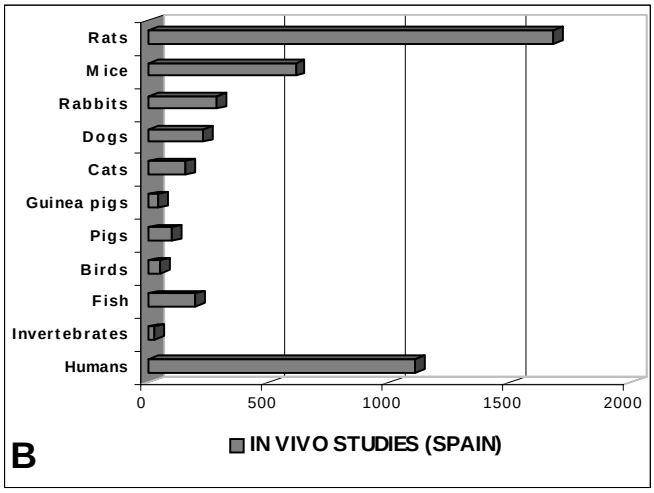


Figure 3. Animal species employed in the publications corresponding to *in vivo* experimental methods in total (A) and for Spanish groups (B) included on Medline database from 1996 to 1998



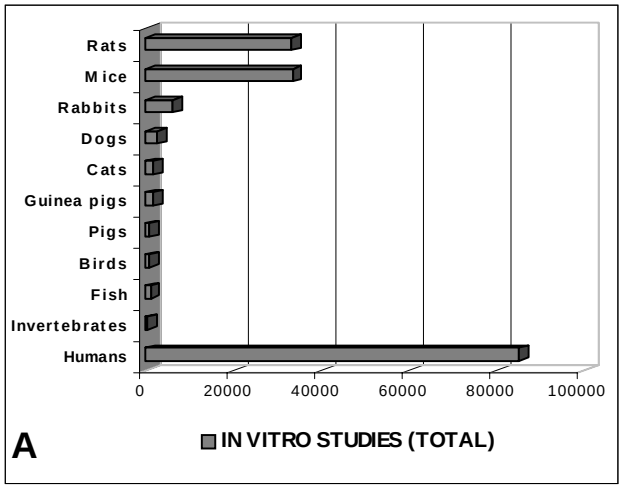


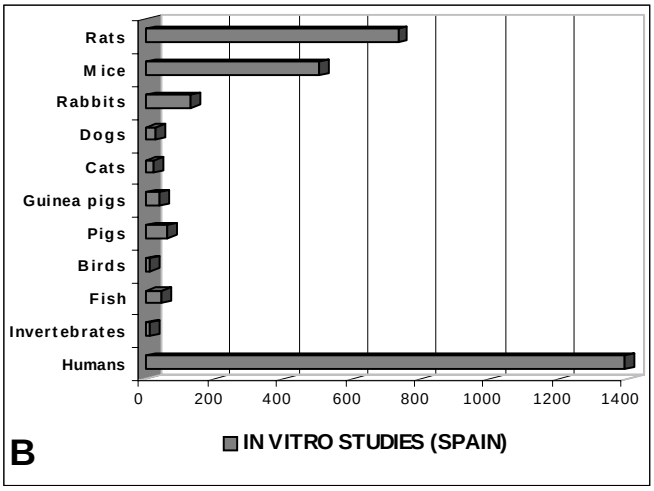
Of the world-wide experimental studies published from 1966 to 1998, 63 % were animal studies, 30 % *in vitro* studies, and 7 % human volunteer studies. Considering only Spanish teams, the ratio was similar, with 62, 29 and 9 %, respectively.

Although the number of alternative methods employed has approached those with animals in the publications, this tendency has been slower in Spain. Worldwide, in the last 3 years, the proportion of animal studies was 52 %, in contrast to 36 % in vitro studies, and 12 % with human volunteers. In Spain, the percentages were 56, 32 and 12 %, respectively.

The number of world-wide publications which described animal use doubled from the 60's to the 90's. In Spain they increased more than 15 times. Alternative studies have grown by more than 5 times world-wide, and by nearly 20 times in Spain.

Figure 4. Animal species employed in the publications corresponding to *in vitro* experimental methods in total (A) and for Spanish groups (B) included on Medline database from 1996 to 1998





An important increase in the ratio of Spanish experimental studies in relation to world-wide production has been produced from 1980 to date: from 0.22 % in 1980 to 2.13 % at present.

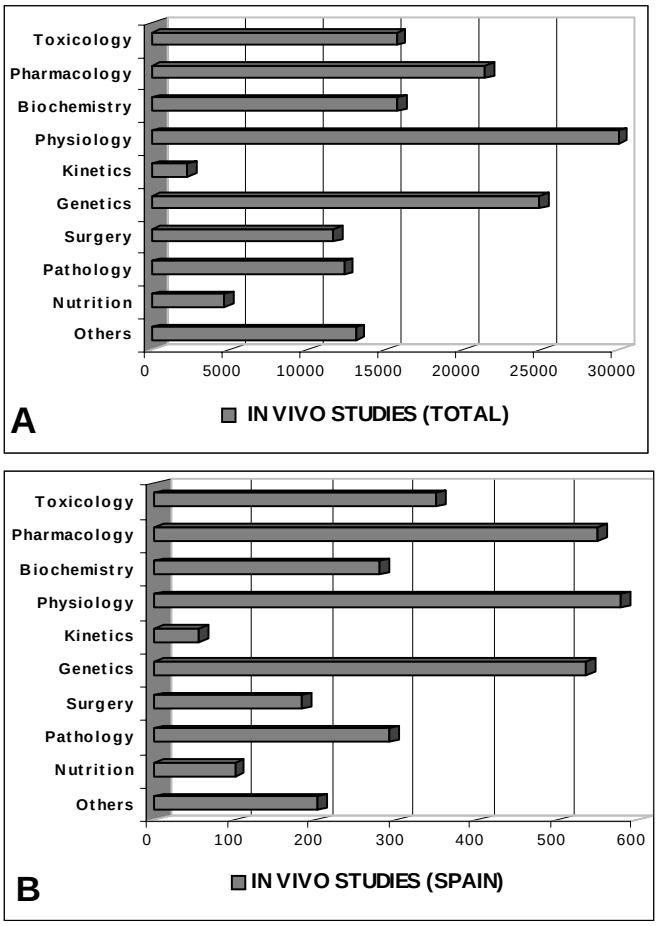
In relation to the animal species most used world-wide in the last 3 years in *in vivo* studies, 33 % were rats, 20 % mice and 25 % humans, while in Spain the percentages were 38, 14 and 25 %, respectively. Likewise in *in vitro* studies 50 % of the biological material came from humans, 20 % from rats and mice, while in Spain the percentages were 48, 24 and 17 % respectively. The data proves that *in vitro* researchers prefer to use human material as a substrate source for assay.

With respect to the type of *in vivo* study, the greater number were performed in physiology (20 %), genetics (16 %), pharmacology (14 %), biochemistry and toxicology (10 %) and surgery and pathology (8 %). Covering only the Spanish case, the studies were 17 % in physiology and pharmacology, 16 % in genetics, 11 % in toxicology, 9 % in pathology and 8 % in biochemistry.

In vitro studies followed a similar pattern as *in vivo* procedures: genetics (22 %), physiology (17 %), pharmacology (13 %), biochemistry (11 %), toxicology (10 %), pathology (8 %) and surgery (6 %). In Spain the distribution was genetics 22 %, physiology and pharmacology 16 %, toxicology 10 %, biochemistry 9 % and pathology 7 %.

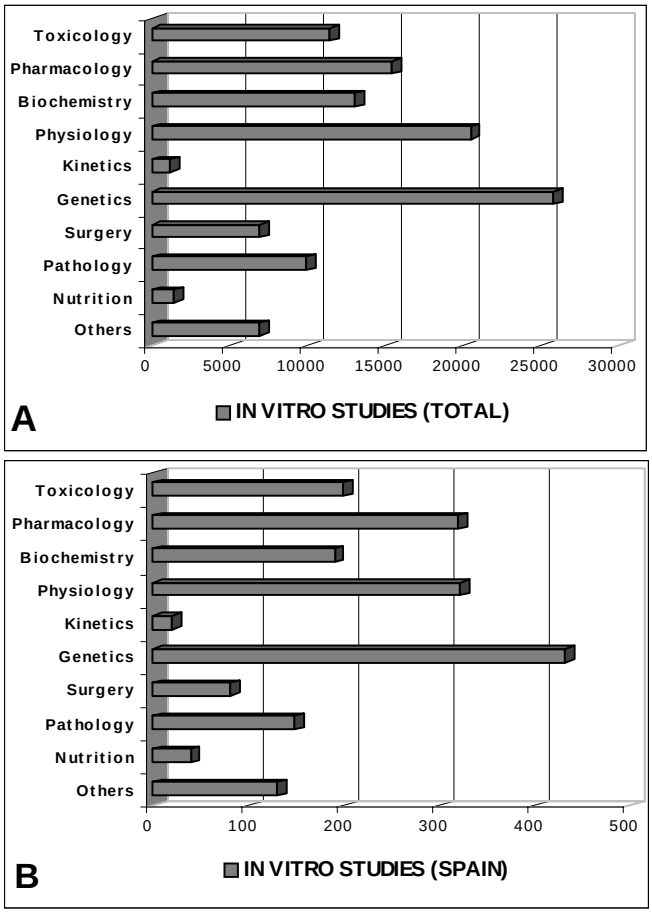
The competitivity of the Spanish hospitals is important; however the Spanish contribution in clinical trials does not reach 1.5 % of the world production. Although this level is well correlated to the GDB, it does not correlate with the scientific and technological development of the Spanish health service, and it should be increased 2 or 3 times. The main reason for this lower development has to do with administrative rigidity, which blocks the direct agreement of contracts with the private sector.

Figure 5. Scientific areas of the publications corresponding to *in vivo* experimental methods in total (A) and for Spanish groups (B) included on Medline database from 1996 to 1998



In conclusion, an important increase in Spanish scientific production has been observed in the investigated areas in relation to global international production, but reaches only 2.13 % at present. The increase in experimental studies is proportionally bigger for publications using alternative methods, but with some delay in Spain (32 in contrast to 36 %). The species most used *in vivo* were rats, mice and humans. *In vitro* researchers preferred humans to rats and mice as a source of material for their assays. The number of studies was parallel *in vivo* and *in vitro*, mainly in the areas of physiology, genetics, pharmacology, biochemistry, toxicology, surgery, and pathology. The important proportion of genetic studies is due to the design of transgenic model systems.

Figure 6. Scientific areas of the publications corresponding to *in vitro* experimental methods in total (A) and for Spanish groups (B) included on Medline database from 1996 to 1998



9 Sources of financing

As stated before, many scientists involved in adopting innovative models in biomedical and environmental research and stimulated by the impressive information which can be obtained working in these fields have also promoted and introduced *in vitro* approaches into the highly conservative field of regulatory toxicology, and also into many different areas. At the same time public institutions have paid attention to *in vitro* approaches, and support has been given to both research in the field and to congresses and meetings. The financing of all these activities has contributed both to technical and scientific advancement.

About 30,000 Spaniards are working in research and development, 12,000 being researchers (40 %), 11,000, technicians, and the remainder, support personnel. Only 23 % of the researchers are working for industry, while the mean is 49 % for the rest of Europe. Although the number of Spanish researchers has doubled in ten years, it represents only 2.7 per 1000 inhabitants, whereas the median in the European Union is 4 researchers per 1000 workers.

As there are more prepared scientists than offers of work (since only a few new positions are created each year), it is very difficult to change from one work-place to another, and the institutions tend to become endogamic, with most of their workers prepared at the workplace. The fulfilment of principles of equality and meritocracy in assessing applicants for a post have recently been discussed (Bosch, 1998a).

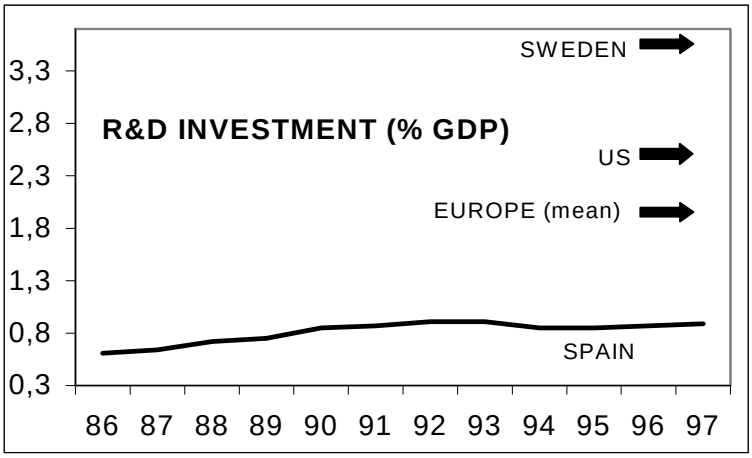
Grisolía (1998) has stated that to promote Spanish science, scientists and institutions should be renewed. He also proposed to strengthening the Interministerial Commission of Science and Technology (CICYT) and to creating a Ministry or Vicepresidency of the Government for Research.

In relation to the European Programmes for the promotion of Science, Research and Development, many Spanish institutions have presented proposals for co-ordinated projects within the IV Framework programme, and 6.2 % of the funding has been returned to Spain. For example, in the Biotechnology Programme 1994-1998, corresponding to the objective of *Prenormative research: in vitro alternatives to animal experiments in Pharmacology-Toxicology*, 11 proposals were approved: one was co-ordinated by a Spanish team, and 3 more Spanish groups were participants in other approved projects. In more detail, two projects were included in topic *711 "In vitro tests for developmental pharmaco-toxicology*"; one related to topic *712 "In vitro tests for neuro-pharmaco-toxicology*"; three for topic *713 "In vitro tests for immuno-pharmaco-toxicology*"; and five to topic *714 "Cell cultures for the development of in vitro tests".*

Figure 7. Evolution of investment in research and development in Spain in relation to the gross domestic product

The total investment in R&D in 1997 in Spain represented 0.85 % of the Gross Domestic Product (GDP), less than in 1992 (0.91 %), and was very low in comparison to the European median (1.9 %). Fortunately, for 1999 an increase was expected to 400,000 million pts (Bosch 1998b).

Spain is situated in position 30 worl-wide in relation to technological innovation. Industrial investment in 1997 was 328,000 million pts, representing a 6 % increase of 18,000 million pts over 1996. However,



these figures represent only 0.42 of the GDP, with no differences in either year. The industry with the largest amount of investment was the pharmaceutical (11 %), followed by TV and radio production and communication equipment (10 %). 80 % was financed by industry, 9 % directly from the public sector, 9 % by contracts, and 6 % from other countries.

9.1.- National Programmes

Roush (1997) reports on the low amount of research funding in animal alternatives by the governments of the US, the UK, and the Netherlands. However, the Dutch Alternative to Animal Experiments Platform invested $1.5 million in 1997, and the German government has funded research to develop alternatives at a rate of $3 to $6 million per year for the past 15 years (Spielmann, 1997).

There is not a fixed amount of investment in Spain for alternative methods. From the answers given to the inventory questionnaire, there are only a few exceptions in which Spanish groups were financed by the specific priorities for alternatives.

The main Spanish National Programmes for Research and Development in areas related to alternative methods are:

1 National Programme of Health, covering biomedical research prioritized according to a list similar to that of the European Biotech Programme.

2 Sectorial Programme for the General Promotion of Knowledge, for fundamental research in areas not prioritized in the previous programme.

3 Fund for Health Research (FIS), related to clinical, experimental/clinical and / or public health, according to the needs of the National Health Service.

4 Other programs from the National Plan for Scientific Research and Technological Development, such as those ralated to environment, food, agriculture, etc

Table 4. Some examples of projects approved in Spain from 1996-1998. Please note that topic 7.2.1 is the only one devoted to the promotion of alternative methods.

TOPIC	YEAR	ORIGINAL TITLE (in Spanish)
PGC	95	Estudio de fenómenos plásticos en un modelo de cerebro

		íntegro *in vitro*
PGC	**97**	**Bases moleculares de las interrelaciones entre factores endocrinos y nutrientes: estudios *in vivo e in vitro.***
PGC	**97**	**Mecanismos antitumorales de la melatonina: control de la proliferación , apoptosis y capacidad invasiva de células tumorales mamarias in vivo e in vitro**
PGC	**97**	**Estudios *in vitro , in vivo* y experimentales sobre mecanismos de desarrollo de la microglia de aves**
1.1	**94**	**Expresión de las moléculas de adhesión en los linfomas cutaneos de células T: modulación de la misma *in vivo* mediante interferón-alfa e *in vitro* mediante interferón –alfa y etretinato.**
1.1	**94**	**Expresión de las moléculas de adhesión en la psoriasis. Modulación de la misma *in vivo* mediante etretinato e *in vitro* mediante etretinato e interferón –alfa**
1.3	**96**	**Terapia génica *in vitro* de una inmunodeficiencia humana de CD3**
2.1.1	**97**	**Estudio *in vivo e in vitro* del papel de CD40 en la apoptosis mediada por la IgM de membrana y FAS en linfocitos B.**
2.2.1	**96**	**Efecto antitumoral de la melatonina . Estudios *in vitro e in vivo***
2.2.3	**97**	**Estudio del efecto radioprotector del GHS *in vitro e in vivo* en células tumorales, epiteliales y fibroblastos.**
3.1.1	**98**	**Anisakis simplex: purificación antigénica y desarrollo de nuevas técnicas de inmunodiagnóstico *in vivo e in vitro***
3.6	**93**	**Influencia de la glicoproteina P-170 inductora de multiresistencia en drogas en los fenomenos de nefrotoxicidad. Farmacología in vitro.**
5.1	**96**	**Efecto comparativo de una dieta rica en grasa monoinsaturada y otra pobre en grasa, sobre el metabolismo de la glucosa *in vivo e in vitro* en hombres y mujeres sanos.**
6.2.1	**96**	**Análisis moleculares y celulares *in vitro* y en modelos animales, con implicaciones en inmunopatología humana.**
7.1.2	**97**	**Caracterización y ensayo *in vivo e in vitro* de la capacidad antitumoral de dos compuestos de azafrán (Crocus stivus L): crocina y un arabinolactano.**
7.2.1 (AM)	**96**	**Estudio farmacológico del mecanismo celular de acción de fármacos antiasmáticos en cultivos celulares de músculo liso de vias aereas humanas**
7.2.1(AM)	**97**	**Desarrollo de nuevas estrategias para el control de la genotoxicidad de fármacos y para el estudio de su relación con el estrés oxidativo**
7.2.1 (AM)	**98**	**Efectos de antioxidantes naturales en modelos de inflamación intestinal en rata**
7.2.2	**96**	**Farmacocinética, metabolismo, biodisponibilidad y residuos de nuevas fluoroquinolonas. Evaluación de la seguridad de uso de animales de consumo humano -pollos broiler.**
7.2.2	**96**	**Estudio de la participación de linfocitos T en las reacciones**

		alérgicas cutáneas a medicamentos. Producción de clones de células T específicas a fármacos.
7.2.2	**96**	**Predicción de la biodisponibilidad en los estudios de desarrollo de fármacos, nuevas fluoroquinolonas**
7.2.2	**98**	**Actividad y estudio del modo de acción leishmanicida y tripanocida de derivados alquilfosfolípidos**
7.2.3	**96**	**Estudio del efecto de los antiinflamatorios no esteroides en la activación del endotelio vascular y su papel en el tratamiento del shock endotóxico.**

From a practical point of view, the National Programme of Health is the only one that prioritizes the use of alternatives. More than ten years ago, the use of new methods in toxicology was removed as an objective and research task due to the inclusion of the Toxicology Programme in the Programme of Pharmacy and Health. Later on, under the scope of Pharmaceutical Research, and following the point "*7.2 Pharmacology and Toxicology of new products of pharmaceutical interest*", the point *"7.2.1 Development and Validation of molecular and cellular alternative models to the use of animals in pharmacology and toxicology"* was included. In the last 3 years, and under the objective 7.2.1., only 3 projects have been approved, with a total cost of 25 million pts, which represents, respectively, 0.8 % of the projects approved and only 0.6 % of the investment of the National Programme of Health.

This data show that the present objective and research task is not very useful in promoting alternatives. From the data it is clear that *in vitro* studies are approved under other objectives. The answers to the questionnaire also confirm that the groups interested in alternatives obtained grants independently of the subject of the alternatives. Two main reasons must be taken into account in relation to the low effectiveness of the grants devoted to alternative methods. The first one is the reduced scope of the objective within the pharmaceutical research area, limited to new pharmaceuticals, with no provision for institutions working with pesticides, cosmetics, food additives, industrial chemicals, etc. The second reason may be the low cultural baggage of reviewers, mainly of academic origin, in relation to the social, political, legislative and logistical impacts of alternatives. In order to better stimulate alternative studies, the definition of the research tasks in the National Programme of Health should include a wider objective in regard to alternative methods, not restricting them to the pharmaceutical area, and prioritizing their impact on the real world as much as possible.

9.2.- Regional programmes

There are many differences in the Spanish regional programmes to be included in the inventory. Apart from the amount invested, the philosophy follows European and National Programmes in some cases, but not in others. Some are very open, but others are extremely restrictive, such as those, for example, that do not consider the scientists working in public service facilities, as researchers.

As an example of the important difference among regions, the industrial investment of only 3 Spanish community regions --Madrid, Catalonia and the Basque Country-- represents 75 % of the total investment in this area.

Table 5. Industrial investment in research and development according to the Spanish regions

Autonomic	**TOTAL (millions**	**%**

Community	pts)	
Madrid	113,061	34.5
Cataluña	92,114	27.1
Pais Vasco	46,475	14.2
Andalucia	15,653	4.8
Valencia	12,051	3.7
Castilla-La Mancha	10,279	3.1
Castilla y León	7,769	2.4
Aragón	6,822	2.1
Galicia	6,102	1.9
Navarra	5,678	1.7
Murcia	3,891	1.2
Asturias	3,259	1.0
Canarias	1,857	0.6
Cantabria	1,442	0.4
La Rioja	1,069	0.3
Extremadura	258	0.1
Baleares	142	0.0

9.3.- Industrial support

The amount of private investment aid in Spain is low in comparison to other countries, approximately 15 % less than the European median. The contracts of the institutions with different companies have been confirmed in the Inventory as a major source of funding.

In recent years, industry has been increasingly using, intramura, *in vitro* methods, at least for preliminary testing, in order to reduce costs and speed up production processes. However, no private open aid or grants directly focused on alternative methods, such as those that exist in many other countries, could be identified in Spain.

Many other activities have been promoted by private entities, acting especially as sponsors or collaborators in the organization of meetings and courses. This small help has been very positive, particularly when it was possible to combine aid from national institutions such as the CICYT, regional institutions, industrial organizations such as Farmaindustria, and other individual organizations.

The main private entities that have collaborated in Spain in the promotion of activities related to alternative methods are: Afora, Boehringer Mannheim, CIDA, Fundació Bosch i Gimpera, Fundación Alive, Fundación Echevarne, Glaxo Wellcome, IFFA-Credo, L'Oreal, Panlab SL, Ropak Europe, SmithKline Beecham and Uriach.

10 The promoters of alternative methods

Different activities have been organized for the promotion and the development of alternative methods in Spain, including the creation of new entities, the interconnection of people by means of internet, and private support for the organization of meetings and courses, etc.

Several scientific societies have included the promotion of alternative methods within their own activities. Among the most relevant can be included:

Spanish Toxicology Society (AET), Spanish Society for the Science of Laboratory Animals (SECAL), Spanish Society of Animal Research (SEEA), Spanish Society of Pharmacology (AEF), Spanish Group of the Chromaffin Cell, the Spanish branch of the Society of Cell Biology, etc.

However, other groups have been created with the main purpose of promoting alternative methods, including GEFTIV, ICLAS/CSIC-WG, GTEMA and REMA.

10.1.- Spanish Group on Pharmaco-Toxicology in vitro (GEFTIV): Informal Group

Establishment and composition: The Grupo Español de Farmacotoxicología in vitro was established in 1992, and has 43 members.

Aims:

The aims of the group are to facilitate contacts and exchanges among the pharmaceutical, chemical and cosmetic industries as well as researchers working in the biomedicine and biotechnology fields at the University, and other research centres that may be interested in these techniques.

Main areas of interest:

Development of cellular models to assess basal cytotoxicity and target-organ toxicity (primary cultures of different organs or tissues , immortalized human cells, cell lines).

Development of tests to assess toxicity, interpretation of the data, and critical analysis of the possibilities and limitations of *in vitro* studies.

Drug metabolism (human and animal species cultured hepatocytes, transient or permanent CYP expressing cellular systems, microsomes).

Mechanisms of toxicity

Production:

A number of a newsletter in 1995, and two books on alternative methods (*In vitro* alternatives to animal pharmacotoxicology; *In vitro* methods in Pharmaceutical research) were published by the Secretary and the Coordinator of the group.

Contact Person:

Dr M Jose Gomez-Lechon (Secretary) and Dr J Vicente Castell (Coordinator)

Unidad de Hepatología Experimental

Centro de Investigación, Hospital Universitario La Fe

Avda Campanar 21
46009 Valencia
Tel: 9563868748 Fax: 9563868718, Email: mjgomez@san.gva.es

Collaborations:
- ETCS, Spanish Branch of the European Tissue Culture Society
- SEBC, Spanish Cell Biology Society
- SECAL, Spanish Society of the Science of Laboratory Animals
- SET, Spanish Toxicology Society

10.2.- THE ICLAS/CSIC Working Group on Complementary Methods (ICLAS/CSIC-WG)

Establishment and composition

In September 1994, the International Council for Laboratory Animal Science (ICLAS), a non-governmental organization for international cooperation in laboratory animal science, created a working group to promote complementary and alternative methods to reduce, refine and replace the use of laboratory animals.

The members of the group are:

Dr. Eduardo de la Peña - Spain (Chair)
Dr. Francisco González Mencio - Cuba
Dr. C.F.M. Hendriksen - The Netherlands
Dr. Kai Pelkonen - Finland
Prof. Horst Spielmann - Germany
Dr. R. Fosse. Norway

Main areas of interest:

A meeting of the ICLAS/CSIC Working Group on complementary methods was held in the Centro Regional de Salud in Talavera de la Reina, Toledo, Spain on April 28-29, 1995 under the chairmanship of Dr. Eduardo de la Peña, ICLAS Scientific Member for Spain.

The objective of this meeting was to obtain a list of recommendations to be presented to the ICLAS Governing Board and General Assembly that was held in Helsinki, Finland, on July 1-7, 1995. Professor Garcia Partida, President of the ICLAS Spanish committee, expressed his gratitude to all those who participated and stressed the significance of this report of the meeting.

The scientific rationale for alternatives development is based upon the 3Rs concept of Russell and Burch--replacement, reduction and refinement. In general, sessions focused on the harmonization of approaches to the development and validation of alternative methods. Validation was defined as the process whereby the relevance and reliability of a procedure are established for a particular purpose.

Several issues related to validation were discussed, including criteria, approaches, and problems associated with previous studies. Several recommendations focusing on the role ICLAS can take in fostering the development and implementation of alternatives were also formulated.

Production:

Publication "ICLAS/CSIC Working Group on Complementary Methods", de la Peña E, Guadaño A, Barrueco C, Repetto G, Gonzalez Menció F, Garcia Partida P, Madrid, 1995

Contact Person:

Dr Eduardo de la Peña
Secretary of the ICLAS-CSIC-CICYT Spanish Committee
CSIC. Centro de Ciencias Medioambientales
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Collaborations:

- ECVAM: European Centre for the Validation of Alternative Methods
- OECD: Organization for Economic Cooperation and Development
  (Dra. E. Valcarce Spanish Coordinator on toxicology methods)
- AET: Spanish Toxicology Society
- ETAC: Group of Alternative Toxicological Methods in Cuba
- EUROTOX: European Society of Toxicology
- CICYT: Interministerial Commission of Science and Technology (E)
- SECAL: Spanish Society for Scientific Laboratory Animals
- SEEA: Spanish Society for Animal Research
- SEMA: Spanish Society of Mutagenesis
- GTEMA- Spanish Group on Alternative Methods

10.3.- GTEMA- Spanish Group on Alternative Methods (AET)

Establishment and composition

After several years of informal contacts between members of the Spanish Toxicology Society, agreement on the convenience of the formal constitution of the group was eventually reached due to the excellent opportunity provided when Spanish participants attended the Working Group on Complementary Methods in1995. The group was formally established in 1995 as a speciality group of the Spanish Toxicology Society (AET), and has 95 members.

Aims:

The main objective of the Grupo de Trabajo Especializado en Métodos Alternativos is to stimulate cooperation and the co-ordination of scientific activities contributing to the development of new experimental methods, *in vivo* and *in vitro*, which will reduce the number of animals used, refine techniques in order to reduce animal suffering, or replace the use of animals altogether (the three "Rs").

Another aim is to stimulate the participation of Spanish research groups in method prevalidation and validation programmes and to promote the regulatory acceptance of alternative methods, particularly *in vitro* toxicity methods.

Main areas of interest:

Main interest areas of GTEMA members are:

- basal cytotoxicity
- organ-specific toxicity: irritation, absorption, neurotoxicity, hepatotoxicity, etc.
- development of model systems
- metabolism
- mechanisms of action and effects: apoptosis, differentiation, oxidative stress.
- mutagenicity
- alternatives in education

Production:

- Stimulation of co-ordinated projects and application for grants related to alternative methods
- Dissemination of information about activities in the field of alternatives in the official journal of the Society: Revista de Toxicología
- GTEMA Newsletter (in Spanish), 4 numbers/year from 1996, distributed to all the members of the Spanish Toxicology Society (400)
- E-mail network: GTEMA Distribution List: e-mail messages, 15 numbers/year, from 1996, distributed to more than 100 members in 7 countries.
- Internet web site: http://tox.umh.es/aet/gtema/

Contact Person:

Dr Guillermo Repetto
Coordinator of GTEMA- Spanish Group on Alternative Methods
National Institute of Toxicology
P. O. Box 863

41080 - Sevilla, Spain.
Tel 34 954371233, Fax 34 954370262, Email: repetto@sev.inaltox.es

Collaborations:
- ECVAM: European Centre for the Validation of Alternative Methods
- OECD: Organization for Economic Cooperation and Development
- AET: Spanish Toxicology Society
- ETAC: Group of Alternative Toxicological Methods in Cuba
- ERGATT: European Research Group for Alternatives in Toxicity Testing
- ESTIV: European Society of Toxicology in Vitro
- EUROTOX: European Society of Toxicology
- CICYT: Interministerial Commission of Science and Technology (E)
- ICLAS/CICYT/CSIC Working Group on Complementary Methods
- SECAL: Spanish Society for the Science of Laboratory Animal
- SEEA: Spanish Society for Animal Research
- SEMA: Spanish Society of Mutagenesis
- ADDA: Spanish Society for the Defence of Animal Rights

10.4.- REMA- Spanish Network for the Development of Alternative Methods

Establishment and composition

- This initiative was proposed at the *Meeting for the Development and Co-ordination with ECVAM of the Spanish groups interested in Alternative Methods,* Glaxo Wellcome, Madrid, October, 1997, with the participation of 30 experts in the field, organized by the Drs de la Peña, Repetto and Gómez Lechón, for the ICLAS/CSIC and GTEMA.
- Formal constitution is scheduled in December 1999.
- The Red Española para el Desarrollo de Métodos Alternativos is not a society, because the members of the network are different societies interested in alternative methods (scientific, animal welfare, etc). Observers from the administration are also included. In this sense, it is similar to platforms created in other European countries (The Netherlands, Belgium)

The designated members of the Promoter Commission were: Drs Eduardo de la Peña, José V. Castell, Guillermo Repetto, Adela López de Cerain, Domingo Gargallo, and Eugenio Vilanova

Aims:

To create a reference point for Spanish scientific associations, interested in alternative methods (in biochemistry, pharmacology, toxicology, environmental, etc), in order to favour and promote joint initiatives.

Main areas of interest:

To potentiate the interchange of ideas, the preparation of professionals, research in the field, bi-directional communication with the administration, the industry, and in general, to increase the representativeness of Spain in international forums. In summary, inform, form and develop activities on alternatives.

Production:

- Gascó P, Barrueco C and Guadaño A (1997) Acta de la Reunión para el Desarrollo y la Coordinación con ECVAM de los grupos españoles interesados en Métodos alternativos.

- REMA (1998) de la Peña E, Castell JV, Repetto G, López de Cerain A, Gargallo D, Vilanova E. Documento de Trabajo sobre la Constitución de la Red Española para el Desarrollo de Métodos Alternativos (REMA*).* Revista de Toxicologia 15: 133-142

- Internet web site: http://tox.umh.es/rema/

Contact Person:

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Srta. Gema Mª Castro
Glaxo Wellcome. C/ Severo Ochoa 2.
Parque Tecnológico de Madrid-Tres Cantos
Apartado de Correos: 36094
28080 Madrid
Email: rema@umh.es
Tel: 918070485 Fax: 918070595

11 Spanish activities related to alternatives

To review the increasing interest and activities developed in Spain in relation to alternatives in the last decade, we have included information, taken and completed from (REMA, 1998), on some of the most relevant examples.

Table 6. Some scientific activities organized in Spain during last decade

DENOMINATION	PLACE, DATE	ORGANIZED BY
Course on culture of animal cells	Valencia, annual from 1974	Dr Javier Cervera, Instituto de Investigaciones Citológicas
Intensive Course on General Toxicology	Sevilla, annual 1980-1992	Dr Manuel Repetto, Instituto Nacional de Toxicología
Course Molecular basis of Clinical Toxicology	Valencia, 1992, 1994 and 1996	Dr J Vicente Castell, Faculty of Medicine
1st Working Days on the Validation of *in vitro* alternative models	Valencia, 1992	Drs Alberto Giráldez, J Vicente Castell and Mª Carmen Fernández Criado
Course Cellular Models for the study in vitro of the toxicity of pharmaceuticals	Barcelona, 1992	Faculty of Medicine UAB and CID (CSIC)
Course on Cellular Biology and Environmental Toxicology	Bilbao, 1994 and 1996	Department of Cellular Biology, University of the Basque Country
2nd Working Days on the Validation of in vitro Alternative models in the pharmaceutical and cosmetic industry	Peñíscola, 1994	Drs Bort, Castell, Donato, Fernández Criado, Gomez Lechón, Guillén, Herrero, Ponsoda and Testar, for the Hospital Universitari la Fe, SECAL and GEFTIV
Workshop on Ecotoxicity tests	Valdeolmos, 1994	Drs J V Tarazona and E de la Peña , for AET
1 Working days about Alternative Toxicological Methods in the Evaluation of Industrial Products	Parque Industrial de Zamudio, Bilbao, 1994	Gaiker
Theoretical and Practical Course on Cell culture, Master in R&D of Pharmaceuticals	Pamplona, 1994-1998	Faculty of Pharmacy, University of Navarra
ICLAS/CSIC Working Group on Complementary Methods	Talavera de la Reina, 1995	Dr Eduardo de la Peña for the ICLAS/CSIC-WGCM
1st Meeting and formal constitution of GTEMA	Tenerife, 1995	Dr Guillermo Repetto for the GTEMA
Course *In vitro* Pharmaco-Toxicology: the use of cellular models on the evaluation of xenobiotics	Barcelona, Valencia, 1995	Dr Xavier Testar for the University of Barcelona
Session on Alternative Methods, 3rd Latin American Congress of	Tenerife, 1995	Dr Guillermo Repetto for the GTEMA

Toxicology		
2nd Working day on Alternative Toxicological Methods in the Evaluation of industrial chemicals	**Parque Industrial de Zamudio, Bilbao, 1996**	**Gaiker**
Course on Cellular and genetic toxicology	**1996, 7, 9 Valencia**	**Drs Manuel Blanco and Consuelo Guerri, Instituto de Investigaciones Citológicas**
Workshop on the use of *in vitro* cellular models for the investigation of molecular mechanisms of toxicity action, EUROTOX-96, chaired by Drs MJ Gómez Lechón and G Repetto	**Alicante, 1996**	**Dr Eugenio Vilanova for the AET, with the collaboration of GTEMA**
Seminar Advances in the Application of in vitro Alternative Methods to the Evaluation of Pharmaceuticals, Cosmetics and Chemicals	**SmithKline Beecham, Tres Cantos, 1997**	**Drs Eduardo de la Peña and Guillermo Repetto for the GTEMA**
Theoretical and Practical Course on Tissue Culture. Application on Pharmacotoxicological, Diagnostical and Therapeutical studies	**Barcelona, Valencia, Madrid, 1997**	**Drs Adela Mazo and Anna M Gómez Foix for the Spanish Branch of ETCS**
International Seminar "The use of animals in Research: Ethical implications"	**University Complutense of Madrid, 1997**	**Dr Juán Carlos Illera, with the collaboration of ICLAS-WGC and GTEMA**
1st Meeting of European Representatives from the National Centres on Alternatives	**ECVAM, Ispra, Italy, July 1997,**	**Dr Julia Fentem, ECVAM. Participation of G Repetto as representant of GTEMA**
Round Table "Alternative methods in Basic and Applied Research" XII Spanish Congress of Toxicology	**Zaragoza, 1997**	**Dr Guillermo Repetto for the GTEMA**
2nd Meeting of GTEMA	**Zaragoza, 1997**	**Dr Guillermo Repetto for the GTEMA**
Meeting for the Development and Coordination with ECVAM of the Spanish groups interested in Alternative Methods	**Glaxo Wellcome, Madrid, 1997**	**Drs de la Peña, Repetto and Gómez Lechón, for the ICLAS/CSIC-WGCM and GTEMA.**
7th International Congress of the European Association for veterinary Pharmacology and Toxicology	**Madrid, 1997**	**Dr Arturo Anadón for the AET**
Workshop Alternative Methods for the evaluation of ecotoxicity. II Iberian Congress of Contamination and Environmental Toxicology	**Leioa, Vizcaya, 1998**	**Dr de la Peña for the ICLAS-WGCM**
Course on Ecotoxicology and Environmental Toxicology	**Internat. University Andalucia, Baeza, 1998**	**Dr Manuel Repetto, Instituto Nacional de Toxicología**
1st International Meeting on Alternative Techniques to Animal	**Barcelona, 1999**	**Asociación Defensa Derechos del Animal, EuroNICHE, Col.**

Experimentation		**Vet. and GTEMA**

12 Interconnecting people: the [3ERRES] GTEMA mailing list on alternatives

In 1996, the GTEMA- Spanish Group on Alternative Methods first implemented its internet activities, apart from the web-site located at http://tox.umh.es/aet/gtema/, by creating an

E-mail-based network. The [3ERRES] GTEMA Distribution List has sent, more than 15 messages per year since 1996, to more than 100 scientists distributed over 7 countries. The messages are mainly written in Spanish.

The network is absolutely open, and does not require membership in any society or group. Anyone wishing to join should request inclusion from the Coordinator of GTEMA, Guillermo Repetto, at the following address: repetto@sev.inaltox.es

The dissemination of information about activities in the field of alternatives includes:

- OECD proposals, guidelines, meetings
- EU proposals, guidelines, meetings
- Validation studies
- ECVAM activities: workshops, task-forces, reports, pre/validation projects...
- US activities: CAAT, ICCVAM
- National activities: AET, SECAL, SEEA, SEF, SEMA...
- World Congresses on Alternatives
- ESTIV / INVITOX
- PIVT
- Others: EUROTOX, IUTOX, SSCP, IVTS, BTS, FELASA...
- INVITTOX protocols
- Opportunities: grants, prizes, projects...
- Internet: information available

13 The preparation of the inventory

In this second part, the report aims to provide information related to individual Spanish scientists and institutions. The strategy for conducting the survey was:

1 Preparation of a database of about 1000 scientists who might be interested in the project. The selection of addresses and names was carried out using the GTEMA database, the experts that have attended meetings related to alternatives in the last 10 years, the members of the Asociación Española de Toxicología, the groups that have obtained grants related to alternative methods (thanks to the help of Dr Ferrandiz, CICYT), etc.

In addition, another database which included CSIC centres, university departments, hospitals, various industries (pharmaceutical, cosmetic, etc), was created for institutions

2 Preparation of questionnaire formats

The questionnaire was prepared to cover most aspects related to alternatives. Its aim was to be simple yet complete, provide valuable, directly usable information, bearing in mind the constraints imposed by issues of confidentiality. The advice of Dr Annett Janusch (ECVAM) about database requirements at this stage is aknowledged.

3 Questionnaires were sent by ordinary mail to more than 1000 researchers and institutions that might be interested in alternatives. In addition, an electronic version of the questionnaire was distributed to about 500 people included on 3 different mailing lists: the GTEMA-list of people interested in alternative methods, TOXICOL-Forum of Toxicology (http://www.rediris.es/list/info/toxicol.html), moderated by Guillermo Repetto, and the list for animal researchers SECAL-L.

4 Data compilation and preparation of the inventory data base

When the questionnaires were received, the information was included in the database.

5 Writing of the report

The original letter and the questionnaire follow:

INSTITUTO NACIONAL DE TOXICOLOGIA
Departamento Territorial **Dr Guillermo Repetto**
Apdo. Postal 863 **Tel: (34) 954371233**
41080-Sevilla **Fax: (34) 954370262**
España **E-mail: repetto@sev.inaltox.es**

Sevilla,10-Octubre-98

Estimado/a amigo/a:

Si estás interesado en el uso de *Métodos Alternativos en experimentación, ensayo o enseñanza*, te invito a participar en el presente proyecto. En el caso de que no lo estés, probablemente algún colega de tu area agradecerá el que le traslades la presente información, lo que te ruego sin más preámbulos.

Estamos preparando para la Comisión Europea un

Inventario de Instituciones y Científicos Españoles Interesados en Métodos Alternativos a la Experimentación Animal (Refinamiento, Reducción o Reemplazo)

que pretende contemplar a investigadores y centros interesados en Métodos Alternativos *in vivo* o *in vitro* que desarrollan sus actividades en campos muy diversos, incluyendo la *Biología, Bioquímica, Cirugía, Diagnóstico, Farmacología, Fisiología, Genética, Patología, Toxicología*, etc.

Con ello, la Comisión Europea quiere identificar expertos y centros para, entre otras cuestiones, poder contratar a laboratorios para la realización de estudios de validación de nuevos métodos.

Una de las bases fundamentales para la preparación del inventario consiste en el procesado de los cuestionarios que hemos enviado a más de 1000 investigadores españoles. En este momento tienes en tus manos la posibilidad de ser incluido en el mismo, simplemente completando a tiempo el cuestionario adjunto. Ha sido diseñado para facilitar su cumplimentación, y en cualquier caso, puedes limitarte a contestar sólo la parte que te corresponda.

La **fecha límite** de recepción de formularios es el **6 de Noviembre de 1998**

Muchas gracias por tu colaboración

Atentamente.

Dr Guillermo Repetto
Coordinador del GTEMA - G. T. E. en Métodos Alternativos (http://tox.umh.es/aet/gtema/)

Moderador de TOXICOL-Foro Virtual de Toxicología (http://www.rediris.es/list/info/toxicol.html)

CUESTIONARIO PARA LA PREPARACION DE UN

INVENTARIO DE INSTITUCIONES Y CIENTIFICOS ESPAÑOLES INTERESADOS EN METODOS ALTERNATIVOS A LA EXPERIMENTACION ANIMAL (REFINAMIENTO, REDUCCION O REEMPLAZO)

para la Comisión Europea, ECVAM, JRC, Environment Institute

Gracias por su colaboración y por facilitar que sus datos figuren en el inventario.

Nota:**Fecha límite, 6 Noviembre 98**

1 DATOS DEL CENTRO:

- Organismo:
- Centro:
- Departamento:
- Denominación del Equipo:
- Dirección:

- Código postal: Ciudad: Provincia:

- Telef: - Fax: Servidor Web: http://

2 ENTIDAD: (marque con una **x** el número que proceda):

1 Universidad
2 Organismo público de investigación
3 Organismo público de servicio (diagnóstico, control, monitorización, peritajes)
4 Organismo privado de investigación
5 Centro hospitalario
6 Industria ¿tipo?
7 Laboratorio privado por contrato
8 Otros:

3 PERSONAL INTEGRANTE DEL EQUIPO: incluir para cada participante al menos los siguientes datos, en hoja aparte si fuera necesario:

1(Persona de contacto)- Nombre y apellidos / Email / Grado (Dr, Ld, I, T, D) y Titulación académica

/ Posición / % horario de dedicación a métodos alternativos / Designaciones y cargos relacionados con MA

2 Nombre y apellidos / Email / Grado (Dr, Ld, I, T, D) y Titulación académica

/ Posición / % horario de dedicación a métodos alternativos / Designaciones y cargos relacionados con MA

3 Nombre y apellidos / Email / Grado (Dr, Ld, I, T, D) y Titulación académica

/ Posición / % horario de dedicación a métodos alternativos / Designaciones y cargos relacionados con MA

4 Nombre y apellidos / Email / Grado (Dr, Ld, I, T, D) y Titulación académica

/ Posición / % horario de dedicación a métodos alternativos / Designaciones y cargos relacionados con MA

5 Nombre y apellidos / Email / Grado (Dr, Ld, I, T, D) y Titulación académica

/ Posición / % horario de dedicación a métodos alternativos / Designaciones y cargos relacionados con MA

Nº Total de personas implicadas en métodos alternativos en el equipo

4 OBJETIVO ¿Cuál es la finalidad de su experimentación?:

1 Investigación básica
2 Investigación aplicada no regulada
3 Evaluación con finalidad reguladora (registro, seguridad, biocompatibilidad, eficacia, riesgo; clasificación y etiquetado, protección del trabajador o paciente)
4 Desarrollo de métodos
5 Validación de métodos
6 Alternativas a los animales en la enseñanza
7Otras:

5 AREAS DE APLICACIÓN: Marque con una **X** sólo las areas de interés del equipo, indicando si es en forma habitual (H) u ocasional (O):

H / O 1 Biología animal, vegetal, humana...(especifique)
H / O 2 Biología celular
H / O 3 Biologia molecular
H / O 4 Bioquímica
H / O 5 Farmacodinámica (especifíquese)
H / O 6 Farmacología (especifíquese)
H / O 7 Fisiología
H / O 8 Biocinética y biotransformación
9 Toxicología:
H / O 91 Mecanismos de toxicidad
H / O 92 Citotoxicidad basal
H / O 93 Toxicidad sistémica aguda
H / O 94 Toxicidad ocular
H / O 95 Irritación y corrosividad dérmica
H / O 96 Nefrotoxicidad
H / O 97 Toxicidad respiratoria

H / O 98 Toxicidad para la reproducción
H / O 99 Neurotoxicidad
H / O 910 Inmunotoxicidad y sensibilización
H / O 911 Hematotoxicidad
H / O 912 Genotoxicidad / mutagenicidad
H / O 913 Carcinogénesis
H / O 914 Fototoxicidad
H / O 915 Hepatotoxicidad
H / O 916 Alteraciones endocrinas
H / O 917 Toxicidad crónica
H / O 918 Ecotoxicidad
H / O 919 Estrategias integradas

H / O 10 Genética
H / O 11 Cirugía
H / O 12 Patología
H / O 13 Producción (especifíquese)
H / O 14 Nutrición
H / O 15 Diagnóstico (especificar)
H / O 16 Monitorización: química, biológica (bioindicadores, biomarcadores)
H / O 17 Control de calidad
H / O 18 Métodos de cultivo
H / O 19 Otros:..

6 PRODUCTOS / COMPUESTOS EVALUADOS: Indíquese los que investigue en forma Habitual (H) u Ocasional (O):

H / O 1 Vacunas
H / O 2 Hormonas
H / O 3 Medicamentos
H / O 4 Cosméticos
H / O 5 Compuestos químicos diversos
H / O 6 Plaguicidas
H / O 7 Drogas de adicción
H / O 8 Toxinas
H / O 9 Accesorios médicos
H / O 10 Biomateriales (biotecnología)
H / O 11 Aditivos alimentarios
H / O 12 Colorantes
H / O 13 Contaminantes ambientales
H / O 14 Residuos
H / O 15 Agentes físicos
H / O 16 Otros:

7 TIPO DE ENSAYOS:

1 Tamizado o criba (screening)
2 Complementario
3 Sustitutivo

8 MODELOS EMPLEADOS:

1 Modelos animales:
- 11 Convencionales. Tipo, reducción, refinamiento.
- 12 Transgénicos. Tipos
- 13 Invertebrados. Tipos

2 Embriones. Tipos
3 Vegetales. Tipos
4 Métodos *in vitro*:
- 41 Microorganismos ¿cuáles?
- 42 Cultivo de órganos, lonchas, ¿tipo?
- 43 Cultivo de explantes, reagregados, órganos reconstituidos... ¿tipo?
- 44 Cultivo primario de células dispersadas ¿célula, especie?
- 45 Cultivo de lineas celulares ¿cuáles?
- 46 Fracciones celulares ¿tipo?

5 Estudios con voluntarios humanos ¿tipo de innovación?
6 Modelos en la enseñanza ¿tipo?
7 Modelos Matemáticos ¿tipo?
8 Otros ¿cuáles?

9 BIOINDICADORES EMPLEADOS:

1 *In vivo* (indicar)

2 *In vitro*:
- 21 Morfología (técnica)
- 22 Viabilidad (técnica)
- 23 Citoesqueleto / Membranas/ Liberacion de enzimas (técnica)
- 24 Proliferacion (método)
- 25 Actividad metabólica . Tipo
- 26 Señalización celular. Tipo
- 27 Acidos nucleicos (efectos)
- 28 Sistemas biotransformadores (Tipos)

29 Sistemas defensivos (Tipos)
30 Indicadores organoespecíficos (Tipos)
31 Otros

10 SISTEMAS EXPERIMENTALES: Incluir algunos de los sistemas empleados. (ej, 1 linea celular de fibroblastos de ratón 3T3, 2 captación de rojo neutro determinada por fotometría, 3 Estudio de fototoxicidad, 4 Sustitutivo):

1 1 Modelo biológico
2 Bioindicador y técnica de determinación
3 Finalidad del estudio
4 Tipo de ensayo

2 1 Modelo biológico
2 Bioindicador y técnica de determinación
3 Finalidad del estudio
4 Tipo de ensayo

3 1 Modelo biológico
2 Bioindicador y técnica de determinación
3 Finalidad del estudio
4 Tipo de ensayo

4 1 Modelo biológico
2 Bioindicador y técnica de determinación
3 Finalidad del estudio
4 Tipo de ensayo

11 GARANTIA DE CALIDAD / GLPs:
¿Dispone el centro de Unidad de Garantía de Calidad? Si / No
¿Cumple el laboratorio la legislación de Buenas Prácticas de Laboratorio? Si/No; ¿cuáles? UE, OECD, FDA

12 PROGRAMAS DE VALIDACION DE METODOS DE ENSAYO:
¿Ha participado en programas previos? Si / No; ¿Cuáles?

¿Está disponible para participar en programas financiados por la Unión Europea? Si / No; ¿de qué tipo?

13 FUENTES DE FINANCIACION: Indíquense las principales fuentes de financiación del equipo en los últimos 5 años, tanto públicas como privadas, regionales, nacionales e internacionales, señalando si procedieron de programas/objetivos prioritarios específicos para Métodos Alternativos:

14 LINEAS DE TRABAJO: Se ruega describir, preferiblemente en inglés y con menos de 50 palabras, las principales lineas de trabajo del equipo:

15 OTRAS: Por favor, incluya cualquier otra información o comentario que considere relevante

* Se agradece el envio de la relación de publicaciones de los últimos 5 años.
AGRADECIMIENTO: Muchas gracias por colaborar facilitando que su equipo sea incluido en el inventario.

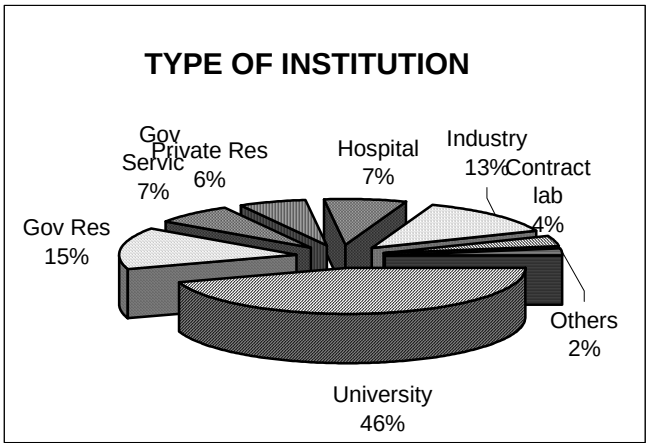
Se ruega remitir **antes del 6 de Noviembre de 1998** a:

Dr Guillermo Repetto
GTEMA- Grupo de Trabajo Especializado en Métodos Alternativos
Http://tox.umh.es/aet/gtema/
Instituto Nacional de Toxicología Tel: 954371233
Apdo Postal 863 Fax: 95437026241080
SEVILLA Email: repetto@us.es

14 Overall evaluation of the inventory

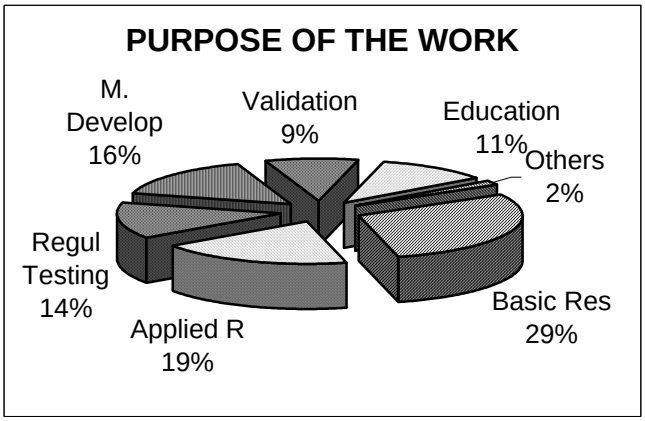
Of the 103 questionnaires received, several were from groups from other countries, so they were included in the inventory, but not in the analysis of the results. The number of Spanish groups interested in alternatives is quite large (98), 75 very competitives, with more than 339 scientists involved, the media being 4.5 scientists per group .

Fig 8. Distribution of the Spanish groups interested in alternatives according to the type



of institution

Fig 9. Distribution of the Spanish groups interested in alternatives according to the main purpose of the work.



In relation to the type of institution, the university was the most active in terms of the number of groups (46 %), followed by governmental research facilities (including CSIC) (15 %), industry (13 %), hospitals and governmental service facilities with 7 %, etc.

The main purpose of the work related to alternatives was basic research (29 %), followed by non-regulated applied research (19 %), development of methods (16 %), regulatory testing (14 %), alternatives in education and training (11 %) and validation of methods (9 %).

Fig 10. Distribution of the Spanish groups interested in alternatives according to the main area of application.

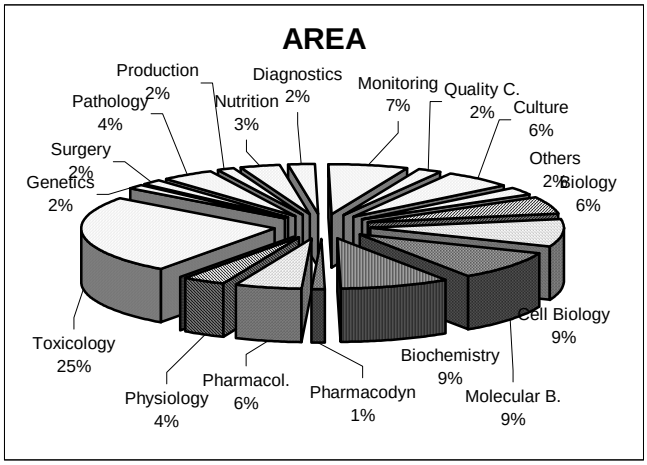
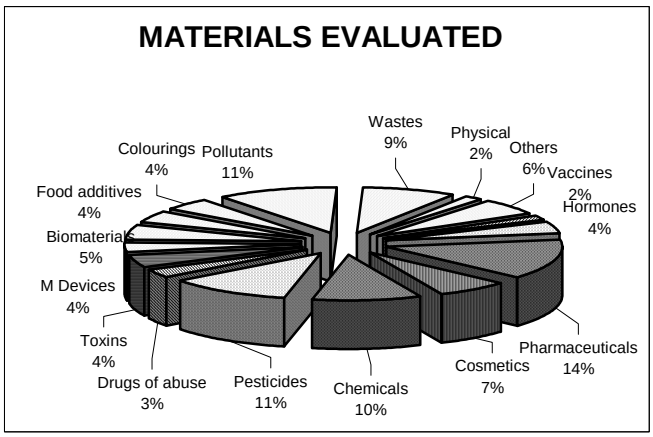


Fig 11. Distribution of the Spanish groups interested in alternatives according to the main materials evaluated



With regard to the main area of application, the most numerous was clearly toxicology (25 %), followed by biochemistry, molecular biology and cell biology (9 %), monitoring (7%), biology and pharmacology (6 %), etc. Nearly all the 20 disciplines of toxicology are represented in similar manner.

Many types of materials are evaluated, including pharmaceuticals (14 %), pesticides and environmental pollutants (11 %), diverse chemical compounds (10 %), wastes (9 %), cosmetics (7 %), vaccines (6 %), etc.

In relation to the type of testing, the results were balanced, with screening (35 %), complementary or adjunct (34 %) and replacement tests (31 %).

Fig 12. Distribution of the Spanish groups interested in alternatives according to the main type of assay performed

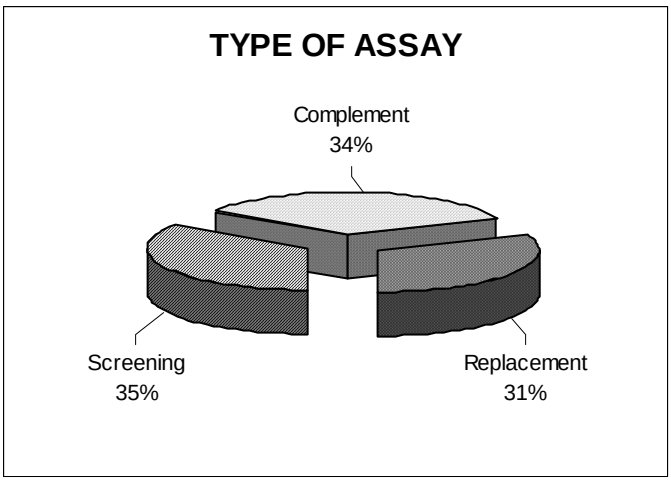
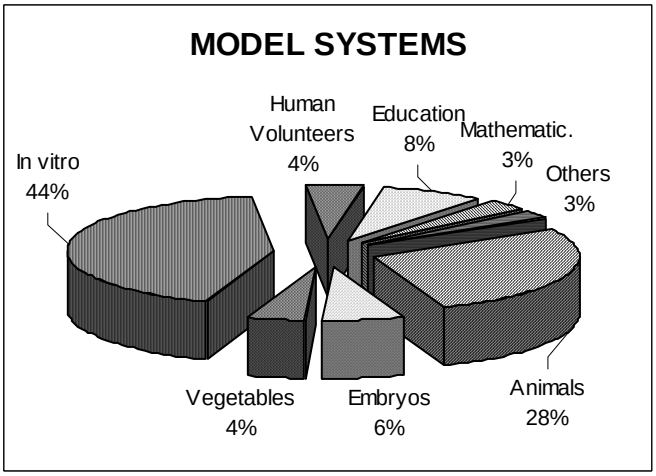


Fig 13. Main model systems used by the Spanish groups interested in alternatives



Regarding the model systems employed, *in vitro* techniques (44 %) were the most often used, followed by animals (28 %), models in education and training (8 %), embryos (6 %), and vegetables and human volunteers (4 %). In so far as the animals used, 63 % of the groups employed conventional animals, while 22 % used invertebrates and 15 % transgenics. Within *in vitro* methods, cell lines were largely used (34 %), followed by primary cultures (25 %), micro-organisms (19 %) and cell-free systems (13 %).

Fig 14. Distribution of animals used *in vivo* by the Spanish groups interested in alternatives

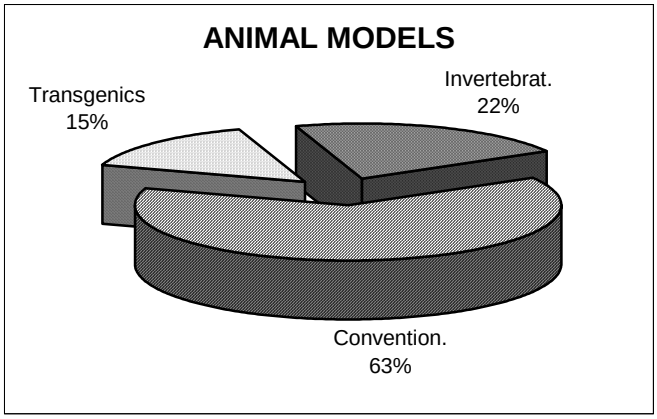
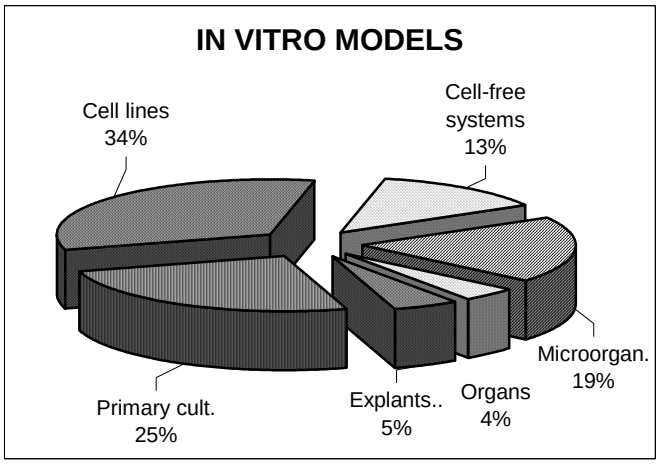


Fig 15. Distribution of *in vitro* models used by the Spanish groups interested in alternatives

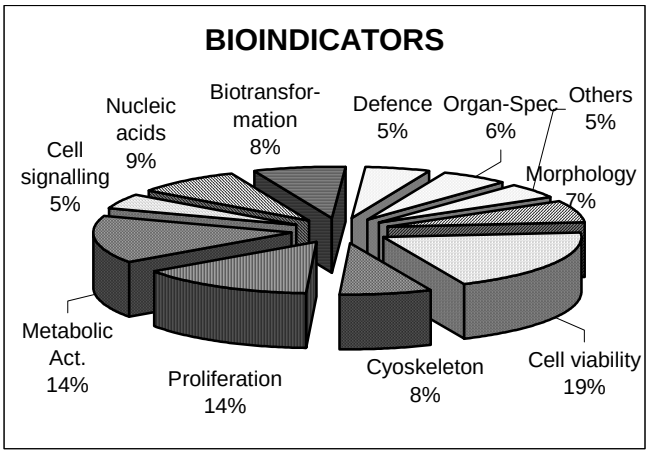


A wide variety of bioindicators were used in *in vitro* models, including cell viability (19 %), cell proliferation (14 %), metabolic activity (14 %), nucleic acids (9 %), cytoskeleton / enzyme release studies (8 %), biotransformation systems (8 %), morphology (7 %), etc

Compliance with Good Laboratory Practices legislation has been affirmed by more than 50 % of the centres. This is probably not absolutely true, but several institutions are also implementing certification by ISO 9000 and accreditation by EN 45001 rules.

Although only a minimum part of the laboratories have participated in previous validation studies, most of then are available to participate in future validation programmes.

Fig 16. Distribution of the bioindicators used in *in vitro* models employed by the Spanish groups interested in alternatives



15 Final comments and future trends

From the overview outlined in this report, the impressive development that alternative methods have undergone in the last ten years in Spain is evident.

The field has become more structured on the institutional ground but also better shaped in its theoretical and scientific aspects. This kind of development undoubtedly represents a positive step and a reliable reference point ifor figuring out possible future trends and related investments in terms of their financial, professional and political aspects. Several aspects should be taken into consideration:

15.1.- The regulations

Only 2 Spanish regions have been able to implement their legislation according to the legal requirements of Directive 86/609/EEC, translated to Spanish regulations in RD 223/1988, which protects laboratory animals and avoids unnecessary studies. Moreover, the rest of the regional governments should be urged to do so as soon as possible, and to make necessary provisions to assure compliance.

The Spanish Ministry of Agriculture should carry out their obligations, as well as possible, in relation to the control of and information to the Commission about animals used for scientific purposes. Non-registered establishments should not be allowed to perform experiments with animals.

15.2.- The sponsors

The total Spanish investment in R&D in Spain should be increased as soon as possible, from the 0.85 of 1997, to reach at least the European median, now situated at 1.9 % of the Gross Domestic Product.

In Spain there is no fixed amount of investment for alternative methods. The answers given to the the survey questionnaire indicate that there are only a few cases in which Spanish groups were financed by the specific priorities for alternatives. There are many differences in the regional programmes, not only in the amount of investment, but also in terms of priorities.

The present objective and research tasks included in the National Programme of Health were not very useful in promoting alternatives. The reduced scope of the objective within the pharmaceutical research area, limited to new pharmaceuticals, providing no opportunities for institutions working with pesticides, cosmetics, food additives, industrial chemicals, etc, should be much wider. To better stimulate alternative studies, its formulation should prioritize the impact of alternatives on the real world as much as possible, in order to provide more stringent evaluation of the projects.

The bureaucracy related to the national programmes delayed unnecessarily each step, from the presentation of proposals, to approval, and the receipt of funding. For this reason, the projects became obsolete before starting, and the teams lost competitivity.

Private help for investment in Spain should be increased at least by 15 % to reach the European median.

Spanish industry should be asked to fund specific grants for alternatives not currently in existence.

All research grants in every scientific area, funded by public or private entities, should require the commitment of the researchers to comply with present legislation about animal protection, including the registration of animal facilities, the preparation of responsible people for performing animal experiments, the prevision of the number of animals to be used, the possibility of causing pain or suffering, alleviation procedures, and the reasons for not using alternative methods if they are available.

15.3.- The promoters

The impressive activities carried out for the promotion and the development of alternative methods in Spain should be encouraged. Different and complementary activities can be promoted by the existing initiatives, including the Spanish Group on Pharmaco-Toxicology *in vitro* (GEFTIV), the ICLAS/CSIC Working Group on Complementary Methods, the GTEMA- Spanish Group on Alternative Methods, and the Spanish Network for the Development of Alternative Methods (REMA).

The interconnection of scientists by means of internet through the [3ERRES] GTEMA mailing list has been proven to be very useful and should be promoted.

15.4.- The scientists and the institutions

An important increase in alternative approaches within Spanish scientific production has been observed since 1966 in relation to overall international production, although, at present, it is only 2.13 % of the whole. The increase in experimental studies is proportionally more important for the publications using alternative methods, but with some delay in Spain (32 % in contrast to 36 %).

Appropiate preparation of researchers and technicians should be encouraged. FELASA recommendations on the education and training of persons working with laboratory animals should be followed, and the creation of active ethical committees should be promoted.

The administrative rigidity of many institutions, which block the direct contact with the private sector should evolve to more flexible systems, providing opportunities to make industrial collaboration easier.

Although only a minimum part of the laboratories have participated in previous validation studies, most of them are available to participate in future validation programmes.

More than 75 very competitive Spanish groups interested in alternatives have been identified, with more than 339 researchers. With respect to the main area of application, the most potent was clearly toxicology (25 %), followed by biochemistry, molecular biology and cell biology (9 %), monitoring (7%), biology and pharmacology (6 %), etc. Nearly all the 20 disciplines of toxicology are represented in similar manner.

15.5.- The European level

The 5th Framework Programme of the European Community for Research, Technological Development and Demonstration Activities (1998/2002) represents an outstanding challenge for *in vitro* toxicology, by offering several opportunities on different grounds to promote and consolidate this activity at the European level

An effective connection with ECVAM is necessary for joint European action pro-alternatives. Initiatives such as the Meeting of Representatives of European Entities Responsible for Alternatives should be organized periodically.

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18 Inventory of institutions

Notes:
[1]To avoid any loss in consistency, some concepts were not translated to English: names of institutions, professional degrees and positions.
[2]According to the tradition in Spain, two surnames are included for each scientist. The first scientist of each group (103) is the contact person.
[3l]The following information is included in experimental systems: biological model system, endpoint and endpoint measurement, aim of the study / area of application, and type of assay.
Prefix for tel and fax is 34

Allmirall Prodesfarma, S.A.

Centro de Investigación

Departamento de Farmacocinética y Metabolismo

Laureà Miró, 408-410

08980 Sant Feliu de Llobregat-- Barcelona

Tel: 932912940, Fax: 932912997, Website: http:// www.almirallprodesfarma.com

Type of Institution: Industrial (pharmaceutical)

Staff:

- Antonio Martínez Tobed, Email: amtobed@almirallprodesfarma.com, Dr, Director Desarrollo Biológico
- Miguel Salvà Coll, Email:msalva@almirallprodesfarma.com, Dr, Técnico Investigación, 70 % time devoted to altern. meth.
- Francisco Jiménez Berbel, D, Ayudante Laboratorio, 70 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in regulatory testing; it performs biochemistry, biokinetics and biotransformation testing on a routine basis. Pharmaceuticals are routinely evaluated. The main use of alternative methods is for complementary and replacement tests.

Model systems: The model systems used are *in vitro* methods - organ culture (rat liver slices) and cell-free systems (S9 fractions, human recombinant P450).
The endpoints employed *in vitro* are biotransformation systems (cytochrome P450, nDP- GTM flavine dependent).

Experimental systems (examples):
1 rat liver microsomes, NADP dependent pharmaceutical biotransformation (by HPLC), metabolic profile, complementary test
2 human recombinant cyt P450-NADP dependent pharmaceutical biotransformation, identification of enzymes responsible for the biotransformation process, complementary test

3 liver microsomes from different animal species, pharmaceutical metabolite isolation by HPLC, identification of metabolites, replacement test
4 rat liver microsomes, P450 and UDP-glucuronyl transferase activities, metabolic enzymes induction, complementary test

Work lines: Comparison of the metabolic profiles of new drug candidates in different animal species using liver microsomes and/or S9 fraction. Isolation and identification of drug metabolites produced by liver microsomes. Identification of the enzymes involved in the metabolism of new drugs. Assessment of drug-metabolizing enzyme induction in rats.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU, OECD

Antonio Puig S.A.
Departamento de Toxicología Cosmética
Potosí 21
08030 Barcelona
Tel: 934007079, Fax: 934007063

Type of Institution: Industrial (cosmetics)

Staff:

- Josep Maria Reig i Carnicé, Email: reig@puig.es, Ld Ciencias Biológicas, Jefe Departamento
- Maria del Mar Recasens, Email: recasens@puig.es, Dr Ciencias Biológicas, Técnico Investigador
- Paz Terraz, Ingeniero Técnico industrial.

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (cosmetics) department is mainly involved in regulatory testing and method development; it performs toxicology (ocular cytotoxicity, dermal irritation and corrosivity, immunotoxicity and sensitisation, phototoxicity) testing on a routine basis, and cell biology, biochemistry, pharmacology, physiology and culture methodology studies at a research level. Cosmetics and essential oils are routinely evaluated.
The main use of alternative methods is for replacement tests.

Model systems: The model systems used are *in vitro* methods -- culture of explants, reaggregates, reconstituted organs (skin), primary culture of dispersed cells (fibroblasts) and cell-free systems (physico-chemical models).
The endpoints employed *in vitro* are cell viability (MTT reduction), cytoskeleton/membranes/enzyme release (IL-1 release).

Experimental systems (examples):
1 human erythrocytes, haemolysis studied by photometry, irritation and phototoxicity,

replacement test
2 skin explants, MTT reduction by spectrophotometry, irritation, photoprotection, replacement test
3 skin explants, IL-1alpha by ELISA, irritation and photometry, replacement test

Work lines: Safety evaluation of cosmetic products by alternative methods to animal testing. Evaluation of sun protection factors. Efficacy evaluation.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the OECD. The team are available to participate in EU validation programmes in relation to alternative methods to the use of animals.

Asociación para la Defensa de los Derechos del Animal

c/ Bailén 164 Local 2

08190 Barcelona

Tel: 93 4591601, Fax: 934590265, Website: http:// www.intercom.es/adda

Type of Institution: society for animal rights

Staff:

- Núria Querol Viñas, Email: 2033491@campus.uab.es, Ld Biología, Especialidad de Biología Cellular y Genética. UAB

Total staff involved in alternative methods is 1 person.
Activities / aims: This society for animal rights is mainly involved in the dissemination of alternatives

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Beafour-Ipsen

Laboratorios Lasa S.A.

Animalario

Crra. Laureà Miró nº 395

08980 S.Feliu de Llobregat-- Barcelona

Tel: 93 6662611, Fax: 93 6851053

Type of Institution: Industrial (pharmaceutical)

Staff:

- Javier Guerrero Bertolín, Email: javier.guerrero@beafour-ipsen.com, Ld Veterinaria, Asesor en bienestar animal, Secretario del Comité, 50 % time devoted to altern. meth., duties related to altern. meth.: review experimental protocols and evaluation of 3 Rs compliance.

Total staff involved in alternative methods is 1 person.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research, non-regulated applied research, regulatory testing and alternatives to the use of animals in education; it performs pharmacodynamics, biokinetics and biotransformation, toxicology testing (acute systemic toxicity, neurotoxicity, endocrine disruption) on a routine basis, and biochemistry, pharmacology, physiology, surgery, pathology and production studies at a research level. Hormones, pharmaceuticals, diverse chemical compounds and biomaterials are routinely evaluated.
The main use of alternative methods is for complementary studies.

Model systems: The model systems used are animal models –conventional and transgenics--, *in vitro* methods -- cell-free systems (microsomes) and education models.
The endpoints employed *in vitro* are metabolic activity biotransformation systems

Work lines: Standardization of refined methods for dehabituation and desensibilization on animals

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU, and FDA in implementation.

Others: The supervision of protocols on ethical committees and education related to the 3 Rs

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Centro de Investigación y Desarrollo Aplicado SAL
CIDA
C.T. Santiga C/Argeters, 6
08130 Sta. Perpetua de Mogoda-- Barcelona
Tel: 937190361, Fax: 937189667, Website: http:// www.cidasal.es
Type of Institution: Private / contract laboratory

Staff:

- Jorge Zapatero Lorenzo, Email: cida@logiccontrol.es, Ld Biología, Director Dpto. Toxicología, 5 % time devoted to altern. meth.
- Cristina Peraire Sirera, Email: cida@logiccontrol.es, Ld Biología, Directora de Estudios Dpto. Farmacología, 50 % time devoted to altern. meth.
- Ricard Molla Palleja, Email: cida@logiccontrol.es, Dr Biología, Dtor. Estudios. Dpto. Toxicología, 40 % time devoted to altern. meth.
- Araceli Tortajada Cervantes, Email: cida@logiccontrol.es, Ld Biología, Dtra. Estudios. Dpto de Toxicología, 5 % time devoted to altern. meth.
- Monserrat de Luna Moreno, Email: cida@logiccontrol.es, Ld Biología, Dtra. Estudios. Dto de Toxicología, 5 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This private / contract laboratory department is mainly involved in

non-regulated applied research, regulatory testing, method development and method validation; it performs toxicology (basal cytotoxicity, ocular cytotoxicity, haematoxicity, genotoxicity / mutagenicity) culture methodology and efficacy of new materials testing on a routine basis. Pharmaceuticals, cosmetics, diverse chemical compounds, medical devices, biomaterials and colourings are routinely evaluated; vaccines, hormones, pesticides and food additives also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models, embryos (HET-CAM), *in vitro* methods - - micro-organisms (*Salmonella typhimurium*, *E. coli*), culture of explants, reaggregates, reconstituted organs, (human spongy bone explants, BCOP- bovine isolated cornea) and cell lines culture (L929, MG63, SIRC, L1210, Lewis lung carcinoma LI, L51784, human osteoblasts, bovine corneal cells).
The endpoints employed are *in vitro* morphology (optical microscopy, scanning electronic microscopy), cell viability (MTT reduction, neutral red uptake, LDH, trypan blue exclusion), enzyme release (LDH leakage), cellular proliferation (cell count (chamber, cell counter)), metabolic activity (mitochondrial (MTT)) and organ-specific indicators (osteocalcine, alkaline phosphatase in osteoclasts).

Experimental systems (examples):
1 L929 mouse fibroblasts, MTT reduction, neutral red uptake by photometry, basal cytotoxicity, replacement test
2 Human osteoblasts, cell adhesion and proliferation, MTT reduction, cell number, protein content, alkaline phosphatase (spectrophotometry), osteocalcine (ELISA), morphology (SEM), physiology, osteointegration, replacement test
3 HET-CAM chick embryo chorioalantoid membrane, membrane alteration (visualization), ocular irritation, replacement test
4 BCOP- Isolated bovine cornea, corneal opacity (optical transmission), permeability (photometry), ocular irritation, replacement test

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU, OECD and the FDA. The team have previously been involved in the following validation programmes on alternative methods: EU/ Home Office Validation Study of Ocular Irritation alternative methods. They are is available to participate in EU validation programmes related to validation of alternative methods

Sources of financing: Self financing, CRAFT project (UE), CIRIT project (Generalitat de Catalunya)

Clínica San Francisco Javier
Avda. Baja Navarra 52
31002 Pamplona-- Navarra
Tel: 93 8231600

Type of Institution: Private research facility

Staff:

- Luis Alfonso Núñez Domínguez, Email: lan@abc.ibernet.coml, Dr.

Total staff involved in alternative methods is 1 person.

Activities / aims: This private research facility department is mainly involved in basic research; it performs toxicology (neurotoxicity) testing on a routine basis, and biochemistry studies at a research level. Drugs of abuse are routinely evaluated; pharmaceuticals also being occasionally studied.

Model systems: The model system used is human volunteers

Experimental systems (examples):
1 psychopathological questionnaires, drug of abuse consumption, consequences of drug abuse

Work lines: Long-term health consequences of drug abuse in human beings, especially cannabis abusers, and among psychiatric patients.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to drugs of abuse screening, and long-term effects.

Consejo Superior de Investigaciones Científicas
Centro de Ciencias Medioambientales
Departamento de Agroecología
Toxicología-Ecotoxicología
Serrano 115 dpdo
28006 Madrid
Tel: 91 5625020, Fax: 91 5640800

Type of Institution: Governmental research facility

Staff:

- Margarita Alía Díaz, Email: arias@cc.csic.es, Dr Farmacia, Investigador Científico, 40 % time devoted to altern. meth.
- José Antonio González Pérez, Dr Ciencias Biológicas, Investigador Contratado, 40 % time devoted to altern. meth.
- Susana Cobacho Arcos, Ld Ciencias Biológicas, Becaria.
- Lucia Irene Herencia Abendaño, Ingeniero Agrónomo, Becaria, 40 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This governmental research facility department is mainly involved in basic research, non-regulated applied research, method development and method validation; it performs toxicology testing (mechanisms of toxicity, reproductive cytotoxicity) on a routine basis and monitoring --chemical, biological-- studies at a research level. Pesticides and environmental pollutants are occasionally evaluated.
The main use of alternative methods is for complementary and replacement studies.

Model systems: The model systems used are animal models -- invertebrates (nematodes) and *in vitro* methods.
The endpoints employed are *in vivo* faunistic study of nematodes and *in vitro* cell viability (loss of sensitive groups).

Experimental systems (examples):
1 nematodes, order Dorylainmida, viability, soil contamination by nitrates, replacement test

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Consejo Superior de Investigaciones Científicas
Centro de Ciencias Medioambientales
Genotoxicología y Mutagénesis Ambiental
Serrano 115 Dpdo
28006 Madrid
Tel: 91 5625020, Fax: 91 5640800
Type of Institution: Governmental research facility

Staff:

- Eduardo de la Peña de Torres, Email: epena@ccma.csic.es, Dr C. Biológicas, 80 % time devoted to altern. meth.
- Ana Guadaño Larrauri, Email: aguadano@ccma.csic.es, Dr C. Biológicas, 80 % time devoted to altern. meth.
- Antonia Martínez López. Ayudante Diplomada CSIC.

Total staff involved in alternative methods is 3 people.

Activities / aims: This governmental research facility department is mainly involved in basic research; it performs toxicology testing (genotoxicity / mutagenicity) on a routine basis. Pesticides and new bioactive substances are routinely evaluated.

Model systems: The model systems used are *in vitro* methods -- micro-organisms (*Salmonella typhimurium*) and primary culture of dispersed cells (peripheral lymphocytes).

Sources of financing: CICYT- PGC, Ministerio de Agricultura

Consejo Superior de Investigaciones Científicas
Centro de Investigaciones Biológicas
Biología Celular y del Desarrollo
Biología Molecular de la Gametogénesis
Velázquez, 144

28006 Madrid
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Type of Institution: Governmental research facility

Staff:

- Jesús del Mazo Martínez, Email: cibjm26@fresno.csic.es, Dr Ciencias Biológicas, Jefe de Grupo, 90 % time devoted to altern. meth.
- Luis Andrés López Fernández, Email: ciba257@fresno.csic.es, Dr Ciencias Biológicas, Becario Postdoctoral, 90 % time devoted to altern. meth.
- Mario Párraga San Román, Email: m.parraga@fresno.csic.es, Dr Ciencias Biológicas, Becario Postdoctoral, 90 % time devoted to altern. meth.
- Edmundo Bonilla González, Email: cibb106@fresno.csic.es, Ld Ciencias Biológicas, Becario Predoctoral, 90 % time devoted to altern. meth.
- Fernando Escolar Antúnez, Email: cibea8s@fresno.csic.es, Técnico de Laboratorio, Personal Técnico, 90 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This governmental research facility department is mainly involved in basic research, non-regulated applied research, method development and method validation; it performs cell biology, molecular biology, toxicology (reproductive cytotoxicity), genetics, production (fertility) and culture methodology testing on a routine basis, and biochemistry studies at a research level. Pharmaceuticals, diverse chemical compounds, pesticides and environmental pollutants are routinely evaluated. The main use of alternative methods is for screening and replacement test.

Model systems: The model systems used are conventional animal models and transgenics-, embryos (mouse) and *in vitro* methods --culture of explants, reaggregates, reconstituted organs (ovary), primary culture of dispersed cells (ovocytes, Sertoli cells, mouse sperm cells) and cell lines culture (15P-1 / Sertoli).
The endpoints employed are *in vivo* gene expression and *in vitro* nucleic acids and organ-specific indicators (stress proteins, gene expression).

Experimental systems (examples):
1 in vitro culture of germ cell lines, differential gene expression, gene expression markers

Work lines: Development of *in vitro* germ cell culture systems of and their use on reprotoxicity tests in mammals.

Quality assurance / Validation programmes: The team have previously been involved in the following alternative method validation programmes: Biotechnology

Sources of financing: UE, Comunidad Autónoma de Madrid, DGICyT

Consejo Superior de Investigaciones Científicas
Centro de Investigación y Desarrollo (CID)

Departamento de Química Ambiental
Ecotoxicología
C/ Jordi Girona
08034 Barcelona
Tel: 934006175, Fax: 932045904, Website: http:// www.cid.csic.es
Type of Institution: Governmental research facility

Staff:

- Cinta Porte Visa*,* Email: cpvqam@cid.csic.es, Dr Biología, Colaborador Cientifico, 50 % time devoted to altern. meth., duties related to altern. meth.: person in charge
- Montserrat Sole Rovira*,* Email: msrqam@cid.cis.es, Dr Biología, Colaboradora Científica Contratada, 20 % time devoted to altern. meth.
- Verónica Borghi*,* Email: vebqam@cid.csic.es, Ld Biología, Becaria Investigación, 80 % time devoted to altern. meth.
- Amaya Albalai Rives*,* Email: aarqam@cid.csic.es, Ld Biología, Contratada, 80 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This governmental research facility department is mainly involved in basic research and method development; it performs toxicology testing (mechanisms of toxicity) and monitoring --chemical, biological-- on a routine basis. Pesticides and environmental pollutants are routinely evaluated.
The main use of alternative methods is for complementary studies.

Model systems: The model systems used are animal models -- invertebrates (mussel, fresh water crayfish),-- and *in vitro* methods - primary culture of dispersed cells (digestive glands, *Mytilus* and *Prokambarus clarkii*) and cell-free systems (microsomes of aquatic organisms).
The endpoints employed *in vitro* are cell viability (photometry), biotransformation systems (cyt P450 (EROD), GST), defence systems (antioxidative enzymes) and AChE inhibition.

Experimental systems (examples):
1 primary culture of mussel digestive glands and gills, eosin-y exclusion by photometry; Ache and carboxylesterases, effects of organophosphate pesticides, complementary test
2 primary culture of the fresh water crayfish *Prokambarus clarkii,* eosin-y exclusion by photometry; Ache and carboxylesterases, effects of organophosphate pesticides, complementary test
3 primary cultures of mussel digestive glands, antioxidant enzymes and GST by spectrophotometry, effects of the pesticide iragrol and possible detoxification, screening
4 primary culture of mussel, viability by spectrophotometry, toxicity of naphthalene sulphonates

Work lines: Study the bioaccumulation and biotransformation of organic contaminants in marine organisms. Identify potential biomarkers of exposure/effect in order to assess the impact of pollution in aquatic ecosystems. Development and use of primary

cell cultures (bivalves and crustaceans) as a tool to investigate the toxicity and mechanism of action of pollutants in aquatic organisms.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: MOPT, Evaluación del impacto del vertido de petróleo del "Aegean Sea" en poblaciones de bivalvos de la costa de La Coruña mediante el uso de índices bioquímicos de estrés" (Marzo 93 - Abril 94); Comunidad Europea-Programa ENVIRONMENT "Biological markers of environmental contamination in marine ecosystems" EV5V-CT94-0550 (Julio 94 - Julio 96); Comunidad Europea - Programa MAST-II- "Risk assessment of organotin antifouling on key benthic organisms of European coastal habitats. MAS2-CT94-099" (Enero 95 - Enero 97); DGICYT "Indices bioquímicos de contaminación ambiental en ecosistemas marinos. Acción Integrada con Portugal.HP94-022" (Octubre 95 - Octubre 96); PLANICYT "Marcadores biológicos de contaminación ambiental en ecosistemas marinos. AMB95-1092-CE" (Octubre 95 - Octubre 96); PLANICYT "Bioacumulación y toxicidad de compuestos organoestánnicos en organismos bentónicos de la costa mediterránea. AMB95-1978-CE" (Enero 96 - Enero 97); FAO-MED POL-Pase II "Biomonitoring of pollution effects in the Barcelona and Valencia coastal areas" (Enero 96 - Enero 97); DGICYT "Cultivos celulares primarios como sistemas modelo para investigar el metabolismo y toxicidad de pesticidas organofosforados. Acción Integrada en el Reino Unido". HB1995-0056 (Abril 96 - Marzo 97); Comunidad Europea-Programa ENVIRONMENT "Biological Markers of environmental contamination in marine ecosystems. Biomar II ENV4-CT96-0300" (Julio 96 - Julio 98); CSIC/PAS "Utilización de proteínas de estrés como biomarcadores de contaminación en zonas costeras 1997/98"; PLANICYT "Marcadores Biológicos de contaminación ambiental en ecosistemas marinos. AMB 97-1800-CE" (Enero 98 - Enero 99).

Consejo Superior de Investigaciones Científicas / UCM
Instituto de Bioquímica (CSIC-UCM)
Facultad de Farmacia
Departamento de Bioquímica, Farmacología y Toxicología
Plaza de Ramón y Cajal s/n
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Type of Institution: University

Staff:

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- Carmen Díez Fernandez, Email: carmen.diez@jrc.it, Dra. Farmacia, Contrato C E ISPRA ECVAM, Italy
- Asunción Zaragoza Castellano, Email: cascales@eucmax.sim.ucm.es, Ld.

Farmacia, Becaria UCM

- David Andres García, Email: cascales@eucmax.sim.ucm.es, Ld. Farmacia, Becario
- Alberto Alvarez Barrientos, Dr. Biología, Técnico Citometría UCM

Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in basic research; it performs cell biology, molecular biology, toxicology (hepatotoxicity) and culture methodology studies on a routine basis. Pharmaceuticals and drugs of abuse are routinely evaluated.

Model systems: The model systems used are animal models and *in vitro* methods -- primary culture of dispersed cells and cell-free systems.
The endpoints employed *in vitro* are cell viability, cellular proliferation, metabolic activity and biotransformation systems.

Work lines: Development of a liver specific *in vitro* model for the identification of non-genotoxic carcinogens and compounds with tumour promoting activity (Sandoz-Novartis). Oxygen free-radicals involved in the mechanisms of xenobiotic-induced hepatotoxicity in murins. Influence of age (FIS 95/0032/01). Modulation of hepatotoxicity by xenobiotic interactions. Ageing effect and influence of dietary antioxidants (CICYT PM 96-0010).

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: SANDOZ (NOVARTIS), 1994; FIS 95/0032/01, 1995-1997; CICYT PM 96-0010, 1997-1999

Consejo Superior de Investigaciones Científicas
Instituto de Investigaciones Biomédicas de Barcelona (IIBAPS)
Departamento de Neuroquímica
Neurotoxicidad in vitro
Jordi Girona 18-26
08034 Barcelona
Tel: 934006152, Fax: 932045904
Type of Institution: Governmental research facility

Staff:

- Cristina Suñol Esquirol, Email: csenqi@cid.csic.es, Dr Ciencias, Ld Ciencias Químicas, Ingeniero Químico IQS, Titulado , 80 % time devoted to altern. meth.
- Elena Fonfria Subiró, Email: efsnqi@cid.csic.es, Ld Farmacia, Becaria, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This governmental research facility department is mainly involved in basic research; it performs toxicology (mechanisms of toxicity, neurotoxicity) testing on a routine basis. Pesticides and environmental pollutants are routinely evaluated.

Model systems: The model systems used are *in vitro* methods - primary culture of dispersed cells (neuronal cells, rat/mouse).
The endpoints employed are *in vitro* cell viability (LDH, MTT reduction) and cell signalling (receptor binding, function, neurotransmitter release).

Experimental systems (examples):
1 primary culture of neurones, uptake and release of neurotransmitter (HPLC, and isotopes); receptor binding and ionic flux, neurotoxicity assays

Work lines: The use of *in vitro* neural systems to study mechanisms of neurotoxicity of environmental xenobiotics in relation to their interaction with specific neural activities, such as uptake and release of neurotransmitters and action through receptors (especially receptors for inhibitory neurotransmitters, like GABA and glycine) by determining their status and function (interaction with binding sites and effects on channels or messenger pathways).
Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to pharmaceutical and toxic agents evaluation in neural *in vitro* systems

Sources of financing: CE-BIOMED, FIS, CICYT, pharmaceutical industry.

Consejo Superior de Investigaciones Científicas
Instituto de Investigaciones Biomédicas de Barcelona
Departamento de Farmacología y Toxicología
Neurotoxicología in vitro
Jordi Girona 18-26
08034 Barcelona
Tel: 934006141, Fax: 932045904

Type of Institution: Governmental research facility

Staff:

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- Rosa Cristofol Martínez, Email: rcmfat@cid.csic.es, Dr Biología, Colaborador Científico, 100 % time devoted to altern. meth.
- Eduardo Rodríguez-Farre, Dr Medicina, Investigador Científico, 25 % time devoted to altern. meth.
- Sergi Gasso Pons, Email: sgpfat@cid.csic.es, Ld Biología, Becario Predoctoral, 100 % time devoted to altern. meth.
- Jordi Sebastia Palleja, Email: jspfat@cid.csic.es, Ld Biología, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 7 people.

Activities / aims: This governmental research facility department is mainly involved in basic research and method development; it performs toxicology (mechanisms of toxicity, neurotoxicity) and culture methodology testing on a routine basis, and cell biology and pharmacology studies at a research level. Pesticides, environmental pollutants, neurotransmitters and neuroactive pharmaceuticals are routinely evaluated; pharmaceuticals also being occasionally studied.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are *in vitro* methods -- organ culture (rat brain slices), primary culture of dispersed cells (neurones, astrocytes, microglia (rat, mice, human)) and cell lines culture (neuroblastoma, PC12).
The endpoints employed *in vitro* are cell viability (propidium iodide, calcein), metabolic activity (MTT reduction, enzyme activities), nucleic acids (apoptosis detection) and organ-specific indicators (neurotransmitter release, oxidative stress).

Experimental systems (examples):
1 primary culture of neurones and/or astrocytes, membrane permeability to propidium iodide, cell viability and pharmaceutical protection, replacement test
2 primary culture of neurones and/or astrocytes, MTT reduction, cell viability and pharmaceutical protection, replacement test
3 primary culture of neurones, oxygen free-radical production, by oxidation of DCFH-DA, oxidative stress, replacement test
4 brain slices, neurotransmitter release, synaptic function, replacement test

Work lines: Neurotoxicity mechanisms of xenobiotics in neuronal cultures

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to neurotoxicity tests

*Sources of financing: "*Evaluación de la Neurotoxicidad selectiva y de los mecanismos de acción de xenobioticos ambientales en modelos neurales *in vitro*" FIS, Ministerio Sanidad y Consumo (No, 97/0656) 1997-1999; "Desarrollo de sistemas neurales *in vitro* para el estudio de los efectos tóxicos de xenobioticos"- FIS, Ministerio Sanidad y Consumo (No, 95/1955) 1995/1996; "Factores de Neurotoxicidad selectiva de xenobioticos" Comisión Interministerial de Ciencia y Tecnología (No SAF 94-0076) 1994-1997; "Development of *in vitro* neural and related immune systems for the study of potentially toxic effects of novel and highly specific compounds during cell differentiation" Programa BIOTECHNOLOGY Commission de Les Comunitats Europees (No BIOT-CT93-0224) 1994-1996. "Ajut per a potenciar els grups de qualitat en el marc del pla de recerca de Catalunya. Comissio Interdepartamental de Recerca i Innovacio Tecnologica (1995GR-00551) 1995; "Desarrollo de sistemas neurales *in vitro* para la identificación de agentes con potencial neurotoxicológico y farmacológico". FIS Ministerio de Sanidad y Consumo (Nº 93/0899E) 1993-1994. "Caracterización de mecanismos de acción y efectos neurotóxicos de policlorocicloalcanos" Comisión Interministerial de Ciencia y Tecnología (No SAL91-0707) 1992-1994.

Consejo Superior de Investigaciones Científicas

Instituto de Parasitología y Biomedicina Lopez Neyra
Departameno de Biología Celular e Inmunología
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18001 Granada
Tel: 958805182, Fax: 958203323, Website: http:// www.ipb.csic.es

Type of Institution: Governmental research facility

Staff:

- Jaime Sancho Lopez, Email: granada@ipb.csic.es, Dr. Ciencias Biológicas, Jefe del Laboratorio y del Departamento.
- Mercedes Zubiaur Marcos, Email: mzubiaur@ipb.csic.es, Dra. Ciencias Biológicas, Investigador Contratado.
- Maria Guirado, Email: mguirado@ipb.csic.es, Ld. Farmacia, Becaria Predoctoral
- Teresa Orta, Email: tereorta@ipb.cisc.es, Ld. Medicina, Becaria Predoctoral

Total staff involved in alternative methods is 4 people.

Activities / aims: This governmental research facility department is mainly involved in basic research; it performs cell biology, molecular biology and biochemistry testing on a routine basis, and pharmacodynamics (inhibition of cell physiological responses) and pathology studies at a research level.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are conventional animal models (immunization), transgenics (experimental model of diseases) and *in vitro* methods -- micro-organisms (bacteria) and cell lines culture (T lymphocyte, fibroblast, myeloid cell lines).
The endpoints employed *in vitro* are cell viability (trypan blue exclusion), cellular proliferation (cell count), cell signalling (tyrosine phosphorylation, intracellular associations, cytoquines), nucleic acids (transfection of kinases and tyrosine-kinases) and flow cytometry (FACS).

Experimental systems (examples):
1 human Jurkat T lymphocytes, tyrosine phosphorylation by immunoprecipitation, western blot, identification of new routes of activation and components
2 cos monkey fibroblasts, cDNAs transfection by FACS, western blot, immunofluorescence, possible interactions and biological effects
3 human Jurkat T lymphocytes cytokines, transcription factors by CAT, ELISA, gel-shift, immunoregulation studies

Work lines: Strategies for blocking the association of signalling proteins containing tandem SH_2 domains with an activation motif present in T cells: in some systems, phosphatidilinositol 3-kinase (PI 3-K) constitutes the major link in the control of DNA synthesis. PI 3-K comprised a p85 regulatory subunit coupled to a p110 catalytic subunit. p85 contains two SH2 domains in tandem and constitutes a putative candidate for interacting with doubly phosphorylated ITAMs. CD38-mediated activation of tyrosine kinase signalling pathways: the lab has characterized some of

the early signalling events triggered by CD38 engagement in Jurkat T cells. CD38 ligation with the specific mAb IB4 induced rapid and transient tyrosine phosphorylation of different patterns of cytoplasmic proteins, including PLC-1, c-Cbl, ZAP-70, Shc, and Erk-2 Mitogen-activated Protein (MAP) kinase.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the the EU. The team are available to participate in EU validation programmes, in relation to Biomed and Biotech.

Sources of financing: "Identificación y clonaje de proteinas intracelulares asociadas al complejo TCR/CD (PB92-0123)", DGICYT; "Estructura y función de los receptores para la fracción Fc de la IgA (94/0666)", FIS; "Estudio de los antígenos HLA en tumores humanos y del complejo CD3-TCR en TIL y linfocitos autólogos (Expte: 95/1505)", FIS; "Estrategia para bloquear la asociación de proteínas que contienen dominios SH2 en tandem con un motivo de activación presente en los linfocitos T (SAF96-0117)", CICYT; "Requerimientos moleculares para la inducción de proliferación y activación celular mediados por CD38. Posible asociación con otros receptores y consecuencias funcionales (Expte: 96/49)", Consejeria de Salud. Junta de Andalucía; Consejería de Salud. Junta de Andalucia. Ayuda de apoyo a grupos de investigación. "Interacción de CD38 con el receptor de alta afinidad para la IgG, CSIC y CNR. "Reconstitution of interactions between signaling molecules and the TCR/CD3 complex, NATO; "Requerimientos moleculares de la señalización mediada por CD38" CSIC, CNR; "Señalización mediada por CD38 e identificación de las kinasas asociadas, CSIC e INSERM; "Señalización del receptor para el antígeno", CSIC y CNRS.

Comité Español ICLAS/CICyT
F. de Veterinaria. Universidad Complutense Madrid
Departamento de Patología Animal
28040 Madrid
Fax: 913943882
Type of Institution: University

Staff:

- Paulino Garcia Partida, Dr, Catedrático de Universidad.

Total staff involved in alternative methods is 1 person.

Activities / aims: This university department is mainly involved in basic research and alternatives to animals in education; it performs toxicology and pathology testing on a routine basis, and toxicology (nephrotoxicity, carcinogenicity, hepatotoxicity) and surgery studies at a research level. Pharmaceuticals are routinely evaluated; biomaterials and environmental pollutants also being occasionally studied.
The main use of alternative methods is for screening.

Model systems: The model systems used are conventional animal models and transgenics, and education models

Work lines: Education and training of scientists using experimental animals. Experimental pathology (oncology, toxicology, metabolism). Primates, alpha-farm-animals.

Quality assurance / Validation programmes: The team have previously been involved in alternative method validation programmes

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Fundación INASMET

Departamento de Adecuación Ambiental

Camino de Portuetxe 12

20009 Donostia-- Guipuzcoa

Tel: 943316173, Fax: 943217560, Website: http:// www.inasmet.es

Type of Institution: Private research facility

Staff:

- Ainhoa Eguizabal Luzuriaga*,* Email: aeguiza@inasmet.es, Ld Ciencias Biológicas.
- Nerea Garagorri Gantxegi, Ld Farmacia.
- Ainara Gordóbil Goñi, Ld Ciencias Biológicas.
- Iñaki Alava Marquínez, Dr Ciencias Químicas.

Total staff involved in alternative methods is 4 people.

Activities / aims: This private research facility department is mainly involved in regulatory testing; it performs toxicology (basal cytotoxicity, ocular cytotoxicity, dermal irritation and corrosivity, genotoxicity / mutagenicity, ecotoxicity) testing on a routine basis. Diverse chemical compounds, environmental pollutants and wastes are routinely evaluated; medical devices and biomaterials also being occasionally studied.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- micro-organisms (Salmonella thyphymurium, *E. coli*.) and cell lines culture (Hela).

Work lines: INASMET is a technological centre whose main areas of activity are "environment" and "materials". The toxicological characterization of industrial wastes and biomaterials is a working tool which makes it possible. It is essential to decide the best way to recover, treat, evaluate and manage industrial wastes and to study the biocompatibility of different materials.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to CA-G0-000, EN 45001, ISO 90001. The team have previously been involved in the following alternative method validation programmes: MAT Programme project Nº 254: Certification of Reference Material for short term mutagenicity tests (1996). Programme BCR measurement analysis: Intercomparison of leach test for stabilized wastes (1993). Several national programmes. They are available to participate in EU validation programmes. The centre performs regulatory *in vivo* and *in vitro* tests and is open to collaboration on the validation of alternative

methods.

Sources of financing: Public contracts, industrial contracts, European projects: Brite, Euram, Environment, Craft, Adapt, Ceca, Innovation, SMT, MAT, Leonardo, BCR.

Others: In the Technological materials centre, the department of Environmental Adaptation and Chemical Technology is working on industrial waste recovery, treatment, evaluation, analysis, toxicology, reuse, etc. The Toxicology Unit performs toxicity, ecotoxicity and mutagenicity assays. The Unit of Biomaterials is also involved on the evaluation of the biocompatibility of dental and medical biomaterials according to UNE-EN 30993-1.

Fundación Valenciana de Investigaciones Biomédicas
Instituto de Investigaciones Citológicas
Departamento de Toxicología Celular
Amadeo de Saboya, 4
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Type of Institution: Private research facility

Staff:

- Consuelo Guerri Sirera, Email: guerri@ochoa.fib.es, Dr Ciencias Biológicas.

Total staff involved in alternative methods is 1 person.

Activities / aims: This private research facility department is mainly involved in basic research; it performs cell biology, molecular biology, biochemistry, toxicology (mechanisms of toxicity, chronic toxicity), pathology and culture methodology studies on a routine basis. Drugs of abuse are routinely evaluated.

Model systems: The model systems used are embryos (rat), and *in vitro* methods -- primary culture of dispersed cells (rat cortical astrocytes and neurones).
The endpoints employed *in vitro* are cytoskeleton/membranes/enzyme release, cellular proliferation, cell signalling, nucleic acids and organ-specific indicators.

Experimental systems (examples):
1 primary culture of astrocytes and neurones, cell proliferation, DNA synthesis, MTT reduction, cytotoxicity, ethanol and other chemicals neurotoxicity
2 primary culture of astrocytes and neurones, DNA fragmentation, cell death by necrosis or apoptosis by 3 DIG-dUTP
3 primary culture of neurones, cytoskeleton proteins, cytoskeleton alterations studied by immunofluorescence and immunotransference

Work lines: Teratogenic effects of ethanol. Toxic effects of alcohol on astroglia development. Cellular and molecular mechanisms of ethanol toxicity. The use of primary cultures of neural cells to study the effects of ethanol on central nervous system development.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU and FDA. The team are available to participate in EU validation programmes.

Sources of financing: Comisión Interministerial de Ciencia y Tecnología, Dirección General de Enseñanza Superior e Investigación Científica, Fondo de Investigaciones Sanitarias de la Seguridad Social, Generalitat Valenciana, Conselleria de Cultura, Educació i ciència, Fundación Ramón Areces.

Fundación Valenciana de Investigaciones Biomédicas
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Departamento de Toxicología Genética
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Type of Institution: Private research facility

Staff:

- Manuel Blanco Pérez, Email: blanco@ochoa.fib.es, Dr. Ing. Agrónomo, Jefe de Departamento, 100 % time devoted to altern. meth.
- Amparo Urios Lluch, Email: urios@ochoa.fib.es, Dra. Ciencias Biológicas, Investigadora, 100 % time devoted to altern. meth.
- Alicia Martínez Romero, Email: martinez@ochoa.fib.es, Ld. Biología, Investigadora, 100 % time devoted to altern. meth.
- Carmen Navarro Rey, Técnico, Ayudante de Laboratorio, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This private research facility department is mainly involved in basic research, non-regulated applied research, method development and method validation; it performs toxicology (genotoxicity / mutagenicity) testing on a routine basis. Pharmaceuticals and diverse chemical compounds are routinely evaluated.
The main use of alternative methods is for screening.

Model systems: The model systems used are *in vitro* methods --micro-organisms (*Salmonella typhimurium*, *E. coli*) and cell-free systems (postmitochondrial fractions (liver, kidney, brain)).
The endpoints employed *in vitro* are nucleic acids (mutations), and biotransformation systems (xenobiotic activation, detoxification, antioxidative systems).

Experimental systems (examples):
1 E coli oxyR-/oxyR+, mutation, mutagenicity induced by oxidative stress
2 postmitochondrial fractions (liver, kidney, brain), mutation, biotransformation / detoxification

Work lines: Development of MUTOXITEST, a bacterial reversion assay specific for oxidative mutagens. The Mutoxitest is based on the use of new Escherichia coli tester strains developed as additions to the WP2 mutagenicity test: strain IC203, deficient in the OxyR function; strain IC206, deficient in the MutY DNA glycosylase. Validation of Mutoxitest. Evaluation of the mutagenicity, resulting from oxidative DNA lesions, of new pharmaceuticals. Prevention of oxidative mutagenesis by chemicals and natural products. Evaluation of antioxidant defenses in fractions from rat liver, kidney or brain.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team have previously been involved in the following alternative method validation programmes: Societé Française de Toxicologie Génetique 1986. They are available to participate in EU validation programmes, in relation to mutagenicity tests directed to the detection of oxidative mutagenesis. Study of antioxidants used as antimutagens.

Sources of financing: "Desarrollo de nuevos ensayos para la detección y estudio del mecanismo de acción de agentes carcinogénicos", CICYT, I+D, 1993-1996; "Desarrollo de nuevas estrategias para el control de la genotoxicidad de fármacos y para el estudio de su relación con el estrés oxidativo", CICYT, I+D, 1997-2000; "Desarrollo de ensayos bacterianos,basados en la utilización de la citometría de flujo, para el control de la genotoxicidad originada por el estrés oxidativo" Generalitat Valenciana, I+D.

Gaiker

Unidad de Bioprocesos

Laboratorio de Toxicología

Parque Tecnológico de Zamudio ed. 202

48170 Zamudio-- Bizkaia

Tel: 944522323, Fax: 944522236, Website: http:// www.bm30.es/gaiker

Type of Institution: Private research facility

Staff:

- Ana Mª Alejandro Vadillo, Email: alejandro@gaiker.es, Ld Cc Biológicas, Dtora. de proyecto, 100 % time devoted to altern. meth., duties related to altern. meth.: Responsable toxicology
- Mª Isabel Rodríguez Llopis, Email: Rodríguez@gaiker.es, Ld Cc Químicas, Dtora. de Proyecto, 35 % time devoted to altern. meth., duties related to altern. meth.: Project responsible
- Francisco Javier Vergara, Email: vergara@gaiker.es, Ingeniero industrial, Dtor. Area de Actividad, 5 % time devoted to altern. meth., duties related to altern. meth.: Director de Laboratorio de Toxicología
- Ainhoa Eguskiza, Email: eguskiza@gaiker.es, Ld Ciencias Biológicas, Analista, 100 % time devoted to altern. meth., duties related to altern. meth.: Analista
- Iñaki Gorostiza, Email: gorostiza@gaiker.es, Ld Ciencias Biológicas, Jefe Unidad Bioprocesos, 15 % time devoted to altern. meth., duties related to altern. meth.:

Dtor. Técnico de Laboratorio

- Francisco Javier Ereño, Email: ereño@gaiker.es, Ld Ciencias Químicas, Investigador del Area de Calidad y Progreso, 40 % time devoted to altern. meth., duties related to altern. meth.: Responsable de la UGC

Total staff involved in alternative methods is 6 people.

Activities / aims: This private research facility department is mainly involved in non-regulated applied research, regulatory testing, method development, method validation and alternatives to animals in education; it performs toxicology (basal cytotoxicity, ocular cytotoxicity, dermal irritation and corrosivity, nephrotoxicity, haematoxicity, genotoxicity / mutagenicity, ecotoxicity) testing on a routine basis and monitoring --chemical, biological-- studies at a research level. Pharmaceuticals, cosmetics, medical devices, environmental pollutants and wastes are routinely evaluated; diverse chemical compounds and food additives also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are vegetables (seeds) and *in vitro* methods -- micro-organisms (*Salmonella typhimurium*, *E. coli*), primary culture of dispersed cells (mice, rat and human tubular and mesangial cells), cell lines culture (3T3, CHO, L929, MCF-7) and proteins: zeine; RBC.
The endpoints employed *in vitro* are cell morphology (microscopy), cell viability (neutral red uptake, MTT reduction), cytoskeleton/membranes/enzyme release, cellular proliferation (total protein content, colony formation), haemolysis and protein solubilization (zeine).

Experimental systems (examples):
1 3T3 fibroblasts, neutral red uptake, total protein, MTT reduction by spectrophotometry, ocular irritation, screening
2 L929, neutral red uptake, by spectrophotometry, biocompatibility of materials, replacement test
3 L929, protein, microscopy, biocompatibility of materials, replacement test
4 kidney tubular cell culture, neutral red uptake, by spectrophotometry, nefrotoxicity, complementary test
5 *Salmonella typhimurium,* reversion of mutation, mutagenicity, replacement test
6 red blood cells, haemolysis, by spectrophotometry, haemocompatibility, ocular irritation replacement test
7 zeine protein, protein solubility, dermal irritation, screening
8 CHO fibroblasts, colony formation, mutagenicity, replacement test

Work lines: The main lines of the Unit are: *in vitro* toxicology, including different cell lines and primary cell cultures for different applications: dermal and ocular irritation, nephrotoxicity and new materials biocompatibility. Mutagenicity and hepatotoxicity tests, as well as assay for environmental toxicology. The introduction of different *in vitro* assays in the industry for screening and / or regulatory purposes. Co-operation with the following industrial sectors: cosmetics, pharmaceuticals, medical devices, environment. Interest in developing and applying methods that will comply with the 3R´s.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating

inside the facility. The institution applies Good Laboratory Practices rules according to the EU; EN 45001. The team are available to participate in EU validation programmes (all types)

Sources of financing: Gobierno Vasco, CICYT, ATYCA, Private industries AAIR, FAIR, BRITE-EURAM, INNOVATION ENVIRONMENT AND CLIMATE, INCO, EUREKA, AVICENA, SMT, BIOTECHNOLOY, CRAFT, ESPRIT, STEP.

Others:- Acreditación en BLP emitida por el ministerio de Sanidad y Consumo para el laboratorio de Toxicología
- Acreditación ENAC (Entidad Nacional de Acreditación) por la norma Europea EN 45001 (Criterios generales para el funcionamieto de los laboratorios de ensayo) a una de las divisiones actuales del Centro, estando las restantes en camino a su reconocimiento por dicho organismo, pero ya trabajando bajo dicha norma.
-Empresa contratista con las Administraciones Públicas.
- Título de idoneidad como Empresa Colaboradora de Verditos del Grupo Tercero.
- Socio del "Grupo de Cromatografía y Técnicas Afines". Real Sociedad Española de Química.
- Socio del "Grupo Espectroquímico". Reales Sociedades Españolas de Física y Química
- Participante en el subcomité de métodos horizontales de análisis de alimentos (SC-4), dentro del Comité Técnico de productos alimenticios (CT-34) de AENOR
- Miembro de la "Water Environment Federation". Sección española ADECAGUA.
- Miembro de la "International Association on Water Quality"
- Miembro del la Asociación para la Revitalización del Bilbao Metropolitano Metrópoli-30.
- Socio del Centro de Cooperación Medioambiental. Fundación Universidad-Empresa. Comisión Europea-Dirección General I Programa ECIIP
- Representante de EITE en el ACLIMA (Agrupación Cluster de Industrias de Medio Ambiente de la CAPV) en calidad de socios de Honor
- Representante del EITE en CEMA (Centro de Empresas de Medio Ambiente de la CAPV), en calidad de socios fundadores
- Miembro Fundador del Centro de Innovación Tecnológica en Medio Ambiente (CITMA)

Glaxo Wellcome S.A.

Tres Cantos

Investigación Area de Quimioterapia

Centro de Investigación Farmacológica

Severo Ochoa, 2

28760 Tres Cantos-- Madrid

Tel: 918070632, Fax: 918070614

Type of Institution: Industrial (pharmaceutical)

Staff:

- Santiago Ferrer Bazaga, Email: sfb44640@GlaxoWellcome.co.uk, Dr Biología, Responsable Centro Investigación Farmacológica, 25 % time devoted to altern.

meth.

- John Sparrowe Gil del Real, Email: js28076@GlaxoWellcome.co.uk, Diplomado en Veterinaria, Ayudante de Investigación, 25 % time devoted to altern. meth.
- Carmen Bravo Jara, FPII, Ayudante de Laboratorio, 25 % time devoted to altern. meth.
- Magdalena Jiménez Vaquero, FPII, Ayudante de Laboratorio , 25 % time devoted to altern. meth.
- Angeles Talavante Sarro, FPII, Ayudante de Laboratorio, 25 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research and non-regulated applied research; it performs pathology testing on a routine basis and diagnostic studies at a research level. Pharmaceuticals and antifungal medicaments are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models.

Work lines: Development of new animal models in fungal infections

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Glaxo Wellcome S.A.
Tres Cantos
Investigación
Farmacología
Severo Ochoa 2
28760 Tres Cantos-- Madrid
Tel: 918070482, Fax: 918070595

Type of Institution: Industrial (pharmaceutical)

Staff:

- Pablo M. Aviles, Email: pa27487@glaxowellcome.co.uk, Ld Farmacia, Investigador, 20 % time devoted to altern. meth.
- Rosaura Sanroman, T, Ayudante, 10 % time devoted to altern. meth.
- Mª Jose Guillen, Email: mjg68314@glaxowellcome.co.uk, Ld Química, Ayudante, 60 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research; it performs pharmacodynamics (antimycotic drugs), pharmacology (antimycotic drugs), biokinetics and biotransformation testing on a routine basis, and surgery studies at a research level. Pharmaceuticals and diverse chemical

compounds are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models, *in vitro* methods -- micro-organisms (fungi)--, and mathematical modelling.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes.

Glaxo Wellcome S.A
Investigación
Biología (Microbiología)
Severo Ochoa 2
28760 Tres Cantos-- Madrid
Tel: 918070485, Fax: 918070595

Type of Institution: Industrial (pharmaceutical)

Staff:

- Domingo Gargallo*,* Email: dgv28867@glaxowellcome.co.uk, Dr Biología, Jefe de Laboratorio, 20 % time devoted to altern. meth., duties related to altern. meth.: Miembro Comisión Promotora de REMA

Total staff involved in alternative methods is 1 person.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research; it cell biology, toxicology (basal cytotoxicity, acute systemic toxicity, nephrotoxicity, haematoxicity, genotoxicity / mutagenicity, hepatotoxicity) testing performs on a routine basis, and surgery and pathology studies at a research level. Pharmaceutical antifungal drugs are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models and *in vitro* methods --micro-organisms (fungi) and primary culture of dispersed cells and cell lines culture.
The endpoints employed are *in vitro* morphology, cell viability, enzyme release, cellular proliferation, metabolic activity and nucleic acids.

Experimental systems (examples):
1 Salmonella, mutation reversion, genotoxicity tests
2 Caco2
3 rat hepatocytes
4 HepG2

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team have previously been involved in alternative method validation programmes. They are available to participate in EU validation programmes.

Glaxo Welcome S.A.
Investigación
Micología
Severo Ochoa 2
28760 Tres Cantos-- Madrid
Tel: 918070483, Fax: 918070595, Website: http:// www.spain.glaxo
Type of Institution: Industrial (pharmaceutical)

Staff:

- Esperanza Herreros Avilés, Email: eh13868@glaxowelcome.co.uk, Dr Farmacia, Investigador Responsable de Toxicología In Vitro , 70 % time devoted to altern. meth.
- María Jesus Almela Armendari, Email: mja27315@glaxowelcome.co.uk, Diplomada en Ciencias Biológicas, Técnico de Laboratorio, 100 % time devoted to altern. meth.
- Sonia Lozano Arias, Email: sl25656@glaxowelcome.co.uk, Auxiliar de Laboratorio, Ayudante de Laboratorio, 50 % time devoted to altern. meth.
- Mireya Martínez, Email: cmm9164@glaxowelcome.co.uk, Técnico de Laboratorio, Ayudante de Laboratorio, 50 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in non-regulated applied research; it performs cell biology, toxicology (basal cytotoxicity, hepatotoxicity) testing on a routine basis, and biokinetics and biotransformation studies at a research level. Pharmaceuticals are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are *in vitro* methods --micro-organisms (yeast, *Salmonella typhimurium*), primary culture of dispersed cells (rat hepatocytes) and cell lines culture (3-4 cell lines per tissue, C6, MDCK, Caco2, HepG2, L6).
The endpoints employed are *in vitro* morphology (microscopic observation), cell viability (neutral red uptake, CVDE, crystal violet), cytoskeleton/membranes/enzyme release (LDH), cellular proliferation (Tyd uptake, Mty), metabolic activity (MTT, alamar blue, ATP) and nucleic acids (DNA content).

Experimental systems (examples):
1 primary culture of rat hepatocytes, intracellular LDH, induction of hepatotoxicity and biotransformation enzymes, metabolism studies, complementary test
2 HepG2 human hepatoma, Mety incorporation, basal cytotoxicity, complementary test, screening
3 Salmonella tyhimurium, reversion of mutation by spectrophotometry, genotoxicity, complementary test
4 Caco cells, HPLC, intestinal absorption

Work lines: Development of new antifungal agents active against systemic infections of *Candida albicans*, other *Candida* spp., *Aspergillus fumigatus* and *Pneumocystis Carinii*, with cidal activity and suitable for oral administration

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes.

Sources of financing: Private industry

Glaxo Wellcome S.A.
Tres Cantos
Investigación
Toxicología
Severo Ochoa, 2
28760 Tres Cantos-- Madrid
Tel: 91 8070466, Fax: 91 8070595

Type of Institution: Industrial (pharmaceutical)

Staff:

- Antonio Martínez Escandell, Email: am17735@glaxowellcome.co.uk, Dr Veterinaria, Investigador, 20 % time devoted to altern. meth.
- Elena Jiménez Navarro, Email: ej15128@glaxowellcome.co.uk, Ld, Investigador, 40 % time devoted to altern. meth.
- Jesus Caballero de Toro, Email: ezz63063@glaxowellcome.co.uk, Ld, Becario Investigación, 30 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research; it performs toxicology (acute systemic toxicity, nephrotoxicity, haematoxicity, hepatotoxicity) testing on a routine basis, and pharmacodynamics, surgery, pathology and diagnostics studies at a research level. Pharmaceuticals and antimycotic agents are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models and *in vitro* methods --micro-organisms (fungi).

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes.

Grupo Ferrer
Centro de Investigación

Departamento de Toxicología
Unidad de Toxicología "in vitro"
Alfonso Romero
08028 Barcelona
Tel: 933392021, Fax: 934112764

Type of Institution: Industrial (pharmaceutical)

Staff:

- Alfonso Romero Vidal, Email: research@ferrer-int-grupo.es, Dr Biología, Director Departamento, 10 % time devoted to altern. meth.
- Francisca Gómez Marin, Ld Biología y Veterinaria, Técnico Superior, 40 % time devoted to altern. meth.
- Josefa Torres Zurano, Técnico de Laboratorio, Laborante, 20 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in regulatory testing; it performs toxicology (carcinogenicity, hepatotoxicity, integrated test strategies) testing on a routine basis. Pharmaceuticals are routinely evaluated.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are animal models -- transgenics (mmTVu-Ha-ras mice; zetaglobin/v-Ha-ras) and *in vitro* methods -- primary culture of dispersed cells (rat hepatocytes) and cell-free systems (microsomes).
The endpoints employed *in vivo* are tumoral frequency; 20 general parameters of general toxicity and *in vitro* cell viability, cellular proliferation and metabolic activity (gluthation, gamma-GT, FA, ALT, AST, glucose, etc).

Experimental systems (examples):
1 primary culture of adult rats, MTT reduction and others, toxicity of chemicals, screening and complementary test.
2 hepatocytes, (pharmaceuticals) compararison of effects, replacement test.

Work lines: Rat hepatocyte primary cultures for the toxicological screening of a new chemical substance series. Verification or research on the mechanistic systems of the toxic effects detected or suggested by *in vivo* toxicological studies.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU

Sources of financing: Self financing

INSALUD
Complejo Hospitalario de Albacete

Unidad de Investigación
C/ Hermanos Falcó s/n
02006 Albacete
Tel: 967597513, Fax: 967243952, Website: http:// www.chospab.es
Type of Institution: Hospital

Staff:

- Damián García Olmo, Email: dgolmo@arrakis.es, Dr Medicina.
- Dolores García Olmo, Email: logol@chospab.es, Ld Veterinaria.
- Esperanza Martínez Navarro, Email: emarnav@chospab.es, Ld Biología.
- Elena Navarro García, Email: eng_jcs@chospab.es, Ld Biología.
- Jesús Ontañón Rodríguez, Email: jontanon@chospab.es, Ld Biología.

Total staff involved in alternative methods is 5 people.

Activities / aims: This hospital department is mainly involved in basic research; it performs cell biology, molecular biology and pathology testing on a routine basis, and surgery studies at a research level. Pharmaceuticals are occasionally studied.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- cell lines culture (DHD/K12- rat colon tumor cell line).

Work lines: Colon adenocarcinoma-metastasis

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: Fondo de Investigaciones Sanitarias (1995-97), Programa I+D en Salud (1997-98), Junta Comunidades Castilla-La Mancha (1995-97)

INSALUD
Hospital Central de Asturias
Unidad Metabolismo Oseo y Mineral
Julián Claveria s/n
33006 Oviedo-- Asturias
Tel: 985106131, Fax: 985106142
Type of Institution: Hospital

Staff:

- Jorge Cannata Andia, Dr Medicina, Jefe de Servicio.
- Carlos Gómez Alonso, Dr Medicina, Adjunto.
- Jose Luis Fernández Martín, Ld Química, Adjunto, 50 % time devoted to altern.

meth.

- Jose Bernardino Díaz López, Ld Medicina, Adjunto.
- Manuel Javes Díaz, Dr Biología, Adjunto, 75 % time devoted to altern. meth.
- Carmen Diaz Corte, Ld Medicina, Becario, 25 % time devoted to altern. meth.
- Ana Weruaga Rey, Lda Medicina, 25 % time devoted to altern. meth.
- Primitiva Menendez, Dr Medicina, Adjunto, 25 % time devoted to altern. meth.

Total staff involved in alternative methods is 8 people.

Activities / aims: This hospital department is mainly involved in basic research; it performs cell biology, molecular biology, biochemistry, toxicology (nephrotoxicity) testing, pathology, quality control and culture methodology studies on a routine basis. Hormones, pharmaceuticals, diverse chemical compounds and drugs of abuse are routinely evaluated.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models (reduction, refinement) and *in vitro* methods -- culture of explants, reaggregates, reconstituted organs (femoral, neck, tibial), primary culture of dispersed cells (human osteoblasts) and cell lines culture (ROJ 17/28, M6-63).
The endpoints employed *in vitro* are cytoskeleton/membranes/enzyme release (alkaline phosphatase, osteocalcein), cellular proliferation (Tyd uptake), metabolic activity and nucleic acids (traduction).

Experimental systems (examples):
1 primary culture of osteoblasts, osteocalcein by RIA, effect of calcitriol, complementary test
2 M6-63 osteoblastic cell line culture, osteocalcein by RIA, effect of aluminum, replacement test
3 primary culture of osteoblasts, alkaline phosphatase, proliferation and osteocalceine, effect of aluminum, replacement test
4 rat parathyroid gland culture, PTH, RNApth, effect of aluminum, replacement test

Work lines: Renal osteodistrophy. Toxicology of aluminum. Bone metabolism.

Sources of financing: Hospital Central de Asturias, FIS, CICYT, Fundación Renal Iñigo Alvarez de Toledo

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Osakidetza
Hospital Aranzazu
Unidad Experimental (UEX)
Avd. Ar. Bequiristain
20014 San Sebastian-- Guipuzcoa
Tel: 943 447061, Fax: 943 460758

Type of Institution: Hospital

Staff:

- Pablo Aldazabal, Email: paldaza@hnsa.es, Ld Medicina, Coordinador EUX

Total staff involved in alternative methods is 1 person.

Activities / aims: This hospital department is mainly involved in basic research and non-regulated applied research; it performs physiology, toxicology (acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity) and nutrition testing on a routine basis, and cell biology, biochemistry and pathology studies at a research level. Biomaterials, wastes, surgery are routinely evaluated; hormones and medical devices also being occasionally studied.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models, *in vitro* methods -- cell lines culture-- , human volunteers (clinical trials phases >III) and education models (animal, video, simulator, mechanical models).
The endpoints employed are *in vivo* (histopathological and physiological parameters) and *in vitro* morphology, cell signalling and defence systems.

Work lines: Experimental surgery, total parenteral nutrition in the rat, immunohystochemistry, neurogenetics, biomaterials.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: FIS, CICYT, Gobierno Vasco, Pharmaceutical Industry.

Servicio Andaluz de Salud
Hospital Carlos Haya
Unidad de Investigación
Camino de Antequera S/N
29010 Málaga
Tel: 909 572992, Fax: 952645796

Type of Institution: Hospital

Staff:

- Miguel Blanca Gómez, Email: mblanca@eurociber.es, Dr Medicina, Jefe Unidad, 20 % time devoted to altern. meth.
- Cristobalina Mayorga Mayorga, Dr Biología, Becario Investigación , 20 % time devoted to altern. meth.
- Manuel Rosal Sánchez, del, Dr Medicina, Becario Investigación , 30 % time devoted to altern. meth., duties related to altern. meth.: Inmunólogo
- Sinfoniano Posadas Mañanes, Ld Químicas, Becario Investigación, 30 % time devoted to altern. meth., duties related to altern. meth.: Responsable Biología Molecular
- Maria Jose Torres Jaen, Dr Medicina, Responsable Clínico (Adjunto), 40 % time

devoted to altern. meth.
Total staff involved in alternative methods is 6 people.

Activities / aims: This hospital department is mainly involved in basic research, method development and method validation; it performs molecular biology, biokinetics, biotransformation and toxicology (mechanisms of toxicity, acute systemic toxicity, dermal irritation, corrosivity, immunotoxicity and sensitisation) testing on a routine basis, and monitoring --chemical, biological-- studies at a research level. Pharmaceuticals are routinely evaluated; cosmetics, diverse chemical compounds, biomaterials, food additives and colourings being also occasionally studied.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are *in vitro* methods - organ culture primary culture of cells (lymphocytes).
The endpoints employed *in vitro* are cellular proliferation, nucleic acids and organ-specific indicators.

Experimental systems (examples):
1 lymphocyte cell lines, biological response
2 lymphocyte cell lines, reaction

Work lines: In vitro methods for quantitating IgE antibodies to betalactams. Adverse reactions to drugs with immunological basis. Studies of the mechanisms involved. Methods for IgE production *in vitro* using peripheral cells from sensitized subjects.

Quality assurance / Validation programmes: The team have previously been involved in the following alternative method validation programmes: validation of RIA and ELISA methods. They are available to participate in EU validation programmes in relation to the evaluation of immunotoxicological methods, metabolic transformation, and detoxification mechanisms.

Sources of financing: Project Biomed, Project Biotech, Project FIS, CICYC, Consejeria Salud, Ministerio Educación y Ciencia, PAI

Others: General interest in the development of *in vitro* methods for the study of immunotoxicological reactions to pharmaceuticals and chemical compounds.

Hospital La Fe
Centro de Investigación
Hepatología Experimental
Avd. Campanar 21
46009 Valencia
Tel: 963868748, Fax: 963868718

Type of Institution: Governmental research facility

Staff:

- Maria Jose Gómez-Lechón, Email: mjgomez@san.gva.es, Dra Biología, Adjunto

Investigación, 100 % time devoted to altern. meth., duties related to altern. meth.: Vocal Junta de ESTIV

- Jose Vicente Castell Ripoll, Email: jose.castell@gong.ci.uv.es, Dr Química y Farmacia, Jefe de Sección, 100 % time devoted to altern. meth., duties related to altern. meth.: Consejo Científico ESTIV, Representante Español en ESAC-ECVAM
- Ramiro Jover, Email: ramiro.jover@uv.es, Dr Biología, Contratado, 100 % time devoted to altern. meth.
- Teresa Donato, Dr Farmacia, Adjunto Interino, 100 % time devoted to altern. meth.
- Xavier Ponsoda, Dr. Biología, Colaborador.

Total staff involved in alternative methods is 11 people.

Activities / aims: This governmental research facility department is mainly involved in basic research, non-regulated applied research and method development; it performs toxicology testing on a routine basis, and cell biology, molecular biology, biochemistry, biokinetics and biotransformation and culture methodology studies at a research level. Pharmaceuticals, cosmetics, diverse chemical compounds, drugs of abuse, biomaterials and food additives are occasionally evaluated.
The main use of alternative methods is for complementary and replacement studies.

Model systems: The model systems used are *in vitro* methods --organ culture (liver), primary culture of dispersed cells (hepatocytes), cell lines culture (several hepatoma cell lines), cell-free systems (microsomes) and human volunteers (metabolism studies).
The endpoints employed *in vitro* are cell viability (MTT reduction, neutral red uptake, XTT reduction, LDH leakage, ATP), metabolic activity (liver), biotransformation systems (phase I [CYPA] and phase II), defence systems (GSH), organ-specific indicators (from liver [glucuronoconjugation, plasmatic proteins]) and toxicity mechanisms (Ca^{+2}, lipid peroxidation).

Experimental systems (examples):
1 primary culture of hepatocytes from several species, hepatic physiological criteria, hepatotoxicity replacement test
2 primary culture of hepatocytes from several species, metabolic profile, HPLC/RMN, metabolism
3 transgenic cell lines expressing only one CYP, metabolism of specific substrate, identification of CYPS related to metabolism
4 primary culture of keratinocytes and fibroblasts, MTT reduction, phototoxicity, photodegradation

Work lines: Hepatotoxicity of drugs in cultured hepatocytes (several species). Mechanisms of hepatotoxicity. Evaluation of new drugs safety. Metabolism of drugs using primary cultured hepatocytes (including human) and CYP expressing transgenic cell lines. Development of stable and competent immortal hepatic cellular models.

Quality assurance / Validation programmes: The team have previously been involved in the following alternative method validation programme: MEIC. They are available to participate in EU validation programmes in relation to Biomed, Biotech, AIR, FLAIR.

Sources of financing: CEE (6 projects): AIR, Biomed I, Biomed II, Biotech; FIS (2); Pharmaceutical industry

Servicio Andaluz de Salud
Hospital Torrecárdenas
Departamento de Medicina Interna
Grupo de Trabajo Investigación Organo-fosforados
Paraje de Torrecardenas s/n
04009 Almeria
Tel: 950212100
Type of Institution: Hospital

Staff:

- Fernando Yelamos Rodríguez, Dr Medicina, 35 % time devoted to altern. meth.
- Francisco Laguez Britones, Dr Medicina, 25 % time devoted to altern. meth.
- D.C. Martín Rubí, Ld Medicina, 15 % time devoted to altern. meth.
- D.L. Blanco Coronado, Dr Medicina, 10 % time devoted to altern. meth.
- Federico Orozco Rodríguez , 10 % time devoted to altern. meth.
- Mª Carmen Marfil Montoya, Ld Medicina, 5 % time devoted to altern. meth.

Total staff involved in alternative methods is 6 people.

Activities / aims: This hospital department is mainly involved in regulatory testing; it performs toxicology (mechanisms of toxicity, basal cytotoxicity, acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity, nephrotoxicity, respiratory cytotoxicity, reproductive cytotoxicity, neurotoxicity, immunotoxicity and sensitisation, haematoxicity, carcinogenicity, phototoxicity, hepatotoxicity, chronic toxicity) and pathology studies on a routine basis. Pesticides are routinely evaluated.

Work lines: Acute and chronic effects of organophosphate pesticides

Institut Catala de la Salut
Hospital Materno Infantil Vall d´ Hebron
Centro de Investig. en Bioquímica y Bio. Molecular
Patología Celular
CIBBIM, H. Materno Infantil Vall d´ Hebron (PI-14)
08035 Barcelona
Tel: 934894066, Fax: 934894064, Website: http:// cdoming@hg.vhebron.es
Type of Institution: Hospital

Staff:

- Carmen Domínguez, Email: cdoming@hg.vhebron.es, Dr Biología, 30 % time devoted to altern. meth.
- María Rosa Leiva, Diplomada en Enfermería, 80 % time devoted to altern. meth.
- Eduard Hidalgo Albert, Ld Farmacia, 30 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This hospital department is mainly involved in non-regulated applied research and method development; it performs cell biology, biochemistry, toxicology (mechanisms of toxicity) and diagnostic (metabolopaties) studies on a routine basis and pharmacodynamics (cytotoxicity of antiseptics) studies at a research level. Pharmaceuticals and metals are occasionally evaluated.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- culture of explants, reaggregates, reconstituted organs, (dermal fibroblasts) and cell lines culture (MDCK).
The endpoints employed *in vitro* are cell viability (MTT and XTT reduction, neutral red uptake) cytoskeleton /membranes/enzyme release (LDH, hexosaminidase), cellular proliferation (cell count, total protein content), metabolic activity (lysosomal, oxidative stress) and defence systems (antioxidative mechanisms).

Work lines: Study of cytotoxicity mechanisms of aluminum and lead in human dermal fibroblasts. Study of the most frequently applied topical antiseptics: antimicrobial activity related to cytotoxicity in cultured human fibroblasts. Growth modulating effects of radical oxygen species.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to the techniques used

Sources of financing: Self financing, pharmaceutical industry.

Instituto Nacional del Consumo
Investigación y Control de Calidad
Ministerio de Sanidad y Consumo
Servicio de Productos Alimenticios
Avda. de Cantabria S/N
28042 Madrid
Tel: 917472333, Fax: 917479517

Type of Institution: Governmental facility for service (diagnostic, control, monitoring)

Staff:

- Jesus Salas Zapatero, Email: istzo350@tsai.es, Ld Veterinaria, Jefe de Servicio de Productos Alimenticios.
- Pilar Moya Esteve, Dr Biología, Unidad de Productos de Origen Animal
- Angel Blazquez de los Riscos, Ld Veterinaria, Unidad Electroquímica y PCR

- Mª Rosa Sarazá Linares, Ld Veterinaria, Unidad Microbiología.
- Mª Teresa González Villa, Ld Biología, Unidad Microbiología.
- Mª del Pilar Clemente Belmonte, Ld Biología, Unidad de Parasitología.
- Raquel Fernández Peiteado, Ld Biología, Unidad de Productos de Origen Animal y Vegetal
- Carlos Arnaiz Ronda, Dr Biología, Director Técnico del Centro de Investigación y Desarrollo

Total staff involved in alternative methods is 8 people.

Activities / aims: This governmental service facility (diagnostic, control, monitoring) department is mainly involved in regulatory testing, method validation, quality control of food and industrial products; it performs toxicology (mechanisms of toxicity, basal cytotoxicity, acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity, nephrotoxicity, respiratory cytotoxicity, reproductive cytotoxicity, neurotoxicity, immunotoxicity and sensitisation, haematoxicity, genotoxicity / mutagenicity, carcinogenicity, hepatotoxicity, endocrine disruption, chronic toxicity, integrated test strategies), nutrition studies and quality control testing on a routine basis. Cosmetics, diverse chemical compounds, pesticides, food additives, colourings and wastes are routinely evaluated.

The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are *in vitro* methods -- micro-organisms.

Work lines: Quality control of food and related products of human use. Evaluation of toxicological tests for the evaluation of pesticides, food additives and food traces. The use of mutagenicity *in vitro* tests applied to quality control activities.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes, in relation to *in vitro* methods for detecting toxic substances in food products

Sources of financing: Self financing

Instituto Nacional de Investigaciónes Agrarias y Alimentarias (INIA).
Ministerio de Agricultura, Pesca y Alimentación.
Centro de Investigación en Sanidad Animal (CISA)
Area de Toxicología del Medio Ambiental (Sanidad Ambiental)
Ecotoxicología *In Vitro*
Finca de las Fuentes y las Setas
28130 Valdeolmos-- Madrid
Tel: 916202300, Fax: 916202247

Type of Institution: Governmental research facility

Staff:

- María Argelia Castaño Calvo*,* Email: castano@inia.es, Dr Ciencias Biológicas, Investigador A3, 90 % time devoted to altern. meth.
- María Jesús Muñoz Reoyo*,* Email: reoyo@inia.es, Dr Ciencias Biológicas, Investigador A3, 80 % time devoted to altern. meth.
- Matilde Carballo Santaolaya*,* Email: carballo@inia.es, Dr Ciencias Biológicas, Investigador A3, 40 % time devoted to altern. meth.
- María Teresa Llorente Rodríguez, Email: mteresa@inia.es, Ld Ciencias Biológicas, Becaria Predoctoral INIA, 100 % time devoted to altern. meth.
- Apolonia Novillo Villajo, Email: novillo@inia.es, Dr Ciencias Biológicas, Contratada Postdoctoral, 100 % time devoted to altern. meth.
- Ana Isabel de la Torre Reoyo*,* Email: torre@inia.es, Dr Ciencias Biológicas, Becaria Postdoctoral INIA, 50 % time devoted to altern. meth.
- Jaime Roset Álvarez*,* Email: roset@inia.es, Ld Ciencias Biológicas, Becario Predoctoral INIA, 50 % time devoted to altern. meth.
- María Luisa Cuéllar Cuéllar, FPII, Ayudante de Laboratorio de Plantilla, 70 % time devoted to altern. meth.

Total staff involved in alternative methods is 8 people.

Activities / aims: This governmental research facility department is mainly involved in non-regulated applied research, method development and method validation; it performs toxicology testing (basal cytotoxicity, reproductive cytotoxicity, genotoxicity / mutagenicity, ecotoxicity), monitoring -chemical, biological studies on a routine basis. Diverse chemical compounds, pesticides, environmental pollutants and wastes are routinely evaluated.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models (sea and freshwater fishes), invertebrates (*Daphnia magna, Folsomia candida, Enchitraeus albidus, Eisenia foetida*), embryos (medaka *Oryzias latipes*), vegetables (seed germination and unicellular algae), and *in vitro* methods - primary culture of dispersed cells (fish *O mykiss* macrophages), and cell lines culture (RTG-2, CHSE-214, EPC).
The endpoints employed are *in vivo* stress parameters, micronuclei induction, metahaemoglobin, retinol, acetylcholinesterase, P-450 induction, macroscopic alterations of development, larva viability, histopathologic alterations in fish species and *in vitro* cell viability (neutral red uptake, exclusion of dyes (trypan blue, ethidium bromide)), cellular proliferation (total protein content (kenacide blue, Bradforf method)) and metabolic activity (ATP intracellular content).

Experimental systems (examples):
1 RTG-2 rainbow trout fibroblast cell line, neutral red uptake, total protein content, ATP, acute cytotoxicity, replacement test
2 RTG-2 rainbow trout fibroblast cell line, micronuclei frequency and cell cycle alterations, by flow cytometry, detection of genotoxicity alterations, replacement test
3 *Daphnia magna* and *Dahpnia pulex,* immobilization and reproduction, acute and chronic toxicity
4 *Chlorella vulgaris,* growth, by spectrophotometry on 96-well, sub-acute toxicity,

replacement test
5 eggs and larva of medaka (*Oryzias latipes),* alterations in development, viability. Stereomicroscopic observation, lethality, teratogenesis, chronic toxicity, replacement test
6 *Folsomia candida* and *Enchitraeus albidus,* alterations in reproduction, acute toxicity, by count and uptake, lethality and reproduction, replacement test

Work lines: Diagnosis and risk assessment of environmental toxicological processes by the development of new miniaturized short-term tests for soil and water (on fish cell lines, algae, invertebrates, multispecies, microcosms test). Invertebrates and fish biomarkers: stress, cytochrome P-450, ACTH, micronuclei frequency by flow cytometry and mutations detection by DNA fingerprinting.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU, OECD and the FDA The team have previously been involved in the following alternative method validation programmes: ring test on reproduction of *Daphnia magna*, MEIC and EDIT (RTG2 cytotoxicity test, and ovoalbumin denaturalization test). They are available to participate in EU validation programmes.

Sources of financing: Project AIR1-AZ92-0036 New *in vitro* approaches for fish protection in aquaculture: application of cell and tissue cultures for pathogen detection and for vaccines development. --Commission of the European Communities 1992-1996; Proyecto CAM AMB COR 0010/94 Valoración ecotoxicológica del Parque Sudeste de la Comunidad de Madrid. Incorporación de bioensayos de toxicidad en programas de seguimiento y control. Comunidad de Madrid 1994-1997; Proyecto AMB 94-0655-C02-01 Desarrollo y validación en condiciones de campo de sistemas de detección y biomarcadores de genotoxicidad en organismos acuáticos CICYT 1994-1997; Proyecto SC98-098-C2 Identificación y valoración de impactos medioambientales de las explotaciones porcinas. Adecuación a las nuevas directivas de la Unión Europea. INIA 1998-2001; Proyecto SC95-091. Desarrollo y validación de sistemas de detección de efluentes y vertidos. INIA 1995-1997; Aspectos toxicológicos y medioambientales relacionados con el regadio, la utilización de aguas residuales y el estudio de sistemas de depuración por lagunaje. IRYDA 1994-1997 (15750); Proyecto AMB 97-0431-C02-01 Valoración ecotoxicológica de efluentes industriales sobre organismos acuáticos. Validación de sistemas alternativos y biomarcadores de exposición de genotoxicidad CICYT 1997-2000 (5006); Caracterización toxicológica de vertidos y aguas residuales de la industria química. Infraestructura y Ecología 1996-1997 (7000)

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Type of Institution: Governmental service facility

Staff:

- Françoise Lhoest Mathijsen, Ld Biología, Facultativo, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 1 person.

Activities / aims: This governmental service facility department (diagnostic, control, monitoring, expert work) is mainly involved in regulatory testing; it performs toxicology (ecotoxicity) testing on a routine basis. Environmental pollutants and wastes are routinely evaluated; pesticides also being occasionally studied.
The main use of alternative methods is for complementary studies.

Model systems: The model systems used are animal models -- invertebrates (*Daphnia magna*) and *in vitro* methods -- micro-organisms (*Photobacterium phosphoreum*).
The endpoints employed are *in vivo* (immobilization) and *in vitro* metabolic activity (bioluminescence inhibition).

Experimental systems (examples):
1 *Daphnia magna ,* immobilization, characterisation of wastes, replacement test
2 *Photobacterium phosphoreum,* bioluminescence inhibition, characterization of wastes, replacement test
3 cell culture, cell viability, cytotoxicity, complementary test

Work lines: Toxicological evaluation of wastes using ecotoxicity assays.Determination and evaluation of acute and chronic toxicity of pharmaceuticals using cell lines

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: Self financing

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Type of Institution: Governmental service facility

Staff:

- Pilar Gascó Alberich, Email: extox@mad.inaltox.es, Dr Farmacia, Jefe Sec Experimentación Toxicológica. Prof Asociado, 20 % time devoted to altern. meth.

- Javier Piga de Lariba, Ld Ciencias Biológicas, Facultativo Sección Experimentación Toxicológica, 20 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This governmental service facility department (diagnostic, control, monitoring, expert work) is mainly involved in non-regulated applied research, regulatory testing and alternatives to animals in education; it performs toxicology (ecotoxicity) testing on a routine basis and toxicology (dermal irritation and corrosivity, hepatotoxicity) studies at a research level. Environmental pollutants and wastes are routinely evaluated; diverse chemical compounds also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models (rat, mouse, rabbit), invertebrates (*Daphnia magna*), vegetables (algae), *in vitro* methods -- micro-organisms (*Photobacterium phosphoreum*) and education models (hepatocytes).
The endpoints employed are *in vivo* (toxicity/ lethality, immobilization, growth) and *in vitro* cell viability (dyes) and
metabolic activity (inhibition of bioluminescence).

Experimental systems (examples):
1 *Daphnia magna,* immobilization, visual, CE50, ecotoxicity
2 *Chlorella vulgaris,* inhibition of growth, by spectrophotometry, CI50, ecotoxicity
3 *Photobacterium phosphoreum,* bioluminescence inhibition, CE50, ecotoxicity

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes related to ecotoxicity and toxicology.

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Type of Institution: Governmental service facility

Staff:

- Carmen Rodríguez Vicente, Dr Ciencias Químicas y Farmacia, Jefe de Servicio, 50 % time devoted to altern. meth.
- Patrocinio Villar López, Ld Ciencias y Farmacia, Jefe Servicio Criminalística, 20 % time devoted to altern. meth.
- Carmen Martínez Sánchez, Dr Medicina, Especialista en Anatomía Patológica,

Facultativo, 10 % time devoted to altern. meth.

- Josefa Valenzuela Moreno, Capacitación para Animales de Laboratorio, Auxiliar de Laboratorio, 80 % time devoted to altern. meth.
- Josefina Barba Quintero, Grado Medio, Auxiliar de Laboratorio, 20 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This governmental service facility department (diagnostic, control, monitoring, expert work) is mainly involved in basic research, regulatory testing and method development; it performs biochemistry and toxicology (mechanisms of toxicity, acute systemic toxicity, hepatotoxicity, chronic toxicity, ecotoxicity) testing on a routine basis. Pharmaceuticals, cosmetics, pesticides, drugs of abuse, environmental pollutants and wastes are routinely evaluated; toxins, food additives and colourings also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models (BrSelf Norway: nefrotoxicity studies), invertebrates (Daphnia magna), vegetables (Algae: *Chlorella vulgaris*), *in vitro* methods - micro-organisms (*Photobacterium phosphoreum*) and human volunteers.
The endpoints employed are *in vivo* (enzyme activities, haematology, biochemical parameters, histological observations, behavioural tests) and *in vitro* metabolic activity (bioluminescence).

Experimental systems (examples):
1 *Photobacterium phosphoreum,* bioluminescence inhibition, ecotoxicological evaluation, replacement test
2 brSelf Norway rats, biochemical evaluation: NAG creatinine, urea, bilirrubin, nephrotoxicity studies, refinement
3 rabbits, ADH, hair accumulation of ethanol, chronic alcoholism refinement
4 fauve Bourgogne red-haired rabbits, hair and cannabis accumulation, toxicokinetics of abused drugs, refinement
5 wistar rats, enzymes. GOT, GPT, transaminases, bilirrubin, pharmaceutical toxicity, refinement
6 rat, rabbit, human, Ache, Che inhibition, interference of pharmaceuticals, refinement

Work lines: The ecotoxicological evaluation of wastes and waters by means of *Photobacterium phosphoreum*, *Daphnia magna* and *Chlorella vulgaris* tests. Toxicokinetic studies of abused drugs on fauve Bourgogne red-haired rabbits and their application for forensic purposes. Nephrotoxicity studies using sensitive models such as the brSelf Norway rats. The hepatotoxic effects of pharmaceuticals on Wistar rats. The neurotoxicity of pesticides.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: Self financing, Junta de Andalucía, food additives and pharmaceutical industries.

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Type of Institution: Governmental service facility (diagnostic, control, monitoring, expert work)

Staff:

- Guillermo Repetto Kuhn, Email: repetto@sev.inaltox.es, Dr Medicina, Facultativo, 50 % time devoted to altern. meth., duties related to altern. meth.: Coordinador del GTEMA, Representante español en ERGATT
- Pilar Sanz Nicolás, Email: biol@sev.inaltox.es, Dr Biología, Jefe Servicio Biología, 10 % time devoted to altern. meth.
- Antonio Garfia, Dr Medicina, Jefe Servicio Anatomía Patológica
- Ana del Peso Bejarano, Ld Farmacia, Facultativo Servicio Química, 30 % time devoted to altern. meth.
- Inmaculada Flores García, Ld Farmacia, Facultativo Servicio Biología, 10 % time devoted to altern. meth.
- Susana Jiménez Fuentes, Técnico Auxiliar de Farmacia, Agente de Laboratorio Servicio Biología, 50 % time devoted to altern. meth.
- Dolores Osuna Guillén, Grado Medio, Auxiliar de Laboratorio Servicio Biología, 50 % time devoted to altern. meth.
- Manuel Salguero Villadiego, Dr Medicina, Especialista Anatomía Patológica, Profesor Universidad, 40 % time devoted to altern. meth.
- Manuel López Soto, Ld Biología, Facultativo de Biología, 50 % time devoted to altern. meth.
- M Rosario Repetto Kuhn, Dr Medicina, Servicio de Información Toxicológica, 10 % time devoted to altern. meth.
- Manuel Repetto Jiménez, Dr Ciencias y Medicina, Director INT-SE, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 10 people.

Activities / aims: This governmental service facility department (diagnostic, control, monitoring and expert work) is mainly involved in basic research, non-regulated applied research, regulatory testing, method development, method validation and alternatives to animals in education; it performs biochemistry and toxicology (mechanisms of toxicity, basal cytotoxicity, acute systemic toxicity, neurotoxicity, ecotoxicity) testing on a routine basis, and cell biology, molecular biology, biokinetics and biotransformation, genetics, pathology, nutrition, diagnostics and monitoring --

chemical, biological-- studies at a research level. Pharmaceuticals, cosmetics, diverse chemical compounds, pesticides, drugs of abuse, environmental pollutants and wastes are routinely evaluated; toxins, food additives and colourings also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are animal models -- invertebrates (crayfish *Procambarus clarkii*, medaka), embryos (chick embryo), vegetables (chloroplasts), *in vitro* methods -- micro-organisms (E coli), organ culture (skin), primary culture of dispersed cells (human lymphocytes), cell lines culture (Neuro 2a, SH-5Y5Y, Vero, RTG-2), cell-free systems (microsomal), human volunteers (monitoring), education models (computer models) and mathematical modelling (toxic effects, education).
The endpoints employed *in vitro* are morphology (optical microscopy, immunohistochemistry), cell viability (neutral red uptake, propidium iodide by spectrophotometry), cytoskeleton/membranes/enzyme release (LDH leakage, hexosaminidase release, neurofilaments), cellular proliferation (total protein content, DNA), metabolic activity (PFK, LDH, ATPase, SDH), nucleic acids (apoptosis / necrosis by TUNEL and gel electrophoresis), biotransformation systems (P450), defence systems (GOR, GST, G6P-DH, SOD) and organ-specific indicators (acetylcholinesterase, enolase, galactosidase, TAU protein).

Experimental systems (examples):
1 SH-SY5Y human differentiated neuroblastoma cells, apoptosis by TUNEL and gel electrophoresis differentiation, cell death mechanisms, replacement test
2 SH-SY5Y human differentiated neuroblastoma cells, acetylcholinesterase, enolase, galactosidase Tau protein, neurofilaments by immunocytochemistry, neurotoxicity mechanisms, replacement test
3 Neuro-2a mouse neuroblastoma cell culture, ATPase, phosphofructokinase, LDH, SDH, metabolic alterations, replacement test
4 Neuro-2a mouse neuroblastoma cell culture, neutral red uptake, MTT reduction, cell proliferation, basal cytotoxicity, replacement test
5 Monkey kidney Vero cell culture, glutathione reductase, glutathione transferase, glucose-6P-DH, SOD, antioxidative mechanisms, replacement test
6 RTG-2 rainbow trout fibroblasts cell proliferation by total protein content, ecotoxicity tests, replacement test
7 rat microsomal fraction, pyrrole adduct formation, by spectrofluorimetry, metabolic activation of diketone neurotoxic solvents, replacement test
8 muscle microsomal fractions of the crayfish *Procambarus clarkii,* acetylcholinesterase, neuropathy-target sterase, ecotoxicity effects of environmental pollutants in invertebrates, replacement test

Work lines: The development and use of *in vitro* methods applied to different materials and areas. The development of models of human neuronal differentiation and the interaction produced by drugs of abuse. The neurotoxic effects of environmental pollutants on mouse and human neuroblastoma cells, as alternative procedures to the use of animals. Interference in chick embryo development is studied. The investigation of chemical induced oxidative stress using Vero monkey kidney cells. The effects produced on very simple vegetal systems, including chloroplasts. As ecotoxicological tests, *in vivo* and *in vitro* cell fractions from the crayfish *Procambarus clarkii* are used in parallel to cultures of fish cell lines (RTG-2).

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: Aid for the consolidation of research groups. Junta de Andalucia. Desarrollo de un modelo *in vitro* con celulas de neuroblastoma para la evaluación de la neurotoxicidad de compuestos orgánicos. Desarrollo de modelos experimentales *in vitro* para la evaluacion de la toxicidad de compuestos metalicos. CICYT. Evaluacion de los efectos de la cocaina sobre celulas de neuroblastoma. Comunidad de Madrid. Studies for the pharmaceutical, cosmetic, and food additive industries. Inventory of Spanish institutions and Scientists interested in Alternative Methods. European Union.

Others: The team provides help for activities organized by GTEMA- Grupo de Trabajo Especializado en métodos Alternativos (http:// tox.umh.es/aet/gtema) and REMA- Red Española de métodos Alternativos (http:// tox.umh.es/rema/).

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Type of Institution: Governmental research facility

Staff:

- Ana I. Ortiz, Dr Veterinaria, Jefe de Servicio, 50 % time devoted to altern. meth.
- Ana Santa Maria, Ingeniero T. Agrícola, Jefe de Sección, 80 % time devoted to altern. meth.
- Alicia Galindo, Ld. Ciencias Bilógicas, Facultativo, 50 % time devoted to altern. meth.
- Maria del Mar Díaz Llorente, Ld. Farmacia, Ax Investigación, 100 % time devoted to altern. meth.
- Ana López Jiménez, Ld. Farmacia, Ax. Investigación, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This governmental research facility department is mainly involved in basic research and non-regulated applied research; it performs toxicology (basal cytotoxicity, genotoxicity / mutagenicity) testing on a routine basis. Diverse chemical compounds, food additives and colourings are routinely evaluated; pharmaceuticals, pesticides and wastes also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are *in vitro* methods -- cell lines culture (CHO-K1, Vero, A-549, Hep-2, NRK, BGM, RD).
The endpoints employed are *in vitro* cell viability, cellular proliferation and metabolic activity.

Experimental systems (examples):
1 Chinese hamster ovary cell line (CHO), neutral red uptake, cytotoxicity, replacement test
2 Chinese hamster ovary cell line (CHO), total protein content, cytotoxicity, replacement test
3 Chinese hamster ovary cell line (CHO), MTT reduction, cytotoxicity, replacement test
4 Chinese hamster ovary cell line (CHO), gene mutation, micronuclei, genotoxicity, replacement test

Work lines: Cytotoxicity assays: neutral red (NR) uptake test; total protein content (kenacide blue (KB)) test, MTT assay; genotoxicity-mutagenicity: gene mutation assay, micronucleus assay.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes.

Sources of financing: Comunidad Autónoma de Madrid, Fondo de Investigación Sanitaria (FIS)

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Type of Institution: Governmental research facility

Staff:

- Ingrid Maive Outschoorn, Email: ioutscho@isciii.es, Dr BSc (St Andrew´s), DEA (Montpellier), Mphill (Columbia), Dr Ciencias , J. Sección Respuesta Inmune, 100 % time devoted to altern. meth., duties related to altern. meth.: FELASA Workshop Participant Utrecht ND
- José Díaz Romero. Dr., Novartis, Suiza.
- Jesus Colino Gutierrez, Dr, Univ Health Sciences, Jones Br, Rd, Bethesda, US

Total staff involved in alternative methods is 3 people.

Activities / aims: This governmental research facility department is mainly involved in basic research, regulatory testing, method development, method validation and

alternatives to animals in education; it performs cell biology, molecular biology, biochemistry, physiology, biokinetics and biotransformation, toxicology (basal cytotoxicity, reproductive, cytotoxicity, immunotoxicity and sensitisation, haematoxicity, hepatotoxicity, endocrine disruption, chronic toxicity, ecotoxicity, integrated test strategies), nutrition, quality control, culture methodology testing studies on a routine basis, and vegetal biology, pathology, diagnostics (autoimmune infections), monitoring --chemical, biological-- studies at a research level. Vaccines, hormones, pharmaceuticals and environmental pollutants are routinely evaluated.

The main use of alternative methods is for screening.

Model systems: The model systems used are conventional animal models (hamster, rabbit, rat, mice, monkey), embryos (mouse, hamster), vegetables (fungi) and *in vitro* methods, including micro-organisms (Candida albicans, yeast, neisseria, coxiella, ricketsia), primary culture of dispersed cells (thymus, thyroid, spleen [mice, human]) and cell-free systems (lymphoid).
The endpoints employed are *in vitro* cellular proliferation (Tyd incorporation), cell signalling (surface marker expression), nucleic acids (DNA proliferation, mitochondrial DNA in ageing models), defence systems (antibodies, cytokines) and organ-specific indicators (tyroglobulin, microsomal).

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules. The team are available to participate in EU validation programmes.

Sources of financing: FIS, Instituto Universitario de Estudios de la Mujer, Universidad Autónoma de Madrid

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Type of Institution: Governmental research facility

Staff:

- Bartolomé Ribas Ozonas, Email: bribas@isciii.es, Dr Medicina y Dr Farmacia, Jefe de Area, 55 % time devoted to altern. meth., duties related to altern. meth.: Director de Estudio
- Mercedes Nuñez García, Email: mnuñez@isciii.es, Ld. Ciencias Biológicas, Facultativo Especialista, 80 % time devoted to altern. meth., duties related to altern. meth.: Responsable Unidad Laboratorio
- Macarena Gamoneda López-Ibor, Email: gamoneda@isciii.es, Ld. Ciencias Químicas, Becaria, 25 % time devoted to altern. meth.

- Miguel González-Doncel, Ld. Ciencias Biológicas, Becario, 40 % time devoted to altern. meth.
- Paloma Mendez Ureña, Auxiliar de Investigación, 30 % time devoted to altern. meth.
- Catalina Andrés Ratón, Auxiliar de Investigación, 40 % time devoted to altern. meth.
- Mercedes Fernández de la Puebla, T. E. S. A., Becaria.
- Isabel Orgaz Ariza, Email: iorgaz@isciii.es, Ld Ciencias Químicas, Jefe de Sección, 80 % time devoted to altern. meth., duties related to altern. meth.: Responsable Unidad Laboratorio
- Javier Méndez González, Email: jmendez@isciii.es, Dr Ciencias Químicas, Jefe de Servicio, 100 % time devoted to altern. meth., duties related to altern. meth.: Responsable Técnico
- Rosario Mancha López-Jurado, Email: rmancha@isciii.es, Ld, Jefe de Sección., duties related to altern. meth.: Responsable Unidad Laboratorio
- Gema Díaz López, Email: gdiaz@isciii.es, Facultativo Especialista, duties related to altern. meth.: Responsable Unidad Laboratorio

Total staff involved in alternative methods is 15 people.

Activities / aims: This governmental research facility department is mainly involved in basic research, non-regulated applied research, regulatory testing, method development, method validation and alternatives to animals in education; it performs pharmacodynamics (dose-response relationships), toxicology (mechanisms of toxicity, basal cytotoxicity, acute systemic toxicity, reproductive cytotoxicity, haematoxicity, genotoxicity / mutagenicity, carcinogenicity, hepatotoxicity and ecotoxicity) studies, monitoring --chemical, biological--, quality control and risk assessment testing on a routine basis, and cell biology and molecular biology studies at a research level. Diverse chemical compounds, environmental pollutants and wastes are routinely evaluated; pesticides and toxins also being occasionally studied.

The main use of alternative methods is for screening and as complementary studies.

Model systems: The model systems used are animal models --conventional, invertebrates (Parasarcoph. argyrostoma, fish, Daphnia, algae, earthworm,vegetables, aerobic micro-organisms, fish embryo, microcrustaceans, -- vegetables (growth and development of seeds) and *in vitro* methods, including micro-organisms (mixed inoculum), primary culture of dispersed cells (human lymphocytes) and cell lines culture (HV-60, neurones).
The endpoints employed are *in vivo* (metallothioneins, G6PDH) and *in vitro* cellular proliferation (J Immunol Methods 65 (1983),55).

Experimental systems (examples):
1 microbial mixed inoculum, respiration inhibition, toxicity evaluation, complementary test
2 rotifers, mortality (CE50), toxicity evaluation, screening
3 microcrustaceans(*Thaminocephalus platyurus*), mortality (CE50), toxicity evaluation,screening
4 medaka fish (*Oryzius latypes*), sublethal effects, embryotoxicity evaluation
5 rats, rabbits, mice, metallothioneine metals in each isoform, by HPLC, capillary

electrophoresis, specific bioindicators
6 rat tissue culture and treatments, glutathione, metallothioneine, redox state evaluation in other sulphur compounds by HPLC, capillary electrophoresis

Work lines: Biomedical effects of electromagnetic fields in extremely low frequency and in radiofrequency under the auspices of two projects and the collaboration of the Department of Physics University Complutense of Madrid, CSIC, and the Clinical Hospital of Madrid. Evaluation and validation of the new proposed technique on a Diptera larvae as alternative method. The evaluation of redox state by different sulphur compounds in degenerative diseases. Risk assessment.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team have previously been involved in the following alternative method validation programmes: ring tests of a method for ready biodegradability (OECD). They are available to participate in EU validation programmes, in relation to validation of the proposed diptera larvae.

Sources of financing: CAM, FIS, CAICYT, Autonomic Community of Madrid and collaboration with ENDESA, CONAMA

Others: The validation of the diptera larvae method is proposed.

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Staff:

- Carmen Barrueco Fdez-Cuervo, Email: cbarrue@isciii.es, Dra Ciencias Biológicas, Técnico de Gestión de Organismos Autónomos, 70 % time devoted to altern. meth., duties related to altern. meth.: Miembro del GTEMA

Total staff involved in alternative methods is 1 person.

Activities / aims: This governmental research facility department is mainly involved in risk assessment; it performs toxicology (genotoxicity / mutagenicity) testing on a routine basis. Pesticides are routinely evaluated.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are *in vitro* methods --micro-organisms (*Salmonella typhimurium*), primary culture of dispersed cells (human lymphocytes), cell lines culture (CHO), cell-free systems (microsomal fraction)-- and human

volunteers (workers exposed to pesticides).
The endpoints employed are *in vivo* induction of chromosomal aberrations, SCE and micronuclei in human lymphocytes from exposed workers and *in vitro* nucleic acids reversion of mutation, gene mutations, structural mutations, SCE and micronuclei and biotransformation systems (rat liver microsomal fractions).

Experimental systems (examples):
1 *Salmonella typhimurium,* reversion of mutation, by count on solid medium, detection of gene mutation as genotoxicity, screening
2 human lymphocytes and CHO cell line, structural chromosomal aberrations, cytogenetic analysis of metaphases, *in vitro* genotoxicity, screening
3 human lymphocytes, micronuclei by cytogenetic study of cells or interphase, detection of clastogens and aneugens, screening
4 human lymphocytes and CHO cell line, SCE- sister chromatid exchange, cytogenic lesions to DNA, screening

Work lines: Genotoxicity evaluation of pesticides using *in vitro* and *in vivo* assays for determining different endpoints (gene mutation, chromosomal aberration and DNA effects). Evaluation of the report for new pesticides registration according to Annex I of Directive 91/414/EEC.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU and OECD. The team are available to participate in EU validation programmes related to genotoxicity assays.

Sources of financing: Instituto de Salud Carlos III, INIA

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Type of Institution: Industrial (pharmaceutical Laboratory)

Staff:

- Carmen González Martín, Dra Ciencias Biológicas, Jefa de Toxicología, Responsable de Unidad, 50 % time devoted to altern. meth.
- Montserrat Gozalo, Lda. Ciencias Biológicas, Técnico Ayudante de Toxicología, 20 % time devoted to altern. meth.
- Eva María Archilla, Formación Profesional FP 2, Auxiliar de Toxicología, 100 % time devoted to altern. meth., duties related to altern. meth.: cell culture

Total staff involved in alternative methods is 3 people.

Activities / aims: This industrial (pharmaceutical laboratory) department is mainly involved in basic research, non-regulated applied research, method development and

method validation; it performs toxicology testing (mechanisms of toxicity, basal cytotoxicity, acute systemic toxicity, nephrotoxicity, hepatotoxicity, chronic toxicity) on a routine basis. Pharmaceuticals are routinely evaluated; physical agents also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement tests.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- cell lines culture (epithelioid (kidney, endothelial)).
The endpoints employed *in vitro* are morphology (HTD by optical and electronic microscopy), cell viability (neutral red uptake, MTT reduction, LDH), cytoskeleton/membranes/enzyme release (LDH release), cellular proliferation (CFA) and cell signalling (immunofluorescence).

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to OECD

Laboratorio de Análisis Dr. Echevarne
Departamento de Genotoxicidad y Oncología molecular
Provenza 312
08037 Barcelona
Tel: 934964444, Fax: 932154838
Type of Institution: Private / contract laboratory

Staff:

- Elisabeth Carbonell Teruel, Email: elisabet.carbonell@blues.uab.es, Dra. Biología (Genética), Directora Unidad de Genotoxicidad, 100 % time devoted to altern. meth.
- Fernando de Cuevillas Matozzi, Email: Fernan_cuevillas@hotmail.com, Dr Químicas, Ldo. Farmacia, Director Unidad Oncología Molecular, 50 % time devoted to altern. meth.
- Carmen Montoriol Sabaté, Lda. Farmacia, Respon. Suplente Unidad Oncología Molecular, 50 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This private / contract laboratory department is mainly involved in non-regulated applied research, regulatory testing and method development; it performs molecular biology, toxicology testing (genotoxicity / mutagenicity), diagnostics, monitoring --chemical, biological-- and culture methodology studies on a routine basis. Pharmaceuticals, cosmetics, diverse chemical compounds, pesticides, medical devices and biomaterials are routinely evaluated; food additives, colourings and environmental pollutants also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are *in vitro* methods - micro-organisms (*Salmonella typhimurium*, *E. coli*), primary culture of dispersed cells (human lymphocytes, breast, colon, lung cells, etc) and cell lines culture (solid tumours, L5178).
The endpoints employed are *in vitro* cell viability (dye uptake), cellular proliferation (flow cytometry), metabolic activity (P450, glutathione S-transferase), nucleic acids (gene mutation (p53), chromosomal breaks, reorganizations (AC, SCE, MN, SCGE)) and defence systems (glycoprotein P).

Experimental systems (examples):
1 *Salmonella typhimurium,* reversion of mutation, genotoxicity, complementary test
2 human peripheral lymphocytes, chromosomal breaks, genotoxicity, complementary test
3 human peripheral lymphocytes or tissues, BRCA1 gene sequence, diagnostics, screening
4 human peripheral lymphocytes, Philadelphia chromosome, diagnostics, screening

Work lines: Development and implementation of methods of clinical interest on solid and haematological tumours. Genotoxicity evaluation of chemicals. Biomonitoring of workers exposed to pollutants.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to OECD. The team have previously been involved in the following alternative method validation programmes: Programas Nacionales de Sanidad y de la CEE. They are available to participate in EU validation programmes (all types): *in vitro* micronuclei assay and comet assay

Sources of financing: "Determinación de la naturaleza del daño genético inducido por distintos agentes genotóxicos utilizando Drossophila y linfocitos humanos como sistema de bioensayo" CICYT, SAF94-0697, 1994-97; genotoxicidad en eucariotas: nuevos estudios sobre sus mecanismos y detección CICyT, SAF95-0813; Colaboración en el "Estudio del diagnóstico de tumores de mama y ovario hereditarios, por secuenciación del gene BRCA1"

Laboratorio Dr. F. Echevarne Analisis S.A.
Unidad de Ensayos Toxico-Biológicos (UETB)
C/ Sant Antoni núm. 11 baixos
08301 Mataro-- Barcelona
Tel: 937907411, Fax: 937907516

Type of Institution: Private / contract laboratory

Staff:

- Lluis Simo I Castells, Ld Ciencias Biológicas, Director Unidad, 20 % time devoted to altern. meth.
- Nuria Alvarez Genoher, Ld Ciencias Biológicas, Subdirector Unidad, 30 % time

devoted to altern. meth.

- Samuel Corcobado Moreno, Téc. Esp. Química, Técnico, 20 % time devoted to altern. meth.
- Juan Rodríguez Ruíz, Téc. Esp. de Laboratorio, Técnico, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This private / contract laboratory department is mainly involved in regulatory testing; it performs toxicology (acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity, genotoxicity / mutagenicity, ecotoxicity) testing on a routine basis. Pharmaceuticals, cosmetics, medical devices, biomaterials, wastes are routinely evaluated; vaccines, hormones, diverse chemical compounds, pesticides, toxins, food additives, colourings and food also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models, *in vitro* methods -- micro-organisms (*Photobacterium phosphoreum*), culture of explants, reaggregates, reconstituted organs, (skintex, eyetex), primary culture of dispersed cells (rbc)-- and human volunteers (adaptation to the situation).
The endpoints employed are *in vivo* death, toxicity, irritation, temperature changes, sensibilization / allergy and *in vitro* morphology, cell viability and metabolic activity enzymes.

Experimental systems (examples):
1 rabbits, temperature, rectal probe, pyrogenicity
2 mice, death, abnormal toxicity (safety)
3 red blood cells, haemolysis, ocular irritation, replacement test
4 micro-organisms, luminescence, wastes, water, replacement test

Work lines: Alternative methods in toxicology

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to OECD. The team are available to participate in EU validation programmes (all types).

Sources of financing: Self financing

Laboratorio Dr Goya Analisis
Goya
Vía Complutense 75
28805 Alcalá de Henares-- Madrid
Tel: 918893410, Fax: 918803319

Type of Institution: Private / contract laboratory

Staff:

- J. Ramón Goya Ramos, Email: rgoya@recol.es, Dr Farmacia, Dr Laboratorio
- Paloma Aberturas, Analista Clínico del Laboratorio. Encargada de los tests de esterilidad y pirógenos

Total staff involved in alternative methods is 2 people.

Activities / aims: This private / contract laboratory department is mainly involved in regulatory testing; it performs biochemistry, toxicology (mechanisms of toxicity, acute systemic toxicity, ocular cytotoxicity, dermal irritation, corrosivity, haematoxicity, integrated test strategies) and culture methodology studies on a routine basis. Pharmaceuticals and cosmetics are routinely evaluated, medical devices also being occasionally studied.
The main use of alternative methods is for complementary studies.

Model systems: The model systems used are animal models –conventional.

Work lines: This is a control laboratory authorized by the Ministry of Health for the control of cosmetics, medical equipment, etc. Therefore toxicity, primary irritation, sterility and pyrogens are the main tests carried out at the laboratory.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: Privates

Laboratorios J. Uriach & Cía

Farmacocinética

c/ Dega Bahi, 59-67

08901 Barcelona

Tel: 933471511, Fax: 932560639, Website: http:// www.uriach.com

Type of Institution: Industrial (pharmaceutical)

Staff:

- Nuria Sales Aragones, Email: rd@uriach.com, D, 80 % time devoted to altern. meth.
- Lourdes Conte Visús, Email: rd@uriach.com, Dr, 80 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in basic research and regulatory testing; it performs pharmacodynamics (*in vitro* metabolism) testing on a routine basis. Pharmaceuticals are routinely evaluated.

Model systems: The model systems used are *in vitro* methods: cell-free systems (microsomes and postmitochrondrial fractions).

The endpoint employed *in vitro* is metabolic activity.

Work lines: The main lines of work are: *in vitro* metabolism by hepatic rat, dog, monkey and human microsomes; Research of the CYP P-450 involved in the metabolism of different drugs; In vitro investigation of potential inhibitory effects of a drug on CYP P-450.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules. The team have previously been involved in the following alternative method validation programmes: Validation for HPLC. They are available to participate in EU validation programmes related to metabolism studies validation.

PROVITAL S.A.
Departamento Técnico
UTTEC-Unidad de Ensayos Cutáneos y Toxicológicos
Ctro Indust. Santiga.
Talleres 6 nº15
08210 Barberá del Vallés-- Barcelona
Tel: 937188012, Fax: 937183830

Type of Institution: Industrial (Cosmetic ingredients. Vegetable extracts)

Staff:

- Lourdes Mayordomo, Email: provital@sumi.es, Ld Biología, Directora UTTEC, 100 % time devoted to altern. meth.
- Ricardo Armengol, Ld Farmacia, Director Técnico, 10 % time devoted to altern. meth.
- Aurora Benaiges, Ld Farmacia, Directora I+D, 30 % time devoted to altern. meth.
- Antonia María Gómez, Dr Ciencias Químicas, Responsable de Cultivos Celulares, 70 % time devoted to altern. meth.
- Desirée Pérez, Técnico Superior de Análisis Químico, Auxiliar de Laboratorio, 10 % time devoted to altern. meth.
- Mónica Puigdellivol, Técnico Especialista, Auxiliar de Laboratorio, 30 % time devoted to altern. meth.

Total staff involved in alternative methods is 6 people.

Activities / aims: This industrial (cosmetic ingredients and vegetable extracts) department is mainly involved in regulatory testing; it performs toxicology (ocular cytotoxicity, dermal irritation and corrosivity) testing on a routine basis, and cell biology and biochemistry studies at a research level. Cosmetics and vegetable extracts are routinely evaluated.
The main use of alternative methods is for screening and replacement test.

Model systems: The model systems used are embryos (chick embryo chorioalantoid

membrane assay (HET-CAM)), *in vitro* methods -- micro-organisms (*Salmonella typhimurium*), organ culture (bovine corneal opacity / permeability (BCOP)), cell lines culture (foreskin fibroblasts) and cell-free systems (hepatic mitochondria).
The endpoints employed are *in vitro* morphology (optical microscopy; lesions to blood vessels (CAM), cornea (BCOP)), metabolic activity (LDH, oxygen consumption) and nucleic acids (mutation reversion).

Experimental systems (examples):
1 chick embryo chorioallantoid membrane assay, blood vessel lesions, ocular irritation, replacement test
2 *Salmonella typhimurium*, mutation reversion, mutagenicity, screening, replacement test
3 hepatic mitochondria, oxygen consumption, metabolic alterations, screening
4 foreskin fibroblasts, LDH activity by spectrophotometry, cytotoxicity, screening, replacement test

Work lines: Provital manufactures ingredients for the cosmetic industry, mainly plant extracts. The aim of the studies is to obtain data on efficacy and safety in order to comply with present legislation. Plant extracts are not included in alternative method validation, and Provital is defining the methods most suited to these products.

Quality assurance / Validation programmes: The team have previously been involved in the following alternative method validation programme: the EC/HO international validation study on alternatives to the Draize eye test. They are available to participate in EU validation programmes.

Sources of financing: Self financing; Ayudas intercambio personal investigador entre industrias y centros públicos de investigación (B); Ayudas I+D para incorporación de doctores a empresas, para cultivos celulares.

Puig Martin Asociados
Les Corts, 36-38
08028 Barcelona
Tel: 934583099, Fax: 934593434
Type of Institution: Distributor

Staff:

- Jorge Puig Martín, Email: pmasoc@datalogic.es, Ingeniero Químico IQS, Director, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 1 person.

Activities / aims: This distributor department is mainly involved in commercialization of *in vitro* tests; it performs toxicology (dermal irritation and corrosivity, immunotoxicity and sensitisation, phototoxicity, hepatotoxicity), cosmetics testing on a routine basis. Cosmetics are routinely evaluated.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are *in vitro* methods: culture of explants, reaggregates, reconstituted organs, cell lines culture (hepatocytes, fibroblasts).
The endpoint employed *in vitro* is cell viability

Experimental systems (examples):
1 rabbit fibroblasts, neutral red uptake, ocular irritation, replacement test
2 human skin explants, dermal irritation, replacement test

Others: The company is the exclusive Spanish distributor of the firm BIOPREDIC (Rennes, France)

Servicio Andaluz de Salud. Consejeria de Salud de Andalucía
Delegación Provincial de Salud de Almeria
Servicio de Salud
Estudio de Plaguicidas
Canónigo Molina Alonso 6-6º2
04004 Almeria
Tel: 950267728

Type of Institution: Governmental service facility (diagnostic, control, monitoring)

Staff:

- Tesifón Parrón Carreño, Dr Medicina.
- Reyes Alvarez Osorio, Dr Medicina.
- Rafael Durban, Ld Farmacia.
- Jose Luis Serrano Ramirez, Ld Medicina y Cirugía.
- Alberto González Ramón, Ld Veterinaria.

Total staff involved in alternative methods is 5 people.

Activities / aims: This governmental service facility department (diagnostic, control, monitoring) is mainly involved in basic research; it performs toxicology (chronic toxicity) testing on a routine basis. Pesticides are routinely evaluated.
The main use of alternative methods is for screening.

Model systems: The model systems used are human volunteers.
The endpoints employed *in vivo* are conventional analysis, cholinesterase, paranitrophenol, lithium and serotonine.

Work lines: Effects of long term exposure to pesticides in fumigation workers: case control studies. Mutagenesis in fumigation workers. Memory alteration in fumigation workers after acute and chronic exposure.

SmithKline Beecham
Centro de Investigación Básica
Department of Molecular Screening Technologies
Natural Product Screening
C/ Santiago Grisolía, 4, 28760 Tres Cantos (Madrid)
Tel: 918039444, Fax: 918039184
Type of institution: Industrial (Pharmaceutical)

Staff:
Emilio Díez, Email: emilio_diez@sbphrd.com, Dr Ciencias Químicas, Associate Director, 20 % time devoted to alternative methods.
Total staff involved in alternatives methods is about 15 persons: 9 doctors, 2 Lic, 4 technicians.

Activities / aims: This Industrial (Pharmaceutical) department is mainly involved in basic research, methods development and validation, search and identification of pharmaceutical from vegetal origen; it performs cell biology, biochemistry, biokinetics and biotransformation, and toxicology testing on a routine basis, and toxicology (basal cytotoxicity) studies at a research level. The materials routinely evaluated are pharmaceuticals, diverse chemical compounds, being also occasionally studied.
The main use of alternatives methods is for screening.

Model systems: The model systems used are *in vitro* methods -- micro-organisms (*E. coli*, *S. aureus*, *C. albicans*, etc), primary culture of dispersed cells (human lymphocytes, monocites, PMS, etc), cell lines culture (mouse, decens of human stable and receptor or enzyme transfected cell lines) and cell-free systems (plasmatic, isolated proteins, recombinant proteins).
The endpoints employed *in vitro* are cell viability (cellular dehydrogenases by photometry and esterases by fluorimetry), celular proliferation (Tyd uptake, filtration) and cell signalling (cAMP, IPS, intracellular calcium with Fluo3 and Fluo 4).

Experimental systems (examples):
1Bacteria subcellular fractions, lucipherase by luminiscence, identification of new antibiotics, screening
2 Mammal cells, cAMP by radioactivity and SPA scintillation proximity assays, identification of new pharmaceuticals, screening
3 Purified recombinant proteins, diverse enpoints determined by flourescence (TRF, HTRF, FLINT, FP), radiometry, luminiscence, photometry, identification of new pharmaceuticals, screening
4 Mammal cells, cell dehydrogenases by photometry and esterases by fluorimetry, cytotoxicity, screening

Work lines: High troughput screening of pharmaceuticals of vegetal origen.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU.

Sources of financing: Own financing, CDTI, Becarios del Ministerio de Educación, Plan IDE (Incorporación de Doctores a Empresas)

Universidad de Alcalá de Henares
Facultad de Farmacia
Departamento de Nutrición y Bromatología
Elementos Minerales y Biodisponibilidad
Crta. Madrid-Barcelona Km 33,6
28871 Alcalá de Henares-- Madrid
Tel: 918855147, Fax: 918854783
Type of Institution: University

Staff:

- Maria Jose González Muñoz, Dr Farmacia, Titular, 30 % time devoted to altern. meth.
- Mª Carmen Martínez Para, Dr Farmacia, Catedrática, 10 % time devoted to altern. meth.
- Isabel Meseguer Soler, Dr Farmacia, 30 % time devoted to altern. meth.
- Mª Victorina Aguliar Vilas, Dr Farmacia, Titular, 40 % time devoted to altern. meth.
- Carmen Jose Mateos Vega, Ld Farmacia, Becaria, 80 % time devoted to altern. meth.

Total staff involved in alternative methods is 7 people.

Activities / aims: This university department is mainly involved in non-regulated applied research; it performs toxicology and nutrition testing on a routine basis, and toxicology (neurotoxicity) studies at a research level. Nutrients are routinely evaluated.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are conventional animal models.

Work lines: Bioavailability of several mineral elements in different food: Cr, Fe, Se, P in breakfast cereals, using an "*in vitro*" and "*in vivo*" method. Role of certain minerals in several neurological diseases: determination of Se in the cerebrospinal fluid of Parkinson and Alzheimer patients.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the FDA. The team are available to participate in EU validation programmes.

Sources of financing: Universidad de Alcalá, FIS

Universidad de Alcalá de Henares
Facultad de Farmacia
Departamento de Nutrición y Bromatología

Métodos Alternativos a la Toxicología in vivo

28871 Madrid

Tel: 918854775, Fax: 918854783

Type of Institution: University

Staff:

- Jose María Cobo Sanz, Email: nbcobo@nutri.alcala.es, Dr Farmacia, Profesor Universidad Alcalá, 50 % time devoted to altern. meth.
- Rafael García-Cañero, Email: rgcanero@hepatexp.cph.es, Dr Ciencias Químicas, Adjunto Jefe Servicio Bioquímica, 90 % time devoted to altern. meth., duties related to altern. meth.: Vocal de la Sociedad Española de Biología Cellular
- Javier Pérez de Diego, Dr Farmacia, Profesor Universidad Alfonso X, 20 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research and method development; it performs biochemistry, toxicology (mechanisms of toxicity, basal cytotoxicity, carcinogenicity, hepatotoxicity, chronic toxicity) and culture methodology studies on a routine basis, and cell biology and molecular biology studies at a research level. Diverse chemical compounds and wastes are routinely evaluated; pharmaceuticals also being occasionally studied.

The main use of alternative methods is for complementary studies.

Model systems: The model systems used are conventional animal models and *in vitro* methods: primary culture of dispersed cells (rat liver cells) and cell lines culture (CHO, HL60, Hela, HepG2).
The endpoints employed *in vitro* are cellular proliferation (fluorescence), metabolic activity (apoptosis), nucleic acids (DNA degradation) and organ-specific indicators (ion uptake, cell membranes).

Experimental systems (examples):
1 HL60 cells, cell cycle and apoptosis, by flow cytometry, gel electrophoresis, DNA degradation, replacement test

Work lines: The most important field is to study the interrelation between different chemicals and cancer and to establish the role of apoptosis as regulator of DNA degradation. Interest in the chemicals that change Na/K across membranes and how this change might affect intracellular pH and stop mitosis.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices. The team are available to participate in EU validation programmes in relation to biochemical methods applied to cell cultures, detection of carcinogenicity, etc.

Sources of financing: Fondo de Investigación Sanitaria, Dirección General de Investigación y Ciencia, Fondos de Investigación de la Universidad Privada, Diversas ayudas para el Desarrollo de Cooperación con otros laboratorios internacionales.

Universidad de Almeria
Escuela Politécnica Superior
Departamento de Biología Aplicada
Nutrición y Alimentación Animal
Campus La Cañada S. Urbano s/n
04120 Almeria
Tel: 950245294, Fax: 950215476, Website: http:// www.ualm.es
Type of Institution: University

Staff:

- Francisco Javier Moyano López, Email: fjmoyano@ualm.es, Dr Ciencias Biológicas, Titular de Universidad, 50 % time devoted to altern. meth.
- Manuel Díaz López, Email: mdiaz@ualm.es, Dr Farmacia, Titular de Universidad, 50 % time devoted to altern. meth.
- Francisco Javier Alarcón López, Email: falarcon@ualm.es, Dr Ciencias Biológicas, Becario Postdoctoral, 75 % time devoted to altern. meth.
- Tomás Martínez Moya, Email: tomas@ualm.es, Ld Veterinaria, Becario Predoctoral, 100 % time devoted to altern. meth.
- Inmaculada Martínez Díaz, Email: imdiaz@ualm.es, Ld Ciencias del Mar, Becaria de Proyecto, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in non-regulated applied research, method development and alternatives to animals in education; it performs physiology and nutrition testing on a routine basis. Protease inhibitors and nutrient digestibility are routinely evaluated.

Model systems: The model systems used are *in vitro* methods -- cell-free systems (digestive enzymes) and education models (computer simulations)
The endpoints employed are *in vitro* metabolic activity (controlled proteins and fatty acids hydrolysis).

Experimental systems (examples):
1 digestive enzyme from fish, protein hydrolysis, digestibility, replacement test
2 ruminant liquid extract, changes in nutrient content, digestibility, replacement test
3 digestive enzyme from fishes, protein degradation studied by electrophoresis, digestibility studies, replacement test
4 digestive enzyme from fish, protease inhibition studied by zymogramme, protease inhibition, complementary test

Work lines: Design and nutritive evaluation of feed sources for marine fish. Design and nutritive evaluation of artificial feed for larval marine fish. Nutritive evaluation of feed for ruminants

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes in relation to digestibility techniques *in vitro*.

Sources of financing: 3 CICYT projects on water culture.

Universidad Autónoma Barcelona
Facultad de Ciencias
Departamento de Genética y Microbiología
Grupo de Mutagénesis
Campus de Bellaterra
08193 Cerdanyola del Valle-- Barcelona
Tel: 935812052, Fax: 935812387, Website: http:// cc.uab.es

Type of Institution: University

Staff:

- Ricardo Marcos Dauder, Email: rmd@cc.uab.es, Biólogo, Catedrático, 50 % time devoted to altern. meth.
- Amadeu Creus Capdevila, Email: a.creus@uab.es, Biólogo, Prof. Titular, 50 % time devoted to altern. meth.
- Noel Xamena López, Email: n.xamena@uab.es, Biólogo, Prof. Titular, 30 % time devoted to altern. meth.
- Antonia Velázquez Henar, Email: a.velazquez@uab.es, Biólogo, Prof. Titular, 30 % time devoted to altern. meth.
- Jordi Surralles Calonge, Email: j.surralles@uab.es, Biólogo, Post. Doct. Contratado,30 % time devoted to altern. meth.

Total staff involved in alternative methods is 10 people.

Activities / aims: This University department is mainly involved in basic research, non-regulated applied research, method development and method validation; it performs toxicology (mechanisms of toxicity, genotoxicity / mutagenicity, carcinogenicity) testing, genetics and monitoring --chemical, biological- studies on a routine basis. Pharmaceuticals, diverse chemical compounds and pesticides are routinely evaluated; environmental pollutants and physical agents also being occasionally studied.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are invertebrate animal models (*Drosophila*), *in vitro* methods -- primary culture of dispersed cells (human lymphocytes), cell lines culture (human lymphoblast cell lines)--, and human volunteers (biomonitoring) studies.
The endpoints employed are *in vivo* genetic and cytogenetic lesions and *in vitro* nucleic acids.

Experimental systems (examples):

1 *Drosophila,* germinal and somatic mutations, genotoxicity, screening and complementary test
2 human lymphocytes, chromosomal mutations, genotoxicity, screening and complementary test
3 human volunteers, chromosomal mutations, genotoxicity, screening and complementary test

Work lines: Genotoxicity testing of different chemicals, mainly pesticides; biomonitoring of people environmentally, occupationally or therapeutically exposed to genotoxicants; molecular basis of chemically induced mutants; improvement of different tests used in genotoxicity testing; molecular epidemiology.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to genotoxic evaluation

Sources of financing: CICyT, CIRIT (Generalitat de Catalunya), Environment Programme (EU) and pharmaceutical industries.

Universidad Autónoma de Barcelona
Unidad de Microbiologia
Genética y Microbiología
Microbiología Molecular y Genética Bacteriana
Edificio Cn
08193 Bellaterra-- Barcelona
Tel: 935812615, Fax: 935812387, Website: http:// ww.uab.es
Type of Institution: University

Staff:

- Montserratt Llagostera Casas, Email: ibm4@cc.uab.es, Dr Biología, Prof. Titular, 20 % time devoted to altern. meth.
- J. Antonio Moreno, Ld Biología, Prof. Ayudante, 20 % time devoted to altern. meth.
- Maribel Cárdenas, Ld Biología, Estudiante Tercer Ciclo, 70 % time devoted to altern. meth.
- Mirle Ferrer, Ld, Becaria ICI, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 4 people.

Activities / aims: This university department is mainly involved in basic research and non-regulated applied research; it performs toxicology (genotoxicity / mutagenicity,) testing on a routine basis. Diverse chemical compounds, pesticides, biomaterials, environmental pollutants and wastes are routinely evaluated.

Model systems: The model systems used are *in vitro* methods: micro-organisms.
The endpoints employed *in vitro* are biotransformation systems (vegetable enzymes: peroxidase, cit P450).

Experimental systems (examples):
1 Ames assay, reversion of mutation, vegetal promutagens activation, complementary test
2 micro-organisms, SOS gene induction, by betagalactosidase activity, comparison with Ames test, complementary test

Work lines: The study of the capability of plants to metabolize environmental pollutants to mutagenic metabolites and the identification of plant enzymatic activities involved in this process.

Quality assurance / Validation programmes: The team have previously been involved in alternative method validation programmes. They are available to participate in EU validation programmes in relation to genotoxicity and environmental mutagenesis

Sources of financing: CE, DGCYT, Generalitat de Catalunya

Universidad Autónoma de Madrid
Facultad de Ciencias
Departamento de Biología
Cantoblanco s/n
28049 Madrid
Tel: 913978248, Fax: 913978344, Website: http:// www.uam.es
Type of Institution: University

Staff:

- María José Hazen de San Juan, Email: mariajose.hazen@uam.es, Dra Biología, Profesor Titular, 80 % time devoted to altern. meth.
- María Luisa Molero Vergara, Dr Biología, Profesor Titular, 80 % time devoted to altern. meth.
- Ana Isabel Pérez Gorroño, Ld Biológia, 40 % time devoted to altern. meth.
- Nuria Ortiz Movilla, Ld Biología, 40 % time devoted to altern. meth.
- Miguel Angel Fernández, Técnico, 25 % time devoted to altern. meth.

Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in non-regulated applied research and method development; it performs cell biology, toxicology (mechanisms of toxicity, basal cytotoxicity, phototoxicity) testing on a routine basis and vegetal biology studies at a research level. Diverse chemical compounds and colourings are routinely evaluated; physical agents also being occasionally studied.
The main use of alternative methods is for replacement studies.

Model systems: The model systems used are *in vitro* methods - cell lines culture (Hela, 3T3 mouse fibroblasts, Vero).

The endpoints employed *in vitro* are cell morphology (scanning and transmission electronic microscopy) and cell viability (MTT reduction, neutral red uptake, total protein content, LDH).

Experimental systems (examples):
1 Hela tumour human cells, basal cytotoxicity by spectrophotometry, photodynamics, replacement test
2 3T3, Vero, basal cytotoxicity by spectrophotometry, photodynamic compounds analysis, replacement test
3 3T3, Vero, basal cytotoxicity by spectrophotometry, participation of oxygen free-radicals, replacement test

Work lines: The major interest of this lab is focused on the development of *in vitro* protocols which allow a rapid and reliable analysis of the photosensitizing properties of chemical compounds. The applications of the work are both to define potential photochemotherapeutic agents and to search for new phototoxic agents.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Universidad de Barcelona
CELLTEC, UB. Parc Científic de Barcelona.
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Laboratory: Department of Cellular Biology. University of Barcelona
Avinguda Diagonal 645 - 08028 Barcelona Tel.: 93 402.15.50 Fax: 93 411.29.67
E-mail: celltec@bio.ub.es WEB: http://celltec.ub.es
Type of Institution*:* University.

Staff:
- Senén Vilaró. E-mail: senen@porthos.bio.ub.es; General Manager. PhD. Professor of Cell Biology.
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- Roser Pagán. E-mail: pagan@porthos.bio.ub.es; Project Manager. PhD. researcher.
- Myriam Fabre. E-mail: mfabre@porthos.bio.ub.es; Project Manager. PhD researcher.
- Àlex Monnà. E-mail: amonna@fbg.ub.es; Promotion. Graduate in Biology.

In addition, there are 3 laboratory technicians and 13 post-graduates students, performing a staff with 22 people involved in alternative methods.

Activities/aims: This university department aims to promote innovation and transfer in the fields of cellular and molecular technology. It performs cell and molecular biology and toxicology testing on a routine basis, biokinetics and biotransformation, pathology,

diagnostics and monitoring –chemical, biological- studies at a research level. Pharmaceuticals, cosmetics and diverse lead compounds are evaluated; is also interested in research related to *in vitro* models of different tissues for transplantation.

Model systems: the model systems used are *in vitro* methods –primary culture of endothelial cells of different species and cell lines of different endothelial cells; primary culture of smooth muscular cells from human aorta (migratory, quiescent, proliferating and confluent phenotypes); cell line of intestinal epithelial cells from human adenocarcinoma; primary culture of dermal fibroblasts from human skin; primary culture of cells from the pigmentary epithelium of the human retina; primary culture of human blood monocytes and cell line of human monocytes from human macrophages; cell line of kidney cells from dog; primary culture of queratinocytes from human skin; primary culture of hepatocytes (adult rat liver, regenerant liver from adult rat, liver from rat embrion and from human liver) and cell line hepatocytes from human hepatic cancer; neurones from human and rat brain; adipocytes from mouse adipose tissue and primary culture of adipocytes from rat and human adipose tissue; and other cellular models (human adrenal gland cells, immortalised human placenta cells, hamster ovary cells and different cell lines with constitutive and stable expression of receptors from the plasmatic membrane that are involved in the development of artheriosclerosis).

Experimental systems (examples):

1. Analysis of the flow of seed molecules through the blood brain barrier on cocultures systems involving endothelial cells and astrocytes as a model of the brain blood barrier.
2. Metabolic, functional and toxicological analysis of the effects of drugs on endothelial cells to study angiogenic molecules.
3. Analysis of the effect of seed molecules on artheriosclerotic processes on HASMC monolayer cultures as a model of artheriosclerosis patology.
4. System of functional screening of pharmacological and cosmetic active principles on cocultures of human keratinocytes and fibroblasts that mimic human skin.
5. Analysis of the functional effect of seed molecules driven to inflammatory, allergic, infectious and arteriosclerotic processes on cultures of Human Monocytes differentiated to macrophage.
6. *In vitro* analysis of renal absorption of seed molecules on polarised cultures of epithelial cellsa and on monolayer cultures of primary hepatocytes to study hepatotoxicity.
7. Analysis of seed molecules effects on differentiation, proliferation or neural toxicity on monolayer cultures of human or rat brain neurones.
8. Analysis of seed molecules on the accumulation of fat in adipocytes (monolayer cultures of rat, mouse or human adipocytes).

Work lines: It offers all biotechnological, pharmaceutical and cosmetics companies the experience and knowledge, concerning development and use of *in vitro* cellular models. The group has a wide experience in the screening and determination of efficacy, metabolism and toxicity of drugs, active principles and elaborated products. CELLTEC UB also offers to the R+D Departments of companies the possibility to obtain its advice and the transfer of its technology. It is developing different activities such as: Assays on *in vitro* cellular models. Research services: Development and validation of new *in vitro* cellular models*;* Research on mechanism of action, toxicity and metabolism of drugs and new molecules; Research on tissue bio-engineering; Cellular *kit* development and validation*;* Technology transfer; Scientific advice.

Quality assurance/Validation programmes: The team is available to participate in EU validation programmes and is in process to implement Good Laboratory Practices.

Sources of financing: National projects (CICYT, MINER, FEDER,CIRIT, public and private foundations, etc.), European projects (Frame Programmes) and contracts with industries (pharmaceutical, cosmetics, biotechnology, etc.).

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- Eulalia Delgado Sureda, Email: jnadal@bio.ub.es, Ld Ciencias Biológicas.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research; it performs toxicology (genotoxicity / mutagenicity, ecotoxicity) testing on a routine basis. pesticides and environmental pollutants are routinely evaluated.

Model systems: The model systems used are invertebrate animal models (earthworm) The endpoint employed *in vivo* is single cell electrophoresis (COMET).

Experimental systems (examples):
1 earthworm coelomocytes, COMET assay, terrestrial ecotoxicity

Work lines: Effect of air pollution on individuals and populations of arthropods, mammals and birds. Biodiversity. Toxic markers include hematology, serum biochemistry, heavy metal analysis, histopathology; respiratory tract: light microscopy, transmission and scanning electron microscopy, microanalysis, image analysis. Soil pollution: single cell gel electrophoresis (Comet test) of vertebrate lymphocytes and earthworm coelomocytes.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules: implementation in course The team have previously been involved in the following validation programme of alternative methods: organochlorated determination. They are available to participate in EU validation programmes.

Sources of financing: Fuerzas Electricas de Cataluña (FECSA) / Fundació Bosch i Gimpera (1992-1995); Fundació La Caixa / Fundació Bosch i Gimpera (1993); Ministerio de Sanidad y Consumo (1994); Plan de Investigacón de Cataluña, GRQ 94-1050 (código del proyecto 02161) (1994); FECSA / Fundació Bosch i Gimpera, proyecto BG2392 (1995); Assesora DGICYT, proyecto PB94-0877 (1995); Plan de Investigación de Cataluña, 1996SGR 00072 (1996); Asesora DGICYT, proyecto PB96-0224 (1997).

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- Manuel Cascalló Piqueras, Email: cpanc@sgenz.bq.ub.es, Ld Bioquímica, Becario, FPI Grupo de Ingeniería Celular y Terapia, 80 % time devoted to altern. meth.
- Joaquín Calbó Angrill, Email: cpanc@sgenz.bq.ub.es, Ld Bioquímica, Becario, FPI Grupo de Ingeniería Celular y Terapia, 80 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and method development; it performs cell biology, molecular biology, toxicology (haematoxicity, genotoxicity, mutagenicity), diagnostics (genetic alterations in tumours) and culture methodology studies on a routine basis. Pharmaceuticals are routinely evaluated.

Model systems: The model systems used are conventional animal models (mice without thymus) and *in vitro* methods: cell lines culture (human pancreatic cell lines (NP9, NP18, NP29, NP31, etc)).
The endpoints employed are *in vivo* analysis of xenotransplant tumours treated with different protocols and *in vitro* morphology (optical and electronic microscopy), cell viability (trypan blue exclusion, MTT reduction) and cellular proliferation (cell count, WST test).

Experimental systems (examples):
1 human pancreatic cell lines: NP-9, NP-29, NP-31, NP-18 and sublines obtained by

metastasis dissemination, propidium iodide by flow cytometry, housecat by microscopy and flow cytometry, anexin-V by fluorescence microscopy and flow cytometry, new therapeutic approaches by suppressor reintroduction.

Work lines: The focus of the research is addressed to the development of new therapeutical approaches based on the combination of genotoxic drugs and the reintroduction of tumour suppressor genes (p53, p16, etc) which potentiates drug action. It is used as a model in the human exocrine pancreatic cancer.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to diagnostic methods and cancer therapy.

Sources of financing: FIS, Maratón del Cáncer (TV3), Plan Nacional de Salud CICYT

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Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in basic research and alternatives to animals in education; it performs human biology, cell biology, molecular biology, biochemistry, nutrition, culture methodology studies on a routine basis. Hormones, biomaterials and nutrients are routinely evaluated.
The main use of alternative methods is for complementary and replacement studies.

Model systems: The model systems used are conventional animal models and transgenics, *in vitro* methods -- primary culture of dispersed cells (human muscle cells), cell lines culture (muscle C2C12), and education models (cell lines, muscle,

kidney).
The endpoint employed is *in vitro* metabolic activity

Experimental systems (examples):
1 primary culture of human muscle cells, metabolism of glucose and lipids, metabolic regulation, replacement and complementary test

Work lines: Development of muscle gene therapy strategies for the treatment of diabetes and inborn myopathies. Viruses with target proteins are constructed and their impact is tested in a primary cultured human muscle model and *in vivo* rodent models.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: CICYT, BIOMED2 Programme

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- Ignacio Díez Martín, Dr Farmacia, Prof. Asociado de Universidad 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 8 people.

Activities / aims: This university department is mainly involved in non-regulated applied research, method validation and alternatives to animals in education; it performs biokinetics, biotransformation and evaluation studies of pharmaceuticals by transdermal route testing on a routine basis. Pharmaceuticals and colourings are routinely evaluated; diverse chemical compounds also being occasionally studied.

Model systems: The model systems used are education models (flux) and mathematical modelling (exponential, linear and non-linear).

Work lines: Transdermal delivery systems, dissolution tests, PK-PD, evaluation of pharmaceutical dosage forms.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: Proyectos de colaboración universidad/empresa (Fundació Bosch-Gimpera), Plan Nacional de I+D

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- Jorge Camarasa García, Email: camarasa@far.ub.es, Dr Farmacia, Catedrático de Farmacología, duties related to altern. meth.: Representante Español en Meeting sobre simulaciones en docencia
- Elena Escubedo Rafa, Email: escubedo@far.ub.es, Dr Farmacia, Profesora Titular de Farmacología.

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in basic research and alternatives to animals in education; it performs pharmacodynamics, pharmacology (molecular pharmacology) and toxicology (neurotoxicity) testing on a routine basis, and molecular biology studies at a research level. Pharmaceuticals and toxins are routinely evaluated.

Model systems: The model systems used are conventional animal models , *in vitro* methods -- primary culture of dispersed cells (rat cerebellar granular cells) and education models (pharmacodynamics simulations).
The endpoints employed *in vitro* are cell viability and cytoskeleton/membranes/enzyme release.

Experimental systems (examples):

1 cerebellar granular cells, propidium iodide, rhodamine 123, neuronal physiology.

Work lines: Study of molecular and cellular aspects on neuronal cell death. Study of different biochemical cell markers, mainly heat shock protein expression, on neurodegerative animal models. Study of the interaction between microglial and lymphocyte cells that can led to neuronal death.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: CICYT, Universidad de Barcelona, private laboratories

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Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in basic research, regulatory testing and alternatives to animals in education; it performs toxicology (ocular, dermal irritation and corrosivity) and cell biology and biochemistry stydies at a research level. Surfactants are routinaly evaluated; diverse chemical compounds also being occasionally studied.

Model systems: The model systems used are chicken embryo chorioalantoid membrane assay (HET-CAM), CAM-TBS Test, RBC test system, RBC photo assay-photohaemolysis and haemoglobin oxidation, haemoglobin denaturation test. The endpoints employed are lesions to blood vessels (CAM), erythrocyte haemolysis and haemoglobin denaturation.

Experimental systems (examples):
1. Chick embryo chorioallantoid membrane assay, blood vessel lesions, ocular irritation, replacement test.
2. Erythrocyte haemolysis to quantify adverse effects of surfactants and detergent products on the cytoplasmic membrane in combination with the damage of liberated

cellular proteins, ocular and dermal irritation replacement test.
3. Phototoxic potentials of chemicals by their ability to disturb the erythrocyte membrane under UV irradiation as a replacement test.

Work lines: The focus of the research is addressed to the development of new in vitro methods to investigate the potential ocular and dermal irritation of surfactants and chemicals.

Quality assurance / Validation programmes: The Quality Assurance Unit of the Barcelona University is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU and OECD. The team are available to participate in EU validation programmes.

Sources of financing: Proyectos de colaboración universidad/empresa (Fundación Bosch-Gimpera), Programa Nacional de Tecnología de Procesos Quimicos CICY

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Grupo de Biología Molecular y Celular de Ciliados

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- Silvia Díaz, Email: jgf00004@teleline.es, Ld Biología, Estudiante Interno .

Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and method development; it performs cell biology, molecular biology, toxicology (mechanisms of toxicity, basal cytotoxicity, chronic toxicity, ecotoxicity) and monitoring -chemical, biological- testing on a routine basis and physiology and genetics studies at a research level. Toxins, environmental pollutants and wastes are routinely evaluated; pesticides also being occasionally studied.

The main use of alternative methods is for screening.

Model systems: The model systems used are vegetables (microalgae) and *in vitro* methods: micro-organisms (ciliated protozoa).
The endpoints employed *in vitro* are morphology, cell viability, cytoskeleton/membranes/enzyme release, cellular proliferation, metabolic activity, nucleic acids and defence systems.

Experimental systems (examples):
1 Tetrahymena thermofila, growth on microplates, by spectrophotometry, toxicity tests of micotoxins, enterotoxins, toxic, replacement test
2 ciliated (*Colpoda steinii* and other species), growth on microplates, using flourochromes, toxicity of heavy metals, replacement test

Work lines: Tetrahymena thermophila as a biosensor to detect mycotoxins and enterotoxins. Ciliated protozoa as bioindicators of heavy metals in soil and aquatic ecosystems.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: DGICYT, Projects CAM, Projects EU, Projects UCM

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Staff:

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Total staff involved in alternative methods is 1 person.

Activities / aims: This university department is mainly involved in basic research, method development, method validation and alternatives to animals in education; it performs biochemistry, pharmacology, physiology, toxicology (reproductive cytotoxicity) studies and production (animal) on a routine basis and molecular biology, biokinetics and biotransformation, toxicology (mechanisms of toxicity, acute systemic toxicity, ocular cytotoxicity, nephrotoxicity, immunotoxicity and sensitisation, haematoxicity, hepatotoxicity), nutrition and culture methodology studies at a research level. Hormones and food additives are routinely evaluated; vaccines and wastes also being occasionally studied.

The main use of alternative methods is for screening.

Model systems: The model systems used are conventional animal models and *in vitro* methods (organ culture).

Work lines: Endocrinology. Food Additives. Reproduction. Immunosupression.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the FDA. The team are available to participate in EU validation programmes in relation to toxicology

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Total staff involved in alternative methods is about 1 person.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and methods development; it performs biochemistry, toxicology (mecanisms of toxicity, ecotoxicity) and monitoring --chemical, biological- studies on a routine basis. Diverse chemical compounds, pesticides and environmental pollutants are routinely evaluated.

The main use of alternatives methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models (wild mouse), invertebrates (bivalve), *in vitro* methods -- cell lines culture (Vero monkey kidney cells).

The endpoints employed are *in vitro* biotransformation systems (glutathion-S-transferase, EROD, GOR) and defence systems (antioxidative enzymes).

Experimental systems (examples):

1 Vero monkey kidney cells, neutral red uptake, MTT reduction, cell proliferation, antioxidative and biotransformation enzymes, biological effects of environmental pollutants

wild mouse liver, antioxidative and biotransformation enzymes, biological effects of environmental compounds

Work lines: The development and use of *in vitro* methods applied to different materials and areas, particularly the investigation of environmental chemicals induced oxidative stress using Vero monkey kidney cells.

Sources of financing: CICyT, Junta de Andalucia, EU

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- Mariana Fernández Cabrera, Ld Ciencias Químicas, Becaria Fundación HUSC

Total staff involved in alternative methods is 12 people.

Activities / aims: This university department is mainly involved in basic research and non-regulated applied research; it performs cell biology, molecular biology, biochemistry, toxicology (endocrine disruption, chronic toxicity), monitoring -chemical, biological- and culture methodology studies on a routine basis. Hormones, diverse chemical compounds, pesticides, environmental pollutants and wastes are routinely evaluated; pharmaceuticals, cosmetics, medical devices and biomaterials also being occasionally studied.
The main use of alternative methods is for screening.

Model systems: The model systems used are *in vitro* methods: cell lines culture (MCF7).
The endpoints employed *in vitro* are cell viability, cellular proliferation and metabolic activity (MTT reduction/SRB).

Experimental systems (examples):
1 E-Screen. MCF7 Cells in culture, cell proliferation, assessment of the total xenoestrogen burden, replacement test
2 MCF7 cells, expression of pS2, estrogenicity
3 MCF7 cells, expression of ER and PgR, estrogenicity
4 Uterine test, organ weight, estrogenicity

Work lines: Endocrine disrupting chemicals (EDCs). Identification of chemicals with hormonal activity. Identification of inadvertent exposure pathways to EDCs in humans. Identification of new chemicals with estrogenic/antiestrogenic activity. Assessment of the total xenoestrogen burden in human samples. Breast cancer and xenoestrogens. Cryptorchidism and male reproductive defects and EDCs. Bisphenols as EDCs.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU, OECD and the FDA. The team have previously been involved in the following alternative method validation programme: collaborative studies of *in vitro* tests for estrogenicity 1997-98. They are available to participate in EU validation programmes in relation to test validation programmes for estrogenicity and endocrine disruption

Sources of financing: UE PL5-1129/CICYT, FIS 1959-95, JA 140/94; 159/96; 231/97

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- Belén Santiago Josefat, Email: sjosefat@unex.es, Ld Ciencias Biológicas, Becaria Predoctoral

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in basic research; it performs cell biology, molecular biology, biochemistry and toxicology (mechanisms of toxicity, carcinogenicity) testing on a routine basis.

Model systems: The model systems used are transgenic animal models (Knock-out), embryos (mouse GD13.5-15.5), *in vitro* methods -- micro-organisms (*E. coli* for cloning and expression of recombinant genes), primary culture of dispersed cells

(mouse neurones, fibroblasts, epithelial glands), cell lines culture (fibroblast: NYH3T3, Swiss 3T3) and cell-free systems (cytosolic, nuclear, microsomes).
The endpoints employed are *in vivo* development of breast gland: cellular and molecular levels (receptors) and in vitro cell morphology (optical and electronic microscopy), cell viability (Trypan blue exclusion; MTT reduction), cellular proliferation (Tyd uptake), cell signalling (inhibitors of MAP and SAP kinases on genes regulated by AHR) and nucleic acids (condensation and fragmentation).

Experimental systems (examples):
1 fibroblast cell lines NIH 3T3, transfection with luciferase and galactosidase, gene regulation at transcription level, complementary test
2 primary culture of mouse cells, viability, proliferation, nucleic acids, role of AHR on proliferation and carcinogenesis, complementary test
3 slices of tissues from cerebella and breast gland, *in situ* hybridization transmission electronic microscopy, levels of gene expression related to AHR, complementary test

Work lines: Role of the aryl hydrocarbon receptor (AHR) in mouse mammary gland carcinogenesis: interaction between AHR and estrogen receptor. Implication of the AHR in viability of cerebellar granule cells: regulation of cytochrome P450s that mediate reactive oxygen species formation and nitrate oxide synthase-derived-nitrogen reactive species.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules. The team are available to participate in EU validation programmes in relation to method development for the identification of genes useful as tumour markers.

Sources of financing: Junta de Extremadura 1997-1999,

Others: Newly formed group

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Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in basic research and alternatives to animals in education; it performs toxicology (nephrotoxicity) and pathology testing on a routine basis, and physiology studies at a research level.

Model systems: The model systems used are conventional animal models (rodents, dogs) and education models (audiovisuals, mechanical models).

Work lines: Design and development of human disease animal models in nephrology and mineral metabolism. Teaching and training of the basic principles of animal experimentation.

Sources of financing: Junta de Castilla y León

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Staff:

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Total staff involved in alternative methods is 8 people.

Activities / aims: This university department is mainly involved in basic research and non-regulated applied research; it performs cell biology testing on a routine basis and molecular biology studies at a research level.

Model systems: The model systems used are *in vitro* methods -- primary culture of dispersed cells (rat and human bone marrow) and human volunteers (bone implantation).
The endpoints employed *in vitro* are morphology (histology, histochemistry, immunocytochemistry, etc.), cellular
proliferation (BrdU, PCNA), metabolic activity (bone formation) and biotransformation systems (biomaterials for bone formation).

Experimental systems (examples):
1 bone marrow cell cultures, bone marrow formation, osteogenic capacity *in vitro*

Work lines: Regeneration in fish. Bone regeneration and bone repair. Osteogenesis

and growth factors.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Sources of financing: DGYCIT, FIS, MAPFRE, Junta de Andalucía (PAI)

Universidad Miguel Hernández
Instituto de Bioingeniería
Unidad de Toxicología y Seguridad Química
Campus de San Juan
03550 San Juan
Tel: 965919477, Fax: 965919484, Website: http:// tox.umh.es

Type of Institution: University

Staff:

- Eugenio Vilanova Gisbert, Email: evilanova@umh.es, Dr Ciencias Químicas, Catedrático, 40 % time devoted to altern. meth., duties related to altern. meth.: Miembro Comisión Promotora de REMA, Presidente Asociación Española de Toxicología
- José Barrilo Antuña
- Maricruz Pellin Mira
- Victoria Carrera González
- Miguel Angel Sogorb
- Maria A. Escudero
- Mónica Bernabeu
- Noelia Ñiguez
- Jorge Estevez
- Adolfo Monroy

Total staff involved in alternative methods is 10 people.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and alternatives to animals in education; it performs biochemistry, biokinetics and biotransformation and toxicology (mechanisms of toxicity, neurotoxicity, ecotoxicity) testing on a routine basis. Pesticides, drugs of abuse, environmental pollutants, wastes and solvents are routinely evaluated.

Model systems: The model systems used are conventional animal models and invertebrates (Daphnia), *in vitro* methods -- primary culture of dispersed cells (bovine chromaffine cells), cell-free systems (membranes, cytosolic), education models (data bases, multimedia) and mathematical modelling (toxicokinetics).

The endpoints employed are *in vivo* general observation, walking alterations, electrophysiology, histopathology, enzyme modifications) and *in vitro* cell viability, biotransformation systems, neurotransmitter secretion and enzyme inhibition.

Experimental systems (examples):
1 chick and human lymphocytes, bovine chromaffine cells, catecholamine secretion, protein phosphorylation ionic current, esterases, mechanisms and effects of esterases inhibition by organophosphate, replacement test
2 Daphnia, enzyme inhibition, death, ecotoxicity model for waste water, replacement test
3 adult hen, locomotion, reflex, enzyme inhibition, delayed neurotoxicity, drastic reduction in animal use
4 slaughterhouse material: extracts from nervous tissue and peripheral nerves, esterase organophosphorylation kinetics, toxic molecular mechanisms, reduction

Work lines: Biotransformation of organophosphorus pesticides; mechanism and role of phosphotriesterases. Characterization of esterases as target for the induction and promotion of toxic neurodegenerative syndromes. The characterization and role of the so called soluble-neuropathy target esterase (S-NTE). Chromaffin cells as model for studying mechanism of molecular interaction and organophosphorus compounds toxicity. Characterization of *Daphnia magna* as model for testing pesticides and other environmental pollutants ecotoxicity. Toxicological evaluation of agrochemical and of industrial solvents.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: FIS, CICYT, industries

Universidad de Murcia
Facultad de Veterinaria
Departamento de Farmacología
Campus de Espinardo
30071 Espinardo-- Murcia
Type of Institution: University

Staff:

- Carlos M. Carceles Rodríguez, Email: carceles@fcu.um.es, Dr Veterinaria, Prof Titular
- Elisa Escudero Pastor, Email: escudero@fcu.um.es, Dra Veterinaria, Prof.Titular U

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in non-regulated applied research; it performs biokinetics and biotransformation testing on a routine basis. Pharmaceuticals are routinely evaluated; wastes also being occasionally studied.
Model systems: The model systems used are conventional animal models and *in vitro*

methods -- micro-organisms (*Hicrococus luteus*).

Work lines: Pharmacokinetics of drugs (antibiotics and anthelmintics) in animals

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to the EU. The team have previously been involved in alternative method validation programmes

Sources of financing: CICYT

Universidad de Murcia
Facultad de Veterinaria
Departamento de Patología Animal (Medicina Animal)
Campus Universitario de Espinardo
30071 Espinardo-- Murcia
Tel: 968363000, Fax: 968364147

Type of Institution: University

Staff:

- Fernando Tecles Vicente, Ld Veterinaria, Becario.
- Jose Joaquín Cerón Madrigal, Email: jjceron@fcu.um.es, Dr. Veterinaria, Profesor Titular.
- Cándido Gutierrez Panizo, Email: cguti@cu.um.es, Dr Veterinaria, Catedrático del Departamento de Patología Animal.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research; it performs toxicology (ecotoxicity) testing on a routine basis. Pesticides and environmental pollutants are routinely evaluated.
The main use of alternative methods is for complementary studies.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- primary culture of dispersed cells (chick embryo neuroblasts).
The endpoints employed are *in vivo* haematology, organic metabolites, blood enzyme activities and *in vitro* metabolic activity (cholinesterase).

Experimental systems (examples):
1 chick embryo neuroblasts culture, cholinesterase inhibition, by spectrophotometry, toxicity of pesticides, complementary test

Work lines: Haematological and biochemical effects of environmental contaminants in animals. Analytical techniques applied in environmental biomonitoring.

Universidad de Murcia
Facultad de Veterinaria y de Medicina
Departamento Toxicología
Toxicología Clínica, Forense, Ambiental y de Residuos
Campus de Espinardo
30100 Murcia
Tel: 968363957, Fax: 968364338

Type of Institution: University

Staff:

- Antonio Juan García Fernández, Email: ajgf@fcu.um.es, Dr Veterinaria, Profesor Titular de Toxicología, 50 % time devoted to altern. meth.
- Diego Romero García, Dr Veterinaria, Contrato de Investigador, 100 % time devoted to altern. meth
- Maximiliano Gómez Zapata, Dr Medicina y Cirugía, Profesor Titular, 100 % time devoted to altern. meth.
- Miguel Motas Guzmán, Email: mmotas@fcu.um.es, Ldo Veterinaria, Becario FPI, 75 % time devoted to altern. meth.
- Pedro José Mojica, Dr Veterinaria, Investigador, 75 % time devoted to altern. meth.
- Isabel Maria Navas Ruiz, Lda Veterinaria, Investigadora, 50 % time devoted to altern. meth.

Total staff involved in alternative methods is 6 people.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research, method development and alternatives to animals in education; it performs toxicology (mechanisms of toxicity, basal cytotoxicity, nephrotoxicity) and monitoring --chemical, biological- studies on a routine basis, and toxicology (ocular cytotoxicity, dermal irritation and corrosivity, reproductive cytotoxicity, immunotoxicity, sensitisation, endocrine disruption, chronic toxicity) studies at a research level. Pesticides, environmental pollutants and wastes are routinely evaluated; cosmetics also being occasionally studied.
The main use of alternative methods is for complementary and replacement studies.

Model systems: The model systems used are conventional animal models (refinement) and *in vitro* methods -- cell lines culture (BF-2, RTG-2, CHO, VERO, PK-15, BGM, EPO).
The endpoints employed *in vivo* are lipid peroxidation, glutathione and related enzymes, ALA-D, LDH and metallothioneins.

Experimental systems (examples):
1 kidney cell lines, neutral red uptake, MTT reduction and LDH, toxicity evaluation, replacement test
2 kidney cell lines, electronic microscopy, risk assessment, complementary test

Quality assurance / Validation programmes: The institution applies Good Laboratory

Practices rules according to the EU. The team are available to participate in EU validation programmes.

Universidad de Navarra
Facultad de Farmacia
Departamento de Bromatología,Tecnología de Alimentos y Toxicología
Unidad de Toxicología
Irunlarrea s/n
31008 Pamplona
Tel: 948425653, Fax: 948425652, Website: http:// www.unav.es

Type of Institution: University

Staff:

- Adela López de Cerain Salsamendi, Email: acerain@unav.es, Dra Biología, Profesora Adjunta, 10 % time devoted to altern. meth., duties related to altern. meth.: Miembro del Comité de Etica para la Experimentación animal de la UNAV
- Olga Ezpeleta Echevarri, Email: oezpeleta@unav.es, Ld Biología, Ayudante, 35 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in basic research; it performs toxicology (mechanisms of toxicity, basal cytotoxicity, genotoxicity / mutagenicity) testing on a routine basis. Toxins are routinely evaluated; pharmaceuticals, cosmetics and medical devices also being occasionally studied.
The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- primary culture of dispersed cells (rat hepatocytes, human lymphocytes), cell lines culture (V79, CHO, MDCK, HT-29, Vero, EVIT-6, MDA-468, etc.
The endpoints employed *in vitro* are cell viability (MTT reduction, neutral red uptake), cytoskeleton /membranes/enzyme release (LDH leakage), cellular proliferation (clonogenicity, total protein content), metabolic activity and biotransformation systems (S9 fractions from tissues induced for cytochromes).

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules.

Universidad del Pais Vasco
Facultad de Ciencias
Departamento de Zoología y Dinámica Celular Animal
Barrio de Sarriena s/n
48013 Bilbao-- Bizkaia
Tel: 944648800, Fax: 944648500

Type of Institution: University

Staff:

- Marta Saloña Bordas, Email: ggpsabom@lg.ehu.es, Dr Biología, Profesora, 20 % time devoted to altern. meth., duties related to altern. meth.: Responsable curso Doctorando, Coord. Zoología 1º

Total staff involved in alternative methods is 1 person.

Activities / aims: This university department is mainly involved in basic research and animals alternatives in education; it performs animal (without experimentation) biology and alternative education models on a routine basis.

Model systems: The model systems used are conventional animal models and invertebrates (arthropods, molluscs, annelid, corrodes), and education models (plastic models, fixed specimens), documentation, photographs and anatomical models.

Work lines: Soil biology / Oribatocenosis: taxonomy, population dynamics, influence on soil structure and processes. Continental waters, oceanic and estuarine systems and bat populations are studied by other department research groups, focused mainly on biogeography, stress and other environmental disturbances, taxonomy and population dynamics.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes, in relation to alternative methods development in education

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Universidad Politécnica de Cataluña
Instituto de Investigación Textil
Laboratorio de Toxicología Ambiental
Colom, 15
08222 Terrasa-- Barcelona
Tel: 937398396, Fax: 937398392

Type of Institution: University

Staff:

- Mª Carmen Riva Juan, Email: riva@iitcit.upc.es, Dr Ciencias Biológicas, Investigador. Jefe de Laboratorio, 25 % time devoted to altern. meth.
- David López Ribas, Email: toxicologia@rackham.upc.es, Ld Ciencias Biológicas, 30 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in non-regulated applied research, regulatory testing and animals alternatives in education; it performs toxicology (ecotoxicity), monitoring -chemical, biological- and quality control studies on

a routine basis, and toxicology (basal cytotoxicity, acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity, hepatotoxicity, chronic toxicity) studies at a research level. Diverse chemical compounds, pesticides, toxins, biomaterials, food additives, colourings, environmental pollutants and wastes are occasionally evaluated. The main use of alternative methods is for screening.

Model systems: The model systems used are *in vitro* methods -- primary culture of dispersed cells and cell lines culture (L-929 mouse), RTG-2O (*Onconhychus mykiss*) The endpoint employed *in vitro* is cell viability.

Experimental systems (examples):
1 RTG-2 *Onconhychus mykiss,* neutral red uptake, total protein content, cytotoxicity, screening
2 L-929 (mouse), cell proliferation, total protein content, cytotoxicity, screening

Work lines: Ecotoxicity, xenobiotic assessment, physiological and histopathological effects. Effects of environmental pollutants on the health of human and aquatic organisms. Bioaccumulation studies. *In vivo* and *in vitro* toxicity tests.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to in-phase of accreditation of 45001. The team are available to participate in EU validation programmes.

Sources of financing: Projects CICYT, MINER, CIRIT, etc.. Contracts with industries related to environmental studies.

Universidad Rovira i Virgili
Facultad de Medicina y Ciencias de la Salud
Departamento de Ciencias Médicas Básicas
Grupo de Farmacobiología Celular
Sant Llorenç 21
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Tel: 977759378, Fax: 977759322, Website: http:// www.urv.es
Type of Institution: University

Staff:

- Jordi Mallol Mirón, Email: jmm@fmcs.urv.es, Dr Medicina y Cirugía, Catedrático Universidad, 50 % time devoted to altern. meth., duties related to altern. meth.: Director de Proyectos
- Jose Luis Paternain Suberviola, Email: jlps@fmcs.urv.es, Dr Biología, Titular Universidad, 75 % time devoted to altern. meth., duties related to altern. meth.: Director de Proyectos
- Montserrat Giralt Batista, Email: mgb@fmcs.urv.es, Dr Medicina y Cirugía, Titular Universidad, 40 % time devoted to altern. meth., duties related to altern. meth.: Desarrollo de Métodos

- Maria Rosa Nogues Llort, Email: rnll@fmcs.urv.es, Dr Farmacia, Titular Universidad Interino, 50 % time devoted to altern. meth., duties related to altern. meth.: Desarrollo de Métodos
- Jaume Folch López, Email: jfl@fmcs.urv.es, Dr Biología, Titular Universidad Interino, 75 % time devoted to altern. meth., duties related to altern. meth.: Desarrollo de Métodos
- Maria Cabré Bargalló, Email: mc@fmcs.urv.es, Ld Biología, Profesor Ayudante, 75 % time devoted to altern. meth., duties related to altern. meth.: Desarrollo de Métodos
- Anna Torres Domingo, Email: atd@fmcs.urv.es, Ld Farmacia, Becaria URV, 100 % time devoted to altern. meth., duties related to altern. meth.: Desarrollo de Métodos

Total staff involved in alternative methods is 7 people.

Activities / aims: This university department is mainly involved in basic research, regulatory testing, method development, method validation and alternatives to animals in education; it performs cell biology, molecular biology, biochemistry, pharmacology, toxicology (basal cytotoxicity, dermal irritation and corrosivity, phototoxicity, hepatotoxicity) and monitoring --chemical, biological- studies on a routine basis. Cosmetics, environmental pollutants and physical agents are routinely evaluated; diverse chemical compounds and toxins also being occasionally studied.
The main use of alternative methods is for screening and replacement studies.

Model systems: The model systems used are conventional animal models (rats reduction) and transgenics (nude rodents), embryos (chick and rat embryo), *in vitro* methods - primary culture of dispersed cells (chick embryo fibroblasts, human kera rat hepatocytes), cell lines culture and cell-free systems- BECAM.
The endpoints employed are *in vivo* (GSH/GSSG, GST, GSHPx, GRed, SOD, CAT, TBARS, induced haemolysis), and *in vitro* cell viability (neutral red uptake, MTT reduction, Hoechst 33342), cytoskeleton / membranes / enzyme release (LDH), cellular proliferation (neutral red uptake, Hoechst 33342, propidium iodide, area measurement), metabolic activity (MTT reduction), cell signalling (quimioluminescence), biotransformation systems (CYP-450), defence systems (GST, GSH, SOD, metalothioneines), and organ-specific indicators (GOT, ALT), BECAM.

Experimental systems (examples):
1 chick embryo fibroblasts, neutral red, MTT reduction, Hoescht 33342, cell viability, replacement test
2 keratinocytes, MTT reduction, metabolic activation, screening and replacement test
3 human erythrocytes, haemolysis, free radical protection, replacement and complementary test
4 rat hepatocytes, LDH, RT-PCR, cellular response to stress, replacement test
5 transgenic line of fibroblasts, neutral red uptake, RT-PCR, toxicity of antineoplasics, replacement test
6 rat embryo, morphology and development, DNA, proteins, embryotoxicity for cadmium, replacement test

Work lines: Monitoring oxygen free-radical (OFR) production and protective systems in human blood.Testing cytotoxicity for cosmetic ingredients. Evaluation of the sun protection factor by *in vitro* and alternative techniques. Biology and cellular response against stress in rat hepatocytes: -studies in hepatocyte culture of the toxicity of

several metals and organic compounds, --cellular response and genetic expression of hepatocytes exposed to toxins, -- *in vitro* studies of hepatocyte protector action, -- studies of semichronic toxicity in co-cultures of hepatocytes and epithelial cells (1-2 weeks), --studies of cellular response to inflammation. Co-cultures of hepatocytes and macrophages. Drug resistance to chemotherapics: studies of toxicity of chemotherapics in transfected cells with over, and under-expression of metallothioneine, cellular response and genetic expression from transfected cells to chemotherapics, induction to drug resistance to chemotherapics by coadyuvant therapy, and studies of resistance mechanisms.

Quality assurance / Validation programmes: The institution applies Good Laboratory Practices rules according to OECD. The team are available to participate in EU validation programmes in relation to cell culture, erythrocytes, lymphocytes and macrophages.

Sources of financing: FISS: 93/322. Estudio del perfil glutatión S-transferasa en piel humana, normal y tumoral. Generalitat de Catalunya 1995SGR/339. Infraestructura para laboratorio de Farmacobiología cellular y desarrollo de métodos alternativos. NOVARTIS C.H. Fundacion Bosch i Gimpera: contratos de investigación para valorar productos cosméticos, mediante técnicas alternativas, entre otras. Generalitat de Catalunya: PIR98, infraestructura para laboratorio de Farmacobiología cellular.

Universidad San Pablo-CEU

Facultad de CC Experimentales y Técnicas

Departamento de Ciencias Biomédicas

Citología-Histología

Urbanización Montepríncipe

28668 Boadilla del Monte-- Madrid

Tel: 913724761, Fax: 913510475, Website: http:// www.ceu.es/

Type of Institution: University

Staff:

- Jose Manuel Pozuelo Gonzalez, Email: jpozuelo@ceu.es, Dr CC Biológicas, Prof. Adjunto, 30 % time devoted to altern. meth.

Total staff involved in alternative methods is 1 person.

Activities / aims: This university department is mainly involved in basic research and non-regulated applied research; it performs pharmacodynamics and toxicology testing on a routine basis, and toxicology (mechanisms of toxicity, genotoxicity / mutagenicity, ecotoxicity) studies at a research level. Vegetable products are routinely evaluated; pharmaceuticals, cosmetics and environmental pollutants also being occasionally studied.
The main use of alternative methods is for screening.

Model systems: The model systems used are *in vitro* methods -- cell lines culture (CHO, HEPG2)

The endpoints employed are *in vitro* cell viability

Experimental systems (examples):
1 CHO cell line, neutral red uptake and MTT reduction, cytotoxicity, screening

Work lines: The study of cytotoxic effects of natural products (plants extracts) and antineoplastic agents with different endpoints.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes.

Sources of financing: Universidad San Pablo-CEU

Universidad San Pablo CEU
Facultad de Ciencias Experimentales y Técnicas
Departamento de Ciencias Biomédicas
Sección de Farmacología-Toxicología
Urb. Montepríncipe Ctra. Boadilla del Monte Km 5,3
28668 Boadilla del Monte-- Madrid
Tel: 913724787, Fax: 913510475
Type of Institution: University

Staff:

- Carmen Pérez García, Email: capegar@ceu.es, Ld Farmacia, Profesora de Toxicología, 5 % time devoted to altern. meth.
- Lidia Morales Goyanes, Email: lmorgoy@ceu.es, Ld Ciencias Biológicas, Profesora de Farmacología, 1 % time devoted to altern. meth.
- Luis Fernando Alguacil Merino, Email: laguacil@ceu.es, Dr CC Biológicas, Profesor Agregado de Farmacología.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research; it performs pharmacodynamics and toxicology testing on a routine basis and toxicology studies at a research level. Pharmaceuticals are routinely evaluated.
The main use of alternative methods is for screening.

Model systems: The model systems used are conventional animal models.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to the EU. The team are available to participate in EU validation programmes.

Universidad de Sevilla

Centro de Producción y Experimentación Animal
Dto. Experimentación Animal
Urb. Espartinillas
41807 Espartinas-- Sevilla
Tel: 95 5711208, Fax: 95 5711264
Type of Institution: University

Staff:

- C. Oscar Pintado Sanjuán, Email: cpiea@svq.servicom.es, Dr Veterinaria, Director Técnico del Servicio de Animales de experimentación, duties related to altern. meth.: Member of ethical Commitee Universidad de Sevilla

Total staff involved in alternative methods is 1 person.

Activities / aims: This University department is mainly involved in basic research; it performs on a routine basis cell biology, molecular biology, biochemistry, pharmacodynamics, pharmacology, physiology, biokinetics and biotransformation, toxicology and surgery studies and at a research level toxicology (genotoxicity / mutagenicity, carcinogenicity, hepatotoxicity, endocrine disruption, chronic toxicity), genetics, production, nutrition, diagnostics and monitoring -chemical, biological- studies.

Model systems: The model systems used are conventional animal models (rat, mouse and rabbit SPF), transgenics- and *in vitro* methods - cell lines culture (embryo).

Work lines: Animal breeding, laboratory animal pathology, policlonal antibodies, monoclonal antibodies, transgenic, knock out, wistar rat, WKY rat, SHR rat, C57BL/6 mouse, Swiss mouse, rabbit.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to research.

Sources of financing: New centre created with Fondos FEDER, University, and for research projects.

Others: Interest in alternative methods applied to basic research, for the promotion of alternatives in the animal room facilities of the university.

Universidad de Sevilla
Facultad de Biología
Departamento de Biología Celular
Cultivo Celular y Radiobiología
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41012 - Sevilla
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Type of institution: University

Staff:

2 Joaquín Piñero Bustamante, Email: pinero@cica.es, Dr Biología, Profesor Titular de Universidad, Biología Celular, 50 % time devoted to altern. meth.

3 Trinidad Ortiz Salles, Email: salles@cica.es, Dr Biología, Profesor Titular de Biología Celular, 50 % time devoted to alt. met.

4 Felipe Cortés Benavides, Email: cortes@cica.es, Dr Biología, Catedrático de Biología Celular, 50 % time devoted to altern. meth.

5 Santiago Mateos Codero, Email: smateo@cica.es, Dr Biología, Profesor Asociado Univers., 50 % time devoted to altern. meth.

6 Inmaculada Dominguez García, Email: idomin@cica.es, Dr Biología, Profesor Asociado de Univ., 50 % time devoted to altern. meth.

Total staff involved in alternative methods is about 5 persons.

Activities / aims: This university department is mainly involved in basic research; it performs cell biology and toxicology (genotoxicity / mutagenicity, carcinogenicity) studies on a routine basis. Diverse chemical compounds and physical agents are routinely evaluated, being environmental pollutants also occasionally studied.

The main use of alternatives methods is for complementary studies.

Model systems: The model systems used are *in vitro* methods -- cell lines culture (radiosensitives, tumor, wild and mutants).

The endpoints employed *in vitro* are cell viability (SRB, flow cytometry), cellular proliferation (cell count, flow cytometry), nucleic acids (degradation, apoptosis) and comet assay.

Experimental systems (examples):

1 radiosensitive and wild cell lines, RX, comet assay, lesion and repair, complementary test

2 cell lines, lesions by restriction enzymes, pulsed field electrophoresis, repair mechanisms, complementary test

3 cell lines, chromosomal aberrations, DNA lesions and repair, complementary test

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Universidad de Sevilla

Facultad de Farmacia

Departamento de Bioquímica, Bromatología, Toxicología y Medicina Legal

Area de Toxicología.

C/ Profesor García González s/n

41012 Sevilla

Tel: 954556762, Fax: 954233765

Type of Institution: University

Staff:

- Ana Mª Cameán Fernández, Email: camean@fafar.us.es, Dr Farmacia, Profesor Titular Universidad, 30 % time devoted to altern. meth.
- Olga Caneo López, Email: caneo@fafar.us.es, Ld Química, Becaria , 30 % time devoted to altern. meth.
- Manuel Repetto Jiménez, Dr Ciencias y Medicina, Profesor Universidad, 10 % time devoted to altern. meth.

Total staff involved in alternative methods is 3 people.

Activities / aims: This university department is mainly involved in basic research; it performs toxicology (hepatotoxicity) testing on a routine basis. Toxins and environmental pollutants are occasionally evaluated.
The main use of alternative methods is for complementary studies.

Model systems: The endpoints employed *in vitro* are cell viability (MTT), and cytoskeleton/membranes/enzyme release (LDH release).

Work lines: Development of analytical methods for the determination of trace metals in biological and environmental samples, food and beverages. Development of chromatographic methods for the determination of toxins from cyanobacteria algae in water and other matrices (fish, molluscs). *In vitro* assays to study the toxic properties of microcystins.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team have previously been involved in alternative method validation programmes. They are available to participate in EU validation programmes.

Sources of financing: Toxicología de metales, Junta de Andalucia; Aplicaciones de las estratégicas quimioterápicas y en especial de las funciones hiperbólicas al estudio de los equilibrios simultáneos y estimación y optimización de parámetros de relevancia y significación analítica y biomédica, CICYT; Efecto de la Hipertensión arterial sobre los sistemas de transporte de monosacáridos en intestino y riñón, FIS; Desarrollo y aplicación de métodos químicos para la determinación de microcistinas en aguas destinadas al consumo, EMASESA; Fundación Avenzoar.

Universidad de Sevilla
Facultad de Medicina
Departamento de Bioquímica Médica y Biología Molecular
Laboratorio de Diabetes Experimental
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Type of Institution: University

Staff:

- Francisco Javier Bedoya Bergua, Email: bedoya@cica.es, Dr Medicina, Prof. T.U, 100 % time devoted to altern. meth.
- Remedios Ramírez Cárdenas, Dra Farmacia, Prof. Escuela Universitaria , 100 % time devoted to altern. meth.
- Julio César Bernabé Ortiz, Ld Biología, Doctorando, 100 % time devoted to altern. meth.
- Juan Tejedo Huamán, Ld Biología, Doctorando, 100 % time devoted to altern. meth.
- Gladys Cahuana Maudo, Ld Biología.

Total staff involved in alternative methods is 5 people.

Activities / aims: This university department is mainly involved in non-regulated applied research; it performs molecular biology and biochemistry testing on a routine basis.

Model systems: The model systems used are *in vitro* methods -- cell lines culture (RINm5F cells).
The endpoints employed are *in vitro* cell viability (cell death).

Experimental systems (examples):
1 RINm5F cell line insuline producers, protein expresssion studied by immunoblotting, cell death, complementary test

Work lines: Mechanisms involved in the destruction of pancreatic ß-cells by the immune system

Sources of financing: FIS, DGCYT

Universidad de Sevilla
Facultad de Farmacia
Departamento de Farmacología
Estudio de Enfermedades Gastroentéricas
Prof. García Gonzalez s/n
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Tel: 954556720, Fax: 954233765
Type of Institution: University

Staff:

- Virginia Motilva Sánchez, Email: motilva@fafar.us.es, Dr Farmacia, Ld Biología, Profesor Titular

Total staff involved in alternative methods is 1 person.

Activities / aims: This university department is mainly involved in basic research; it performs molecular biology and pharmacological studies at a research level. Pharmaceuticals are routinely evaluated.

Model systems: The model systems used are conventional animal models and *in vitro* methods -- cell lines culture (CACO II colon cells).

Work lines: Application of natural products in gastric digestion pathology. Mechanisms of pharmaceutical gastric protection.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: "Investigación y desarrollo de nuevos fármacos útiles en patología digestiva" Programa ALFA Comisión Europea, Dirección General IB., Preproyecto; Subprograma B2 Relaciones exteriores: América Latina nº 4,0108,7, Nombre de la Red: RIMEPAD. 1997-1998; "Investigación y desarrollo de nuevos fármacos útiles en patología digestiva" Programa ALFA Comisión Europea, Dirección General IB., Preproyecto; Subprograma B2 Relaciones exteriores: América Latina nº6,0210,8, RIMEPAD 1998-1999; "Mechanisms involved in the gastroprotective effect mediated by Ibuprofen/L-arginine" Proyecto de Investigación Universidad de Sevilla-Laboratorios Zambon S.A. (Zambon Group), 1996-1998; "Mecanismos patogénicos de las lesiones gastrointestinales inducidas por dexketoprofeno vs ketoprofeno racémico. Modificaciones de las funciones absortivas del intestino delgado proximal" 1997-1998; "Mecanismos implicados en los efectos de metamizol sobre la mucosa gástrica" Proyecto de investigación Universidad de Sevilla-Laboratorios Boehringer Ingelheim, 1997-1998; Programa de Investigación y desarrollo Tecnológico de la Junta de Andalucía. Grupo de Investigación, Facultad de Farmacia Universidad de Sevilla. Area 30, Código 3119, 1988-92 y 1993-94; Programa de Investigación y Desarrollo Tecnológico de la Junta de Andalucía. Grupo de Investigación Mecanismos implicados en la gastropatia yatrogénica. Facultad de Farmacia Universidad de Sevilla. Area 30 Código CTS259, 1995-1997.

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Type of Institution: University

Staff:

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Universidad, 80 % time devoted to altern. meth., duties related to altern. meth.: Investigador Principal Proyecto CICyT

- Rosa María Villanueva Camañas, Email: rosa.m.villanueva@uf.es, Dr, Profesor Titular Universidad, 80 % time devoted to altern. meth., duties related to altern. meth.: Investigador Proyecto Investigación CICyT
- Salvador Sagrado Vives, Email: salvador.sagrado@uf.es, Dr, Profesor Titular Universidad, 80 % time devoted to altern. meth., duties related to altern. meth.: Investigador Proyecto Investigación CICyT
- Yolanda Martín Biosca, Email: yolanda.martin@uf.es, Dr, 80 % time devoted to altern. meth., duties related to altern. meth.: Investigador Proyecto Investigación CICyT
- Jose María Sanchis Mallol, Dr, 50 % time devoted to altern. meth.
- Laura Escuder Gilabert, Doctoranda, 100 % time devoted to altern. meth.
- Mónica Molero Monfort, Doctoranda, 100 % time devoted to altern. meth.
- Carmen Quiñones Torrelo, Doctoranda, 100 % time devoted to altern. meth.

Total staff involved in alternative methods is 8 people.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and method development; it performs biokinetics and biotransformation and toxicology testing on a routine basis. Pharmaceuticals are routinely evaluated.

Model systems: The model systems used are mathematical modelling (liner/non-linear regression, chromatographic retention).
The endpoints employed *in vitro* are studied by HPLC

Experimental systems (examples):
1 micellar liquid , chromatography

Work lines: Quantitative retention-structure (hydrophobicity), retention-activity, retentition-pharmacokinetic studies by micellar liquid chromatography. Development and validation of empirical models used in prediction of new molecule properties/activities.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: Proyecto CICyT Plan Nacional de Salud y Farmacia: SAF 96-1709

Universidad de Valencia
Facultad de Medicina
Departamento de Bioquímica y Biología Molecular
Centro de Citometría y Citogenética

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Type of institution: University

Staff:

- José Enrique O´Connor Blasco Email: jose.e.oconnor@uv.es, Dr Farmacia, Profesor Titular de Universidad, 50 % time devoted to altern. meth.
- Guadalupe Herrera Martín, Email: jose.e.oconnor@uv.es, Lda en Ciencias Biológicas, Becaria de Investigación, 100 % time devoted to altern. meth.
- Efrain Huerga Pérez, Ldo en Bioquímica, Becario de Investigación, 50 % time devoted to altern. meth.

Total staff involved in alternative methods is about 3 persons.

Activities / aims: This university department is mainly involved in basic research, non-regulated applied research and development of methods; it performs cell biology, biochemistry, toxicology (mechanisms of toxicity, basal cytotoxicity, neurotoxicity, genotoxicity / mutagenicity) testing on a routine basis, and diagnostics (immunological or coagulation alterations) and monitoring --chemical, biological- studies at a research level. Diverse chemical compounds are routinely evaluated; pesticides and toxins are also ocassionally evaluated.

The main use of alternatives methods is for screening, complementary and replacement studies.

Model systems: The model systems used are *in vitro* methods -- micro-organisms (WP2 modified *E coli*), primary culture of dispersed cells (human and rat isolated lymphocytes), cell lines culture (N13 rat hepatoma, Neuro-2a mouse neuroblastoma cells, L-132 human lung fibroblasts, human Jurkat leukemic, MOLT-4) and cell-free systems (isolated nuclei or mitochondria).

The endpoints employed are *in vitro* morphology (flow cytometry, laser dispersion), cell viability (flow cytometry by fluorochromes uptake), cytoskeleton / membranes / enzyme release (flow cytometry), celular proliferation (flow cytometry by DNA quantification and BrDU uptake), metabolic activity (mitochondrial activity, lipids, free-radicals), cell signalling (Ca^{+2} movements, pH, membrane potential), nucleic acids (aneuploidia, micronuclei, mutagenesis, SCE) and biotransformation systems (gluthation, GST, CYP450).

Experimental systems (examples):
1 WP2 *E coli* with mutations on oxidative stress control genes, H_2O_2 intracellular levels, gluthation, detection and quantification oxidative stress, screening and replacement test
2 cell lines culture: Neuro-2a, N13 rat hepatoma, cell viability, cytoplasmic and

mitochondrial membrane potential, intracellular calcium levels, lipids, superoxide, peroxide, GSH, cell cycle, DNA ploidia, *in vitro* cytotoxicity (neurotoxicity, hepatotoxicity), screening and replacement studies

3 human lymphocytes culture, early (CD69) or late (CD25) antigen expression, adhesion molecules, flow cytometry, *in vitro* or *ex vivo* immunotoxicity study, screening and complementary studies

Work lines: The group is involved in the development of flow cytometric assays based upon functional parameters (signaling, metabolism, cell proliferation) to detect and quantify cytotoxicity using prokaryotic and eukariotic organisms as indicators / targets.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: Fondo de Investigaciones Sanitarias, Consellería de Educación, Izasa, S. A, C. E. Durviz, S.L, Ingelheim Diagnóstica y Tecnológica

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- Rubén Seara Escudero, Ld CC Químicas, Doctorando.

Total staff involved in alternative methods is 4 people.

Activities / aims: This university department is mainly involved in basic research; it performs toxicology (immunotoxicity and sensitisation, endocrine disruption) testing on a routine basis and biokinetic and biotransformation studies at a research level. Hormones and metals are routinely evaluated.
The main use of alternative methods is for complementary and replacement studies.

Model systems: The model systems used are conventional animal models and *in vitro*

methods -- primary culture of dispersed cells (hepatocytes, splenocytes).
The endpoints employed are *in vivo* parameters for characterizing the secretion of hormones from adenohypophysis, catecholamines and indolamine levels and their metabolites in different brain regions and *in vitro* cell viability (LDH, trypan blue), cellular proliferation (ODC) and organ-specific indicators (lymphocyte phenotype).

Experimental systems (examples):
1 rat lymphocyte culture, lymphocyte subpopulations by flow cytometry, cytokines and interferon, by ELISA, immunotoxicity of heavy metals and pesticides, replacement test
2 primary cultures of trout hepatocytes, cell viability by trypan blue, and LDH leakage, protection of Zn and Se of the toxicity produced by Cd, complementary test
3 primary cultures of trout hepatocytes, determination of metabolites by HPLC with BR detector., study of biotransformation of xenobiotics, complementary test

Work lines: Neuroendocrine toxicology, immunotoxicology, toxicology of metals.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes in relation to the V Programme on Health and Nutrition

Sources of financing: "Mejora de la producción piscícola: influencia de la contaminación medioambiental por cadmio" Fundación La Caixa, 1995; "Estudio *in vitro* de la acumulación de xenobioticos medioambientales utilizando el hepatocito como modelo cellular", Conselleria de Educación y Ordenación Universitaria de la Xunta de Galicia, 1992-1994 "Cronofarmacología de la ciclosporina", Sandoz, Pharma, SAE, 1994-1995 ; Liberación de octreotida en el sistema nervioso central" Sandoz, Pharma, SAE 1994-1995; Mecanismo de acción del interferon gamma en los ganglios linfáticos submaxilares. Papel de la inervación autonómica" DGICYT PB-94-260, 1995-1998; Efectos del cadmio sobre la secreción adenohipofisaria: papel del sistema inmunológico y neuromoduladores hipotalámicos" Xunta de Galicia, 1996-1998

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- Justo Martín Martín, Email: spinola@cece.es, Biólogo Especialidad Botánica y Zoología, Profesor Titular.
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- Manuela Segovia, Email: spinola@cece.es, Ld. Estadística, Responsable del área de análisis estadísticos.
- Manuel Jiménez López, Ldo Veterinaria, Responsable del área de patología.

Total staff involved in alternative methods is 7 people.

Activities / aims: This university department is mainly involved in regulatory testing and alternatives to animals in education; it performs toxicology (neurotoxicity, ecotoxicity), pathology, diagnostics, monitoring --chemical, biological studies at a research level. Pesticides, toxins and environmental pollutants are routinely evaluated; diverse chemical compounds and wastes also being occasionally studied.

Model systems: The model systems used are invertebrate conventional animal models, vegetables, *in vitro* methods -- micro-organisms, education models and mathematical modelling.
The endpoints employed *in vivo* are applied to sea water invertebrates and birds of prey.

Work lines: The team is basically focussed on the long term effects of pesticides and pollutants in the ecosystem, and would like to restrict the research to protected areas or utterly degraded zones.

Quality assurance / Validation programmes: The team are available to participate in EU validation programmes.

Sources of financing: Self financing

Others: The team has just started work, after preparing the study basis.

OTHER COUNTRIES:

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Departamento de Toxicología
Toxicología In Vitro
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ARGENTINA

Type of Institution: Industrial (pharmaceutical, Asoc. Banco Argentino de Células)

Staff:

- Susana García Franco*,* Email: susa@fartox.edu.ar, Dr Químico Biólogo, Jefe de Toxicología Genética, 50 % time devoted to altern. meth., duties related to altern. meth.: Jefe Toxicología In Vitro
- Baltasar Serrano*,* Email: bos@fartox.edu.ar, Técnico Químico y Farmaceútico.

Total staff involved in alternative methods is 4 people.

Activities / aims: This industrial department (pharmaceutical, Asoc. Banco Argentino de Células) is mainly involved in basic research, regulatory testing and method development; it performs pharmacodynamics, pharmacology, toxicology (basal cytotoxicity, acute systemic toxicity, genotoxicity / mutagenicity, hepatotoxicity, chronic toxicity), quality control, and culture methodology studies on a routine basis. Pharmaceuticals and medical devices are routinely evaluated; cosmetics, diverse chemical compounds, pesticides, biomaterials, food additives and environmental pollutants also being occasionally studied.
The main use of alternative methods is for screening, complementary and replacement studies.

Model systems: The model systems used are conventional animal models (rat, mice, dog) and *in vitro* methods - micro-organisms (*Salmonella typhimurium* (Ames test)), primary culture of dispersed cells (lymphocytes), cell lines culture (CHO, L929,3T3, wish) and cell-free systems (liver microsomes).
The endpoints employed *in vitro* are morphology (microscopical examination), cell viability (trypan blue exclusion, crystal violet), cytoskeleton/membranes/enzyme release (MTT), cellular proliferation (clonogenicity) and nucleic acids.

Experimental systems (examples):
1 L 929, cytotoxicity in agar overlay, cytotoxicity, replacement test
2 CHO, cell viability by count, cytotoxicity, replacement test
3 CHO, cell proliferation by clonogenicity, cytotoxicity, complementary test
4 CHO, enzyme release, characterisation, complementary test

Work lines: The centre is devoted to the pharmacological and toxicological screening of chemicals and medical devices, and interested in improving the enzymatic preparation, by using any other alternative than rat liver. Besides, the group is seeking for replace some of the *in vivo* techniques used nowadays by others already validated *in vitro* ones. It plans to incorporate some ecotoxicology *in vitro* techniques for research purposes in the University.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution is implementing Good Laboratory Practices rules according to the FDA. The team have previously been involved in the following alternative method validation programme: Regional patron of measles vaccine. They are available to participate in EU validation programmes in relation to collaborative studies

Sources of financing: Self financing

Others: Organization of scientific meetings on the use of *in vitro* techniques as alternatives to laboratory animals.

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Type of Institution: University

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- Carlos Barrios Guerra, Email: cbarrios@udec.cl, Dr, Profesor Titular.
- Gisella Rios, Email: grios@udec.cl, MSc, Instructor, 20 % time devoted to altern. meth.

Total staff involved in alternative methods is 2 people.

Activities / aims: This university department is mainly involved in alternatives to animals in education; it performs toxicology (acute systemic toxicity) testing on a routine basis. Environmental pollutants are routinely evaluated.
The main use of alternative methods is for screening.

Model systems: The model systems used are conventional invertebrate animal models.
The endpoints employed are *in vitro* organ-specific indicators.

Work lines: The Laboratory of Toxicology (LTOX) is an academic organization. The main objetive of LTOX is to perform and stimulate research in the area of academic and occupational toxicology. Academic and occupational toxicology have changed considerably in the past decade. In both the emphasis has shifted from attention to scientific content, and process regulation plays an important role.

Sources of financing: Dirección de Investigación Universidad de Concepción, CONICYT; IAEA

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Type of institutions: Governmental, Industrial (pharmaceutical)

Staff:

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- Mª Julia Martínez Guerra, Email: moron@fcmsa.sld.cu, Especialista en Toxicología, 25 % time devoted to altern. meth., Duties related to AM: J´Grupo de Toxicología del Hosp. Salvador Allende
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- Emilio Monteagudo Jimenez, Email: emilio@utex.vcl., Dr en Medicina Veterinaria, Especialista en Toxicología, 15 % time devoted to altern. meth., Duties related to AM: J´Grupo Toxicología Alternativa Unidad de Toxicología Experimental Fac. Ciencias
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- Marcia Freeman Perez Ld en Ciencias Biológicas, J´Grupo de Teratogénesis, 20 % time devoted to altern. meth., Duties related to AM: J´Grupo de estudios Toxicológicos Alternativos del Centro Nacional de Toxicología
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- Lisseth García García, Email: ovidio@censa.edu.cu, Ld Ciencias Farmaceúticas, Especialista en Toxicología, 40 % time devoted to altern. meth., Duties related to AM: J´Grupo de Toxicología Alternativa del Centro Nacional de Sanidad Animal CENSA

Total staff involved in alternative methods is about 11 persons.

Activities / aims: This group of industrial (Pharmaceutical) departments are mainly involved in basic research, regulatory testing, methods development and methods validation; they performs toxicology (mecanisms of toxicity, acute systemic toxicity, ocular cytotoxicity, dermal irritation and corrosivity, immunotoxicity and sensitisation, genotoxicity / mutagenicity) and quality control studies on a routine basis, and culture methodology studies at a research level. Vaccines, hormones, pharmaceuticals, cosmetics, diverse chemical compounds, pesticides, medical devices and biomaterials are routinely evaluated, being colourings, environmental pollutants and wastes also occasionally studied.

Model systems: The model systems used are conventional animal models (CTA, FDP, OECD), invertebrates (Artemia Salina MEIC.), embryos (hen) and *in vitro* methods -- primary culture of dispersed cells (L-929).

Experimental systems (examples):

1 L-929 cell line, cytotoxicity of lids and flasks used for pharmaceuticals, USP XXIII, replacement test

2 dog and rat red blood cells, lysis and coagulation, ocular irritation of surfactants (Invittox 37), replacement test

3 chick embryo chorioalantoid membrane, lysis and coagulation, ocular irritation (Invittox 96), replacement test

4 rat, acute toxicity, classification according to EU, reduction test

Work lines: RBC Invittox protocol 37, ATC protocol OECD, the shrimps larvae for the study of the biological activity of natural plants (MEIC), HET-CAM test (Invittox protocol 96), cell culture phototoxicity test (44), 3T3 NRU phototoxicity (78).

Quality assurance / Validation programmes: A Quality Assurance Unit is operanting inside the facilities. The institutions applies Good Laboratory Practices rules according to the UE, OECD, FDA, CECMED and CCEM. The teams have been previously involved in alternatives methods validation programmes. They are available to participate in EU validation programmes related to inter and intralaboratory ring tests.

Others: Financing for preclinical toxicological studies

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- Ovidio Figueroa Hernández*,* Email: ovidio@censa.edu.cu, Dr Medicina Veterinaria, Investigador Agregado, 60 % time devoted to altern. meth.
- Evangelina Marrero Faz*,* Email: jesus@censa.edu.cu, Dra Medicina Humana PhD Medicina Veterinaria, Investigador Titular. Jefe de la Division de Investigación y Desarrollo de medicamentos, 40 % time devoted to altern. meth.
- Gleybis Melchor Orta*,* Email: jesus@censa.edu.es, Ld Ciencias Farmaceúticas, Aspirante a Investigador, 60 % time devoted to altern. meth.

Total staff involved in alternative methods is 6 people.

Activities / aims: This university department is mainly involved in basic research, regulatory testing, method development, method validation and alternatives to animals in education; it performs pharmacodynamics, toxicology (mechanisms of toxicity, acute systemic toxicity, ocular cytotoxicity, genotoxicity / mutagenicity), surgery and culture methodology studies on a routine basis. Vaccines, pharmaceuticals, cosmetics and wastes are routinely evaluated; diverse chemical compounds, pesticides, toxins, food additives and environmental pollutants also being occasionally studied.
The main use of alternative methods is for screening, complementary studies.

Model systems: The model systems used are conventional animal models (respiratory distress S, pulmonary surfactants test) and *in vitro* methods -- organ culture (isolated lung).

Experimental systems (examples):
1 red blood cells, haemolysis, by spectrophotometry, ocular irritation, replacement test
2 chick embryo chorioallantoic membrane assay, morphological observation staining and, ocular irritation, replacement test

Work lines: To prevent and contribute to the health of plants and animals including the human beings. Different drugs and food are evaluated before animal consumption. Evaluation Toxicology Department. The principal tests are focused on toxicology and ecotoxicology. Accredited by the main authority in Cuba, CECMED.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The institution applies Good Laboratory Practices rules according to OECD, FDA. The team have previously been involved in the following alternative method validation programmes: Validation of homeless test as alternative to Draize assay, and acute toxic class, and Het-CAM, in a ring test in Cuba. They are available to participate in EU validation programmes in relation to alternative methods on ocular irritation using cell cultures; phototoxicity tests, etc.

Sources of financing: Self financing, Programa Intercampus (AECI) for the development of a course on alternatives in toxicology and ocular Irritation (Univ Barcelona)

SWITZERLAND

Novartis Pharma AG

Toxicology-Pathology Department

Immuno-dermatotoxicity (Experimental Toxicology)

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Total staff involved in alternative methods is 1 person.

Activities / aims: This industrial (pharmaceutical) department is mainly involved in regulatory testing; it performs toxicology (mechanisms of toxicity, dermal irritation and corrosivity, immunotoxicity and sensitisation, phototoxicity) testing on a routine basis, and cell biology and molecular biology studies at a research level. Pharmaceuticals are routinely evaluated; diverse chemical compounds also being occasionally studied. The main use of alternative methods is for screening and complementary studies.

Model systems: The model systems used are conventional animal models (popliteal lymph node assay) and *in vitro* methods -- culture of explants, reaggregates, reconstituted organs, (human reconstituted skin (SkinEthic)), primary culture of dispersed cells (human keratinocytes, endothelial, epithelial, lanh) and cell lines culture (Hela T).
The endpoints employed are *in vivo* (lymph node assay: weight, proliferation, surface markers (FACS), cytokines (ELISA, PCR)) and *in vitro* cell morphology, cell viability (LDH, MTT reduction), cytoskeleton/membranes/enzyme release (LDH leakage), cellular proliferation (BrdU, 3H-Thy), metabolic activity (MTT reduction), cell signalling (cytokines) and nucleic acids (gene expression).

Experimental systems (examples):
1 Skin Ethic, LDH, morphology, cytokines, dermal irritation, complementary test
2 Murine popliteal lymph node assay, proliferation, weight, immunoactivation, replacement test

Work lines: Safety assessment of compounds and formulations in development regarding their irritant and /or phototoxic potential and / or sensitizing potential. Investigation of immunoactivation / immunotoxicity effects of compounds in development.

Quality assurance / Validation programmes: A Quality Assurance Unit is operating inside the facility. The team are available to participate in EU validation programmes in relation to immunotoxicity and dermatotoxicity.

19 Inventory of scientists

According to the tradition in Spain, two surnames are included for each scientist (339) interested in alternative methods. *Denotes the contact person of each team.

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21 Internet resources on alternatives

Due to the increasing importance of world-wide web internet resources, some important links on alternative and complementary methods used for research, education and testing are included, mostly taken from the homepage of GTEMA.

21.1. Entities:

- GTEMA- Grupo de Trabajo Especializado en Métodos Alternativos, http://tox.umh.es/aet/gtema/
- REMA - Red Española para el Desarrollo de Métodos Alternativos, http://tox.umh.es/rema/
- ECVAM- European Centre for the Validation of Alternative Methods, http://www.ei.jrc.it/ecvam/
- ICCVAM- Interagency Coordinating Commitee on the Validation of Alternative Methods (US), http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.html, http://iccvam.niehs.nih.gov/
- ERGATT- European Research Group for Alternatives in Toxicity Testing, http://embryo.ib.amwaw.edu.pl/~dslado/ergatt/ergatt1.htm
- ESTIV- European Society of Toxicology in Vitro, http://www.xs4all.nl/~shorbach/estiv/index.html
- ICLAS- The International Council for Laboratory Animal Science, http://www.iclas.org/
- FRAME: Fund for the Replacement of Animals in Medical Experiments (UK), http://www.frame-uk.demon.co.uk/
- AWIC- Animal Welfare Information Center US, http://www.nal.usda.gov/awic/awic.htm
- Industrial in Vitro Toxicology Group, http://www.invitro.org/news.htm
- CAAT- John Hopkins Center for Alternatives to Animal Testing, http://infonet.welch.jhu.edu/~caat
- AALAS, American Association for laboratory Animal Science, http://www.aalas.org
- NCA- Netherlands Centre for Alternatives, http://www.pdk.dgk.ruu.nl/nca/
- UC Center for Animal Alternatives, http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm
- UFAW- Universities Federation for Animal Welfare, http://www.users.dircon.co.uk/~ufaw3/
- ECACC- European Collection of Cell Cultures, http://www.camr.org.uk
- SECAL- Sociedad Española para las Ciencias del Animal de Laboratorio, http://www.secal.es
- ADDA- Asociación para la Defensa de los Derechos de los Animales, http://www.intercom.es/adda/

21.2. Data bases on alternatives:

- INVITTOX -Data base on protocols of in vitro experimental methods

(FRAME/ERGATT/ECVAM), http://embryo.ib.amwaw.edu.pl/invittox/, http://www.invittox.com/

- ALTWEB: The Alternatives to Animal Testing Web Site, http://altweb.jhsph.edu
- OECD list of protocols for toxicity assessment , http://www.oecd.org/ehs/test/testlist.htm
- Alternatives databases, http://oslovet.veths.no/databases.html
- Alternatives on line by D Sladowski, http://embryo.ib.amwaw.edu.pl/~dslado/invitro/Online5a.htm
- MEIC- Multicenter Evaluation of in vitro Cytotoxicity, http://www.ctlu.se/CTLU_HOME.html
- Bioethicsline, http://igm.nlm.nih.gov
- Biosis, http://www.biosis.org/
- Dimdi, http://gripsdb.dimdi.de/engl/usercode.htm
- Infotrieve, http://www.infotrieve.com
- Virtual dissection of a frog: focused to undergraduate students, Vfrog, http://www-itg.lbl.gov/vfrog/, Net-Frog, http://teach.virginia.edu/go/frog
- Sheep brain dissection, http://www.exploratorium.edu/memory/braindissection/
- NetVet, the electronic zoo, http://netvet.wustl.edu/e-zoo.htm
- Alternatives to Skin Irritation Testing in Animals, http://www.invitroderm.com/
- Norina database: audiovisuals inventory, http://oslovet.veths.no/NORINA/
- World animal net, http://worldanimal.net
- Toxicology in vitro, http://www.elsevier.nl:80/estoc/publications/store/3/08872333/
- ATLA - Alternatives to Laboratory Animals, http://www.frame-uk.demon.co.uk/atlahome.htm
- 2 World Congress on Alternatives, http://131.211.172.21/wca.dir/wca.htm
- 3rd World Congress on Alternatives and Animal Use in the Life Sciences, http://www.frame-uk.demon.co.uk/congress/index.htm
- EuroNICHE- European network of Individuals and campaigns for Humane Education, http://www.euroniche.internetworking.de/
- The Whole Mouse Catalog, http://www.rodentia.com/wmc/
- CLIVE- Computer Aided Learning in Veterinary Education, http://www.clive.ed.ac.uk/

21.3. Other web sites:

- Chemfinder, http://www.chemfinder.com/
- Toxline, http://igm.nlm.nih.gov/
- Medline, http://www.healthgate.co.uk/medline/search-adv.shtml
- Internet Grateful Med, http://igm.nlm.nih.gov/
- TOXNET (Toxicology data network), http://toxnet.nlm.nih.gov/servlets/simple-search
- Toxikon, http://toxikon.er.uic.edu/
- International Chemical Safety Cards, http://www.cdc.gov/niosh/ipcs/icstart.html
- Organization for the Economic Cooperation and Development , http://www.oecd.org/
- European Chemical Bureau, http://www.ei.jrc.it/report/ecb.html
- The International Agency for Research on Cancer (IARC), http://www.iarc.fr/
- US Environmental Protection Agency, http://epa.gov
- European Union, http://europa.eu.int/index-es.htm
- AET- Asociación Española de Toxicología, http://tox.umh.es/aet/
- EUROTOX- European Society of Toxicology, http://www.uta.fi/eurotox/

- SETAC- Society of Environmental Toxicology and Chemistry, http://www.setac.org
- Instituto Nacional de Toxicología, http://www.mju.es/toxicologia/intframe.html
- CICyT- Comisión Interministerial de Ciencia y Tecnología, http://www.cicyt.es/
- CORDIS – Community Research and Development Information Service, http://www.cordis.lu/
- V Framework Programme, http://www.cordis.lu/fp5/home.html, http://sost.cicyt.es/programa.htm, http://www.cordis.lu/fifth/src/pr-en-8.htm
- European Programmes, http://www.uv.es/cde/GFC/
- Ministerio de Educación y Ciencia, http://www.seui.mec.es.

21.4. Distribution lists:

- [3ERRES] Red Electrónica de Comunicación sobre Alternativas (GTEMA), http://tox.umh.es/aet/gtema/GTEMA2.html
- ESTIV-L, http://www.xs4all.nl/~shorbach/estiv/index.html
- AR- News, http://arrs.envirolink.org/maiLists/ar-news.html
- COMPMED, Comparative Medicine List, http://www.aalas.org
- WAN-general, http://worldanimal.net
- DISEVEN – Lista de información de eventos, http://www.rediris.es/diseven/)
- FCR-INT – Lista de información de becas y ayudas, http://www.fcr.es/siab/
- FARMACOL - Foro de Farmacología, http://www.rediris.es/list/info/farmacol.html
- SECAL-L - Lista de distribución electrónica de la SECAL, http://www.hulp.es/secal/
- TOXICOL- Foro de Toxicología, http://www.rediris.es/list/info/toxicol.html

22 Abbreviations

AET: Asociación Española de Toxicología, Spanish Toxicology Society
CICyT: Comisión Interministerial de Ciencia y Tecnología, Interministerial Commission of Science and Technology
CSIC: Consejo Superior de Investigaciones Científicas, Spanish Research Council
ECVAM: European Centre for the Validation of Alternative Methods
ERGATT: European Research Group for Alternatives in Toxicity Testing
ESTIV: European Society of Toxicology in Vitro
ETCS: European Tissue Culture Society
EU: European Union
EUROTOX: European Society of Toxicology
FELASA: Federation of European Laboratory Animal Science Association
FIS: Fondo de Investigaciones Sanitarias, Spanish Fund for Health Research
GEFTIV: Grupo Español de Farmacotoxicología in vitro, Spanish Group of Pharmacotoxicology in vitro
GTEMA: Grupo de Trabajo Especializado en Métodos Alternativos (AET), GTEMA-Spanish Group on Alternative Methods
ICLAS: International Council Laboratory Animal Science
ICLAS/CSIC-WGCM: ICLAS/CSIC Working Group on Complementary Methods
OECD: Organization for the Economic Cooperation and Development
REMA: Red Española para el Desarrollo de Métodos Alternativos, Spanish Network for the Development of Alternative Methods
SECAL: Sociedad Española para las Ciencias del Animal de Laboratorio, Spanish Society for the Science of Laboratory Animals
SEEA: Sociedad Española de Experimentación Animal, Spanish Society of Animal Research