European Commission

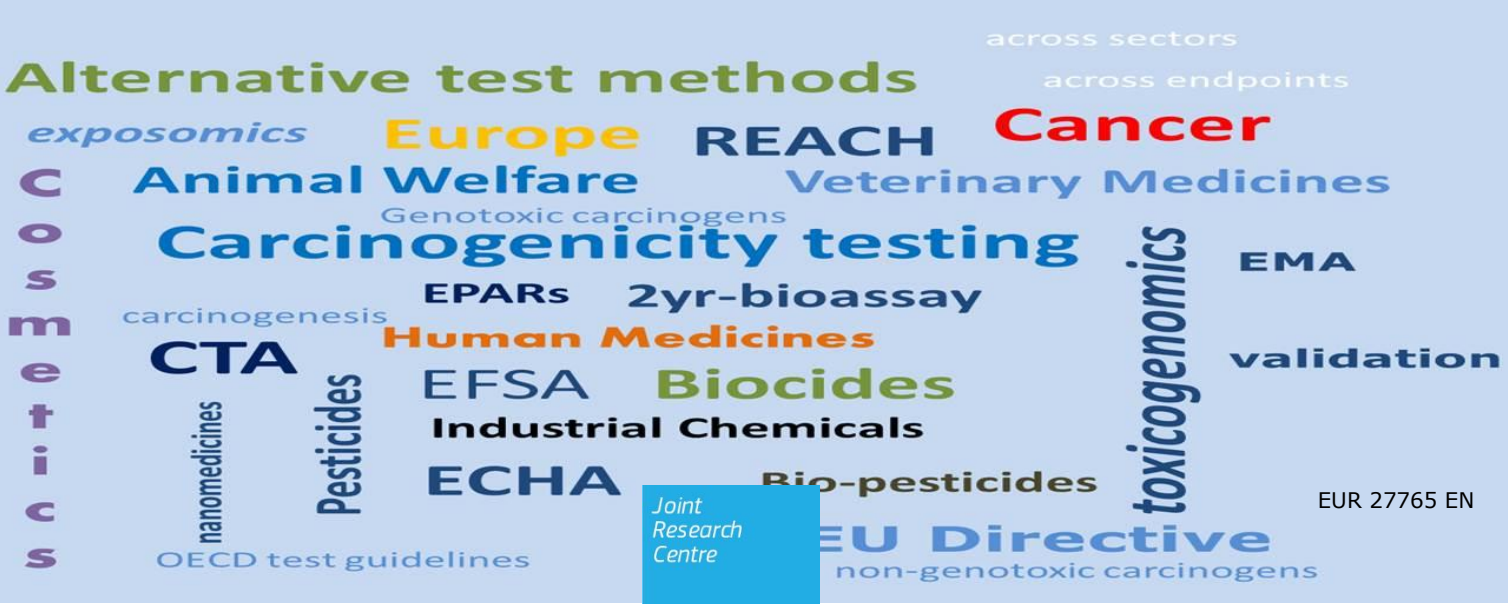
JRC TECHNICAL REPORTS

Analysis of carcinogenicity testing for regulatory purposes in the European Union

Review of the current demand of in vivo carcinogenicity studies across sectors

Federica Madia, Andrew Worth and Raffaella Corvi

2016



Joint Research Centre

EUR 27765 EN

Analysis of carcinogenicity testing for regulatory purposes in the European Union

Contact information
Name: Raffaella Corvi
Address: Joint Research Centre, EURL ECVAM
E-mail: raffaella.corvi@ec.europa.eu
Tel.: +39 0332 785266

JRC Science Hub
https://ec.europa.eu/jrc

JRC100609

EUR 27765 EN

ISBN 978-92-79-56876-3

ISSN 1831-9424 (online)

doi:10.2788/547846 (online)

How to cite: MADIA F., WORTH A. and CORVI R., Analysis of carcinogenicity testing for regulatory purposes in the European Union. Luxembourg, European Commission 2016. EUR 27765 EN. ISBN 978-92-79-56876-3. Available from: doi:10.2788/547846

Table of contents

Foreword

The evaluation of the carcinogenic potential of substances and the methods used differ substantially across sectors. Despite differences in testing schemes, the two-year bioassay study in rodents represents the standard element. However, the validity of the two-year bioassay has been the subject of considerable debate in the last decade. Issues are uncertainty on the relevance to humans; time and resource demand; high animal burden and ethical concerns.

In order to provide context for a broad initiative aimed at exploring opportunities to improve the whole process of carcinogenicity assessment and to lower its impact on animal use and, further the outcome of the Workshop 'Carcinogenicity brain-storming' held at the JRC-Ispra in October 2014, The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has carried out and analysis of carcinogenicity testing across sectors in the European Union.

Results from this analysis are expected to inform initiatives aimed at: a) reducing the need for animal use where animal testing is still a requirement; b) ensuring the adequate hazard identification and characterization in sectors where animal use is banned or limited; and c) where existing methods are not suitable.

Acknowledgements

The authors would like to thank Dr Peter Kasper (BfArM, DE) and Dr Lutz Mueller (F. Hoffmann-La Roche Ltd, DE) who kindly shared valuable information regarding carcinogenicity testing within the pharmaceutical sector, provided helpful discussion and commenting of the report;

Dr Andrea Terron (EFSA) for commenting the section related to pesticides;

Drs Xavier Compas and Panagiotis (Panos) Karamertzanis who kindly provided data from the ECHA public database on registered substances, which included information for carcinogenicity studies;

Dr Rabea Graepel, colleague from Systems Toxicology Unit, for data collection from the Bundesministerium für Ernährung und Landwirtschaft (BMEL) database;

Dr Susanne Louhimies Satu, who kindly provided information from DG ENV related to animal use for scientific purpose in Europe.

Abstract

The approaches for evaluating the carcinogenic potential of substances, including whether carcinogenicity studies should be conducted, differ substantially across sectors. Despite variations in testing schemes, the two-year bioassay study in rodents represents the standard element across all sectors.

The validity of the two-year bioassay has however been questioned in the last decade. Uncertainty is associated with the extrapolation of data from rodents to humans. Furthermore, these studies are extremely time and resource-consuming and the high animal burden has raised ethical concerns. For all these reasons, there is a strong demand for alternative strategies and methods in this area. The development of new *in vitro* methods for carcinogenicity testing, however, has progressed slowly and those available are far from being accepted for regulatory decision making, especially when evaluating the carcinogenicity of non-genotoxic chemicals or specific classes of compounds such as biologicals, microorganisms and nanomaterials.

The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has carried out an analysis of carcinogenicity testing requirements and assessment approaches across different sectors. This consisted of: a systematic review of the different regulatory testing schemes; an analysis of the number of animals used per sector; an estimation of the number of carcinogenicity and genotoxicity studies conducted or waived in respect of the number of substances authorized per sector per year; a review of the type of justifications for waiving the two-year bioassay.

Results from this analysis will provide context for initiatives aimed at: 1) reducing the need for animal use where animal testing is still a requirement; 2) ensuring an adequate hazard identification and characterization in sectors where animal use is banned or limited; and 3) where existing methods are not suitable.

1. Introduction

The incidence of cancer has been estimated as being approximately 3,420,000 new cases per year in the European Union (EU) (data are from population-based Member States Cancer Registries [1]) and approximately 14 million new cases per year worldwide with a projection to 19.3 million by 2025 [2]. These figures have been rising constantly over recent decades making cancer one of the major public health issues and burden of disease, in terms of social impact, quality of life and economic impact, including cost of illness, phase-specific and long-term and indirect costs [2-5].

Despite the advances in research/innovation and activities aimed at cancer prevention, the complexity of the disease together with its multi-faceted causes makes its defeat a slow process.

Carcinogenesis, the process of initiating and promoting cancer, is considered to be a multi-hit/multi-step process from the transition of normal cells into cancer cells via a sequence of complex biological interactions. Cells are no longer fully responsive to signals that regulate differentiation, proliferation, survival or death. Changes affecting normal cells are the results of interactions between one's genetic features, diet, life-style, hormonal balance, environment and xenobiotics. Xenobiotic insults include: physical stressors such as UV and ionizing radiations; chemicals including natural and man-made substances and their derivatives; biological ones such as viruses-, bacteria- and parasites-induced infections (e.g. HIV, hepatitis C/B, HPV and *H. pylori*).

Aging also plays a predominant role in the development of several types of cancer. In fact, the incidence of cancer rises dramatically in people aged 50 or older, most likely due to a build-up of adverse effects over time. Accumulation of cellular and DNA damage, induced by multiple insults is often combined with age-dependent deficiency of cellular and DNA repair mechanisms.

Along with the variety of factors involved in cancer development, chemical substances induce cancer disease through a variety of mechanisms. Agents can act by directly causing alteration to DNA structure (genotoxic) and thereby affecting the integrity of the genome or indirectly, affecting its architecture and/or functionality (epigenetic, e.g. alternative states of gene expression, complex hyper- or hypo-methylation of DNA, histone modifications, inducing changes in protein folding and nucleosomal remodelling, RNA interference) or by specific mechanisms that do not involve genotoxicity (non-genotoxic, e.g. hormonal dysregulation, immune suppression, inflammation, etc.) [6].

Hence, a thorough evaluation of the carcinogenic potential of substances, which considers all the changes in physiology at cellular and molecular level in animals and humans together with relevant data on exposure, represents an important step forward to prevention and ultimately to safety of human health.

Thousands of chemicals are in common use on a daily-basis, but only a portion of them have undergone significant toxicological evaluation. Based on the International Agency for Research on Cancer (IARC) classification scheme [7], 118 chemicals have been classified as 'known human carcinogens' (Group 1), more than a double instead, 369 (Group 2A-2B) are classified as 'probably or possibly' human carcinogens (from IARC website as for 22-02-2016) [7-9]. Yet, many others have not been evaluated for their long-term impact on human health.

An initial screening of substances included in *Annex VI* of the CLP Regulation [10], which ensures that the hazards presented by substances are clearly communicated to workers and consumers in the European Union, reported 406 substances classified as CMRs (carcinogens/mutagens/toxic to reproduction; Carc. 1A or Carc. 1B, and/or Muta. 1A or Muta. 1B, and/or Repr. 1A or Repr. 1B) among the substances registered with ECHA and 665 matches among those notified (which includes the registered substances) [11].

Carcinogenicity evaluation represents an essential component of the safety assessment of all types of substances, relevant to European and International legislation aimed at the protection of human health. In fact, cancer risk assessment forms the basis for prevention and intervention, along with several initiatives in the area of surveillance and social impact where the World Health Organization (WHO) approaches (e.g. The STEPwise Approach to Chronic Disease Risk Factor Surveillance; The WHO Global InfoBase) complement those of the European Commission. For instance, European activities related to breast cancer are aimed to ensuring the implementation of evidence-based practices throughout the entire breast cancer prevention programs, including screening and diagnosis; to implementing population-based cancer registries in order to obtain information from all new cases and to provide a basis for research on cancer causes and outcome; to ensuring and harmonizing quality of breast cancer services across European Countries [12-14].

Substances are defined as carcinogenic if after inhalation, ingestion, dermal application or injection they induce (malignant) tumours, increase their incidence or malignancy, or shorten the time of tumour occurrence.

Carcinogenicity assessment generally requires the conduct of either a carcinogenicity *in vivo* study [OECD TG 451, 15] or a combined chronic toxicity/carcinogenicity test [OECD 453, 16]. Introduced as a regulatory requirement in the early '60s, the two-year bioassay in rodents is widely regarded as the 'Gold standard' and it represents the traditional approach of conducting long term study for the evaluation of cancer hazard and potency. Its adequacy to predict cancer risk in humans, however, has been the subject of considerable debate [17-18].

In order to provide context for a broad initiative aimed at exploring opportunities to improve the whole process of cancer hazard identification and to lower its impact on animal use and before embarking on any new activity, a two-level approach has been considered critical.

First, and the main goal of this report, is to review the actual demand of animal-based carcinogenicity testing across sectors, based on: different approaches used by each sector to satisfy regulatory requirements; the actual number of animals/experiments performed for regulatory assessment of carcinogenicity; the number of chemical product authorizations released within the EU market. This work will be followed by a comprehensive review and analysis of the relevance of, and possible gaps in, the current available testing paradigm.

2. Material & Methods

For a systematic evaluation of carcinogenicity testing in the EU and its impact on animal use, the information available was approached at different levels:

- First level of study aimed at reviewing the regulations and policies currently adopted in the European Union and the definition of regulatory requirements for carcinogenicity testing across different sectors.

- The number of animal used in the European Union formed the second level of investigation. The primary source of information was represented by the DG ENV reports then, reports from The Home Office of Statistics were also consulted for an overview on the number of experimental procedures as performed in the UK. Those reports provided a rough estimation of the number of animal used for scientific purposes in Europe in the past decade and specific to toxicological and safety purposes and to the trend across sectors.

- A third level of investigation referred to the analysis of authorization dossiers for each substance across different sectors, and an in-depth analysis of two different years 2011 and 2014. The aim was to sourcing out information relating the demand and actual performance of studies of carcinogenicity and genotoxicity to satisfy regulatory requirements. This latter study was performed without taking into account the number of animals used.

Additionally, since carcinogenic potential cannot be evaluated without evaluating DNA damage, the latter being an essential component of carcinogenesis, it was also decided to collect information on *in vitro* and *in vivo* genotoxicity studies.

2.1 Regulatory requirements for carcinogenicity testing across sectors

Information on current policies related to consumers, environment health and food safety legislations can be directly retrieved from the European Commission website [19]. This includes Regulations and Directives adopted in regard of the different products, that are made available on the EU market and related requirements for regulatory testing, including carcinogenicity,

Information on carcinogenicity testing approaches specific to each sector was obtained from the guidance documents (SCCS Notes of guidance, ECHA guidance documents, etc.) of different EU Agencies (DG GROW, DG SANTE, EFSA, EMA, ECHA) in charge of the evaluation and authorization of products. These documents are in support of the legislations and guide the fulfilment of testing requirements.

Information on testing of carcinogenicity has been also retrieved from those guidelines published by International Organizations in charge of harmonization of guidance documents. Those are: the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the Trilateral (EU-Japan-USA) Programme aimed at harmonising technical requirements for veterinary product registration (VICH). For testing procedures, the Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals for health effects have been consulted (Table 1). The above documents were also consulted in relation to waiving opportunities in the different sectors.

2.2 Data on number of animals used for scientific purposes

The European Commission publishes, on a three-year basis, a report on the number of animals used for experimental and other scientific purposes across the Member States (MS).

The latest available report, published by the European Directorate General for the Environment (DG ENV) is from 2013 [20] and summarizes the number of animals used in 2011 by 27 MS (Table 1). Data on purposes of the experiments, percentage and actual number of animals per toxicity endpoint and type of product were collected and reported.

The Home Office Statistics in the UK of scientific procedures (intended as number of experiments) on living animals are published yearly but they consider procedures performed in UK only. Both the European and the UK documents were consulted to retrieve information on the number of animals used and experiments performed for different purposes and specifically for genotoxicity and carcinogenicity testing between2004 and 2014 [21-23].

2.3 Data on carcinogenicity and genotoxicity studies across sectors

The actual number and type of carcinogenicity and genotoxicity studies is scattered across different databases; this prompted us to systematically collect data for each sector. The sectors considered in this review include: industrial chemicals, biocidal products, medicines for human and veterinary use, pesticides, and cosmetics.

We made use only of information publicly available from the competent authorities responsible for evaluating and authorising the marketing of products within Europe (Table 1). First, the analysis on the number of authorizations for different sectors was performed. Secondly, information on the number and type of studies performed for carcinogenicity and genotoxicity endpoint was retrieved from the registration dossiers.

The review of available dossiers did not include the exact number of animals used for the relative studies, for this reason data reported from the registration dossiers might not completely overlap with those collected from the DG ENV Report [20] on the number of animals used for carcinogenicity and genotoxicity testing.

- For industrial chemicals, specific toxicological endpoint information was retrieved from the REACH dossiers database stored in the European Chemicals Agency (ECHA) dissemination website (Table 1), since its first launch in 2009. In addition, the ECHA 2nd report on 'The Use of Alternatives to Testing on Animals for the REACH Regulation' [24] was consulted.

- Similarly, information on biocidal products was retrieved from the ECHA dissemination website as on March 10, 2015, from the database on biocides.

- Human and veterinary medicines data were retrieved from the European Medicines Agency (EMA). The Agency publishes a European Public Assessment Report (EPAR) for every human and veterinary medicine for which a central marketing authorisation by the European Commission is granted or refused following an assessment by the EMA's Committees for Medicinal Products for Human Use (CHMP) or for Veterinary Use (CVMP), respectively. EPARs are full scientific assessment peer reviewed reports.

- The EU pesticide database is hosted on the DG SANTE website. For pesticides, detailed information regarding toxicity testing was retrieved, when available, from the European Food Safety Authority (EFSA) Draft Assessment Reports (volume 3 Annex B/6), where studies on carcinogenicity, *in vitro* and *in vivo* genotoxicity are reported for each substance and published on EFSA website.

- Information on cosmetics was retrieved from the EU Scientific Committee on Consumer Products [http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm]. When preparing its policy and proposals relating to consumer safety, public health and environment, the Commission relies on independent Scientific Committees to provide it with sound scientific advice and draws its attention to new and emerging problems. The Scientific Committees can call on additional expertise from a pool of scientific advisors and a database of experts. The Committee provides Opinions on health and safety risks (chemical, biological, mechanical and other physical risks) of non-food consumer products and services among those, cosmetic products and their ingredients.

  It is worth noting that SCCS opinions concern mainly particular classes of cosmetics such as hair dyes, preservatives, nanomaterials, UV filters, CMRs, border-line products (those is not clear between countries whether a particular product is a cosmetic product under cosmetics legislation or it falls under other sectorial legislation) which enter specific Annexes of the EC Regulation 1223/2009 [25].

Data on:	DG - Agency - Organization	Publicly available information
Number of Animals used in EU	European Commission DG ENV	http://ec.europa.eu/environment/chemicals/lab_animals/home_en.htm
Statistics in UK	Home Office Gov UK	https://www.gov.uk/government/policies/ensuring-research-and-testing-using-animals-is-safe-and-reasonable
Pesticides	European Food Safety Authority (EFSA)	http://www.efsa.europa.eu/en/publications.htm
Human medicines	European Medicines Agency (EMA) & ICH	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/epar_search.jsp&mid=WC0b01ac058001d124 http://www.ich.org/home.html
Veterinary medicines	European Medicines Agency (EMA) & VICH	http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/landing/vet_epar_search.jsp&mid=WC0b01ac058001fa1c http://www.vichsec.org/what-is-vich.html
Biocides	European Chemicals Agency (ECHA)	http://echa.europa.eu/regulations/biocidal-products-regulation; http://echa.europa.eu/information-on-chemicals/active-substance-suppliers
Industrial chemicals - REACH	European Chemicals Agency (ECHA)	http://echa.europa.eu/search-chemicals; http://echa.europa.eu/information-on-chemicals/testing-proposals/current
Cosmetics Ingredients	European Commission DG SANTE - Scientific Committee on Consumer Safety (SCCS) European Commission Impact Assessment on the animal testing provisions in regulation 1223/2009 on cosmetics	http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52013SC0066
Legislations	European Commission EURLEX	http://ec.europa.eu/legislation/index_en.htm
Test Guidelines on Carcinogenicity (internationally agreed testing methods)	Organisation for Economic Co-operation and Development (OECD)	http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicalsandrelateddocuments.htm
Number of animals foreseen to be used for research projects in Germany (Zebet/BfR database)[1]	Bundesministerium für Ernährung und Landwirtschaft (BMEL)	https://www.animaltestinfo.de/

Table 1. Source of information List of agencies, competent authorities and organizations websites consulted for data gathering.

[1] Database on foreseen number of procedures (e.g. cancer studies) and animal usage related to research projects proposals within the Academia environment, mainly (as on May 29, 2015).

2.4 Methodology

Firstly, a definition of regulation and policies currently in place at EU level for each sector were reviewed as well as guidance documents on carcinogenicity testing and available test method guidelines internationally agreed (Results, section 3.1).

Information on the number of animals for scientific purposes used from 1996 to 2011 was reported as from the DG ENV Reports with specific emphasis on regulatory toxicology and safety and on carcinogenicity and mutagenicity testing across sectors (Results, section 3.2).

The number of product authorizations per sector per year was also considered. Data from years 1999-2014 were recorded, whenever available (Results, section 3.3).

Year 2011 and year 2014 were chosen for an in-depth analysis. This was based on the most recent data, published in 2013 and referring to the animals used in 2011 (DG ENV Report 2013) and on the basis of REACH registered chemicals data, reported by ECHA in a recently published report [24], also referring to data of year 2011 and 2014. Where possible, we considered all the new Authorizations/Approvals within each sector per year between 2004 and 2014. The above approach was chosen in order to harmonize data collected so far. The reference time-period for the analysis was linked to the available number of animals used for scientific purposes, collected within the seven reports published by the European Directorate General for the Environment (DG ENV) (Results, sections 3.4-3.8).

It is important to bear in mind that data reported within each consulted document refer to studies performed 2-3 years (long-term studies, especially) ahead of final publication. Thus, the impact on animals used for carcinogenicity testing does not take into account ongoing studies.

While reviewing the authorizations/approval reports across the different product sectors, we searched for new active ingredients, renewals of existing substances, new formulations, and whether the drug/substance was approved for more indications or new use. We examined the freely-accessible earliest versions of the review documents, whether Scientific Opinions of Scientific Committees or final Public Assessment Reports. Information on carcinogenicity studies and *in vitro* and *in vivo* genotoxicity studies were retrieved per substance, including number of performed and waived studies with their related justifications.

3. Results

3.1 Mapping carcinogenicity testing requirements across sectors

The approaches for evaluating the carcinogenic potential of substances differ substantially across various sectors: industrial chemicals, biocides, pharmaceuticals, pesticides, etc. For each sector, the different regulations set out recommended data-driven decision pathways to determine whether carcinogenicity studies should be conducted; these rules have been laid down on the basis of human health risk of exposure, specific concerns, available information gathered by other toxicity tests and on the basis of the final use of each compound (Figure 1).

Despite the availability of a number of testing schemes and guidelines, the two-year bioassays in rodents represent the standard option across all sectors for the evaluation of cancer hazard (Table 2). However, these studies, the in-life portion of which alone lasts 24 months, are extremely time- and resource-consuming (one million Euro/chemical, approximately). Moreover, in the last decade their predictive capacity has been strongly questioned due to its poor human specificity in identifying human non-carcinogens and extrapolating data from rodents to humans [26-34]. Knight et al. had even questioned the validity of EPA human carcinogenicity classification in cases where it was solely based on conventional rodent bioassay [30-32].

Test Method	OECD Test guideline	Species/Number	Objective of the Study	Duration of the Study
Carcinogenicity Studies	TG. 451 (B. 32)	Rats and mice (50-65/sex/group) Non-rodents (mainly dog) (4-6/sex/group)	Observe test animals for a major portion of their life span for the development of neoplastic lesions during or after exposure to various doses of a test substance	Normally 24 months for rodents. For specific strains of mice, duration of 18 months may be more appropriate [15].
Combined Chronic toxicity/ carcinogenicity Studies	TG. 453 (B.33)	Rat (10/sex/group) chronic phase; and (50/sex/group) carcinogenicity phase	Identify carcinogenic and the majority of chronic effects and determine dose-response relationships following prolonged and repeated exposure.	Normally 12 months for the chronic phase, and 24 months for the carcinogenicity phase [16].
Chronic Toxicity Studies*	TG. 452	Rodents (20/sex/group) and non-rodents (4/sex/group)	Characterize the profile of a substance in a mammalian species following prolonged and repeated exposure.	Normally 12 months but, 6- or 9- month-studies are also performed [35].

Table 2. Internationally agreed testing methods for carcinogenicity. Data were retrieved from the OECD website. The above methods are used by industry, governments and laboratories for the regulatory safety testing of chemicals. *The chronic toxicity study is not aimed specifically at testing carcinogenicity but, it can be used for early detection of neoplastic lesions.

Above all, the high animal burden with an extensive use of rodents per study (approx. 850 animals) has raised ethical concerns and a strong demand for alternative carcinogenicity strategies and methods.

In addition, the EU Directive on animal welfare, which includes an explicit reference (Article 4) to the 3Rs principles and the use of alternative test methods [36], had an important impact on the regulatory requirements for carcinogenicity testing in the EU. The majority of legislations and guidance documents related to toxicity testing, hence carcinogenicity, have been revised accordingly in the past years (Figure 1), and some new opportunities for enhancing the use of alternative test methods have been introduced.

Considering the link between damage to DNA and cancer development, the genotoxic hazard of substances is assessed before embarking into any type of carcinogenicity studies. Genotoxicity is assessed through a battery of *in vitro* and *in vivo* tests (gene mutations in bacteria and mammalian cells; chromosomal aberrations, micronuclei formation, unscheduled DNA synthesis or DNA damage in mammalian cells and in rodents) (Figure 2). The resulting outcome can guide follow-up testing, i.e. of the long term carcinogenicity study in rodents or the justification for waiving it [37, EURL ECVAM Strategy on genotoxicity testing].

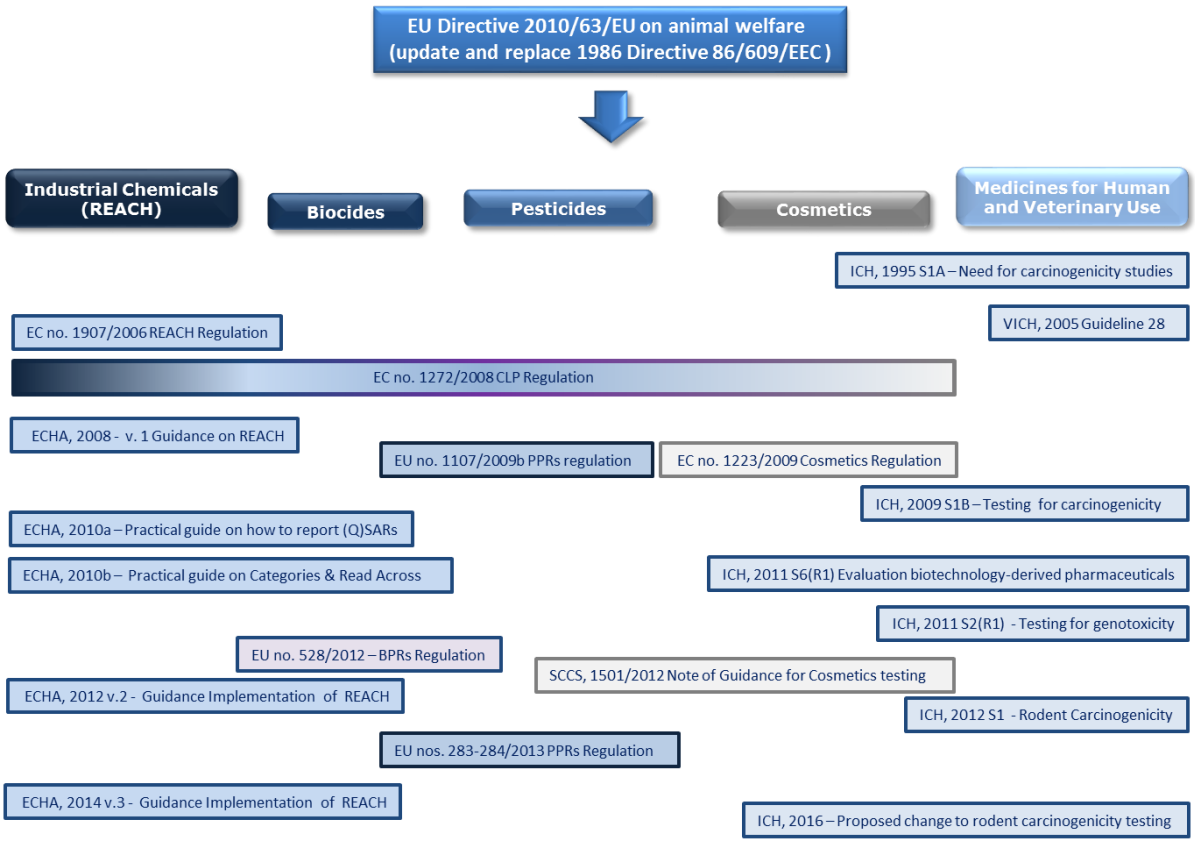


Figure 1. Legislations and guidance documents across sectors in the European Union. The panel summarizes the most relevant pieces of legislations and documents guiding animal use and safety testing specific to carcinogenicity of substances across different sectors. All documents are publicly available and information can be retrieved through the links reported in Table 1.

The requirements for testing of **Industrial Chemicals**, regulated by REACH [38], are based on a tiered approach. Substances manufactured or imported in Europe in quantities less than 1 tonne per year are not tested. Those in the range of 1 to 10 tonnes per year are tested for limited to local toxic effects. Substances which fall between 10 and 1000 tonnes per year are tested for more extensive toxicological endpoints. A carcinogenicity study "may be proposed by the Registrant or may be required by ECHA" for chemicals at high tonnage level of production (Annex X, ≥

1000t/y) if: the substance has a widespread dispersive use or there is evidence of frequent or long-term human exposure; and the substance is classified as mutagens category 3 (GHS category 2) or there is evidence from the repeated dose study/ies that the substance is able to induce hyperplasia and/or preneoplastic lesions [39, 40]. However, REACH also requires that carcinogenic substances at all tonnage levels be identified as substances of high concern, taking into account information from all available relevant sources (non-human and human non-testing and testing data) [41, 42], which can inform on hazard identification, underlying modes of action or carcinogenic potency [43]. Also, the classification and labelling as listed in *Annex VI* of CLP Regulation [10] is legally binding and can trigger further assessment under REACH whether the substance is considered of high concern (Figure 2).

The **Biocidal Products** legislation [44] requires carcinogenicity testing for all new active substances, unless those are classified as mutagens category 1A and 1B (Figure 2). A combined carcinogenicity and chronic repeat-dose toxicity study in the rat, possibly through the oral route plus a second carcinogenicity study in a second rodent species, the mouse, are usually performed. It is necessary to conduct toxicity studies by the oral route for evaluation of consumer safety of active substances that may end up in food or feed [44].

New **Human Medicines** are generally tested for their potential to induce cancer in patients when duration of treatment is continuous for at least 6 months or intermittent for a chronic or recurrent condition (e.g. depression, anxiety and allergies) or for defined specific causes of concern arising from other investigations [45-50] (Figure 2). Some of the factors considered raising concerns are: 1) previous demonstration of carcinogenic potential in the product class that is considered relevant to humans; 2) structure-activity relationship suggesting carcinogenic risk, 3) evidence of neoplastic lesions from repeated dose toxicity studies; 4) long-term retention of parent compound or metabolites resulting in local tissue reactions or pathophysiological response.

For new small-molecule therapeutics, traditional new chemical entities, the safety assessment of carcinogenicity consists in long term studies (two-year bioassay studies) conducted in rats and mice or one long term study in rats plus alternative 6-month mouse transgenic models (rasH2, p53, etc.). The latter being increasingly performed in recent years.

In certain cases, the two-year bioassay may not be required on the basis of specific characteristic of the drug, indication of use, patient population, route of exposure [47-49; 51, 52].

- This is the case of unequivocally genotoxic compounds which are presumed to be trans-species carcinogens, implying hazard to humans. Though, carcinogenicity testing may be necessary when administration to human is chronic.
- In specific conditions, the intrinsic hazard of a substance is overruled by the patient 'status' and/or even disease burden. For medicines developed to treat life-threatening or severely debilitating diseases, carcinogenicity testing is either not done at all or is conducted post-approval in order to speed up the availability of the drug. This can be the case of anti-cancer drugs aimed to ameliorate patient's conditions with metastatic tumours.
- Waiving can be applied also for certain classes of compounds applied topically when there is no concern of significant systemic exposure.

For new biotechnology-derived pharmaceuticals, biotherapeutics, the two-year bioassay is often not scientifically applicable or technically feasible. The class of bio therapeutics includes: proteins and peptides, their derivatives and products of which they are components. They can be derived from cell cultures or be produced using recombinant

DNA technology including production by transgenic plants and animals. Cytokines, plasminogen activators, recombinant plasma factors, growth factors, fusion proteins, enzymes, receptors, hormones and monoclonal antibodies are some examples. The intended uses can be *in vivo* diagnostics, therapeutic or prophylactic [53]. For the biotherapeutics characteristics such as, no direct genotoxicity, no formation of active or genotoxic metabolites and their target specificity, the main concern is that these substances may increase the incidence or the growth rate of a specific neoplasm or group of neoplasms. The assessment of carcinogenicity may be needed depending upon duration of clinical dosing, patient population and/or biologic activity of the product. *In vitro* mechanistic studies can be conducted or specific indices analysis (e.g. cell proliferation) can be included in repeated dose toxicity studies [53].

Carcinogenicity studies are not performed in vaccines according to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines [54] and the Guideline on adjuvants in vaccines for human use [55]. The study is not performed in categories of drugs for gene therapy, cellular blood components, vitamins, etc.

Two-year bioassay does not need to be carried out when testing generic or biosimilar medicines (for definition see section 3.6.1). The recently updated version of guidance on similar biological medicinal products containing biotechnology-derived proteins as active substance [56] states in fact that: *'If the biosimilar comparability exercise for the physicochemical and biological characteristics and the non-clinical in vitro studies (see step 1) are considered satisfactory and no issues are identified as potentially relevant (in step 2) which would block direct entrance into humans, an in vivo animal study is usually not considered necessary'*. Also: *'…Studies regarding safety pharmacology, reproduction toxicology, and carcinogenicity are not required for non-clinical testing of biosimilars'*.

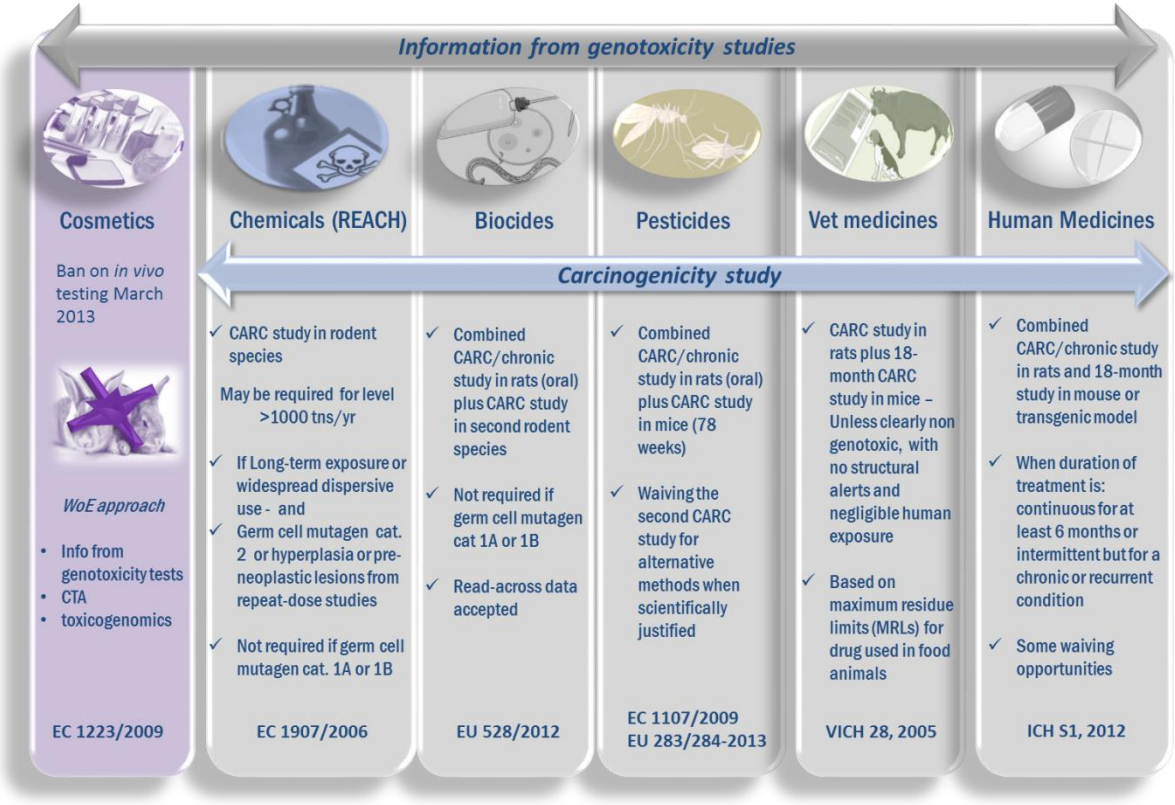


Figure 2. Testing requirements for the assessment of carcinogenicity across sectors. Principal requirements set for carcinogenicity testing as reported within regulations or guidelines for the different sectors (for hazard categories for germ cell mutagens, Globally Harmonized System GHS Classification was used).

Carcinogenicity is also a key component of the safety assessment of **veterinary medicines** that are mostly tested for this endpoint as the human counterparts.

However, there are major concerns for veterinary products used in animals which provide food to human consumers, as drug residues may remain in food of animal origin. The VICH Guideline no. 28, [57] states that the decision on the need of a carcinogenicity for **residues of veterinary drugs** in food for humans study should be based on 1) the results of genotoxicity tests, 2) structure-activity relationships, and 3) findings in systemic toxicity tests that may be relevant to neoplasia in longer term studies and any known species-specificity of the mechanism of toxicity. In addition, to ensure safety of human consumer, the European Commission in consultation with EMA has published guidance on the evaluation of user safety and on the establishment of maximum residue limits (MRLs) for drugs in foodstuffs of animal origin, the procedures of which are laid down in those Regulations [58, 59], harmonized specifically with the Committee for Medicinal Products for Veterinary Use (CVMP) [60].

Carcinogenicity bioassays consisting of a two-year rat study and an 18-month mouse study are generally recommended. With appropriate scientific justification, carcinogenicity studies may be carried out in one rodent species, preferably the rat (Figure 2). A positive response in either test species should be considered indicative of carcinogenic potential.

Clearly genotoxic compounds are regarded as to bear carcinogenic potential unless there is convincing evidence that this is not the case. Clearly negative results for genotoxicity should usually be taken as sufficient evidence of a lack of carcinogenic potential via a genotoxic mechanism. Hence, when substances show to be devoid of mutagenic/genotoxic potential in a suitable battery of tests, and show no structural alerts, carcinogenicity testing can be waived.

Non-genotoxic carcinogens are generally believed to exhibit a threshold dose for carcinogenicity and human exposure to residues of veterinary drugs is low, thus non-genotoxic compounds should not be routinely tested for carcinogenicity. For extremely low level of residues, and consequently negligible human exposure, carcinogenicity can be avoided for those compounds. Nevertheless, cancer bioassays are required if: 1) the compound is known to be animal or human carcinogen, 2) available systemic toxicity studies identify potentially preneoplastic lesions or findings indicative of neoplasia, or 3) systemic toxicity studies indicate that compound may be associated with effects known to be linked with epigenetic mechanisms of carcinogenicity that are relevant to humans [57].

Some opportunities for waiving the carcinogenicity testing are in place for veterinary drugs. As for human medicines, generics (Article 13 of Directive 82 [61]), vaccines or biotechnology derived pharmaceuticals are usually not tested. New formulations of known ingredients used for human medicines are also not tested for carcinogenic potential and the information from single active ingredients is accepted. Also, data requirements can be reduced when a substance is classified as MUMS (for minor use minor species/limited market) [62]. In this case, species other than major food-producing animals (cattle, sheep, pigs, chickens, salmons) and major companion animal species (cats and dogs) are considered as minor.

Based on the rules laid down for the authorization and the making available on the market of **Pesticides - Plant Protection Products** (PPPRs) [63-65], carcinogenicity study is required for all new active substances (Figure 2). Only *'If in exceptional circumstances it is claimed that such testing is unnecessary, that claim shall be fully justified'*. A long-term oral toxicity study and a long-term carcinogenicity study (two years) of the active substance shall be conducted using rat as test species; where possible these studies shall be combined. A second carcinogenicity study (78 weeks) of the active substance shall be conducted using mouse as test species, unless it can be scientifically justified that this is not necessary. In such cases, scientifically validated

alternative models may be used instead of a second carcinogenicity study. Studies can be preceded by range-finding studies in both species and always by a comprehensive battery of *in vitro* and *in vivo* genotoxicity tests.

As of March 2013, *in vivo* testing is no longer permitted for **Cosmetic Ingredients and Products** [25, 66] (Figure 2). The Cosmetics Regulation [25] prohibits the placing on the market of cosmetic products containing ingredients, which have been tested on animals to meet the requirements for toxicity testing. Some chemical ingredients may, however, also need to be registered under REACH [67]. The relationship between the marketing ban and the REACH information requirements is as follows: A) Registrants of substances that are exclusively used in cosmetics may not perform animal testing to meet the information requirements of the REACH human health endpoints, with the exception of tests that are conducted to assess the risks to workers exposed to the substance. 'Workers' in this context, refers to those involved in the production or handling of chemicals on an industrial site, not to professional users using cosmetic products as part of their business (e.g. hairdressers); B) Registrants of substances that are used for a number of purposes, and not solely in cosmetics, are permitted to perform animal testing, as a last resort, for all human health endpoints, C) Registrants are permitted to perform animal testing, as a last resort, for all environmental endpoints.

The decision on the carcinogenic potential of mutagenic or genotoxic substances may be made on the outcome of *in vitro* mutagenicity tests. A positive *in vitro* result in mutagenicity testing is seen as indicative for the carcinogenic potential of substances. *In vitro* mutagenicity tests are quite well developed, although they suffer from a low specificity. Due to the relation between mutations and cancer, these genotoxicity tests can be seen as a pre-screening for carcinogenicity. A positive result in one of the genotoxicity tests may be indicative for considering a substance as putatively carcinogenic. Usually these substances are not further pursued in development.

At present, generally accepted alternative *in vitro* methods for carcinogenicity with OECD Test Guidelines are not available. There are promising *in vitro* approaches which may be helpful to recognise genotoxic as well as non-genotoxic carcinogenic substances. For instance, the Cell Transformation Assay (CTA) may provide additional information when used as a follow-up assay for confirmation of *in vitro* positive results from genotoxicity assays, typically as part of a weight of evidence assessment. It has been demonstrated that the CTA has the ability to also detect some non-genotoxic carcinogens; however the exact performance in this regard is still under evaluation.

Research is on-going with regard to *in vitro* toxicogenomic-based tests for the detection of mutagens, genotoxic carcinogens, and particularly non-genotoxic carcinogens. Global gene expression profiling via microarray technology and gene patterns covering diverse mechanisms of substance-induced genotoxicity can be extracted. These gene patterns/biomarkers can be further used as a follow-up of positive findings of the standard *in vitro* mutagenicity/genotoxicity testing battery. In addition to *in vitro* mutagenicity/genotoxicity tests, data from *in vitro* tests combined with toxicogenomics may also be considered in a weight of evidence approach [66].

3.2 Animals used for experimental and scientific purposes in the EU

Since 1986, the EU has had in place specific legislation covering the use of animals for scientific purposes [68, 69]. On 22 September 2010 the EU adopted Directive 2010/63/EU [68], which updated and replaced the *1986 Directive 86/609/EEC* on the protection of animals used for scientific purposes. The aim of the new Directive is to strengthen legislation, and improve the welfare of those animals still needed to be used, as well as to firmly anchor the principle of the 3Rs[2], to Replace, Reduce and Refine the use of animals in the legislation. Directive 2010/63/EU took full effect on 1 January 2013 and it now represents an integral part of the hazard identification and risk assessment process.

The Directorate General for the Environment (DG ENV) has been reporting on a three-year basis statistics in regard of the actual number of animals used in Europe in different sectors and for different purposes. Data have been collected by each Member State (MS) since 1996. The 7th report relating to year 2011 is the most recently available at EU level [20]. Since the adoption of Directive 2010/63/EU the statistical reporting requirements have been fully revised. MSs are currently busy in implementing the new data collection rules in the national reports covering year 2014, which they are required to make publicly available as per the Directive [68]. EU statistics on animal use report is foreseen not before November 2019.

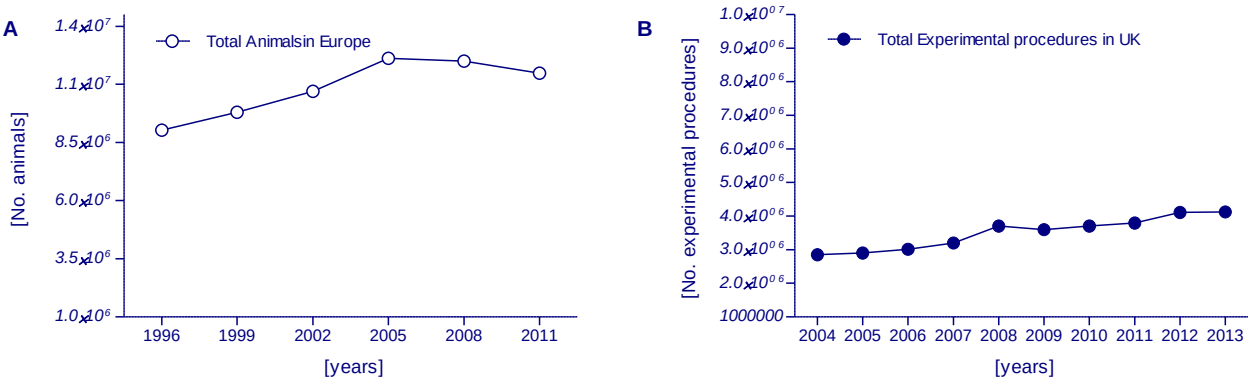


Figure 3 Animals used in Europe. (A) Total number of animals used for scientific purposes in Europe from 1996 to 2011, as reported in DG ENV Reports. Data are not normalized for the number of MSs participating to the survey. (B) Statistics in UK showing yearly-basis data on the number of scientific procedures involving animals performed in UK in the last decade, 2004-2014.

The DG ENV reports provide an estimate of the number of animals used within different sectors and in different scientific areas, in Europe (Figure 3). Over the years the methodology applied for data collection and the surveys sent to each MS underwent constant modifications and some categories overlapped with each other (i.e. test type or specific purposes); also participants to the surveys changed over the years (the number of MS increased up to 27). Nevertheless, the number of animals used for scientific purposes showed an increase up to year 2005, reaching approximately 12 million per

[2] Replacement: substitution of insentient material for conscious living higher animals. Reduction: reduction in the number of animals used to obtain information of a given amount and precision. Refinement: any decrease in the incidence or severity of in humane procedures applied to those animals, which still have to be used.

year, a slow decrease instead to 11 million is observed between 2005 and 2011 (Figure 3A).

Areas of animal use can vary significantly. Animals are employed for basic research, for pharmaceuticals research and development, for production and quality control of human and veterinary medicines and medical devices, for toxicological and safety assessment, for diagnosis of diseases, education and training (Figure 4A).

It is worth noting that 50% of animals used in Europe (Figure 4A) are employed for biological studies/basic research; this signifies almost 6 million animals, mostly rodents. Thirty per cent approximately of all animals are used for toxicology testing, safety pharmacology and production and quality control of medicines (vaccines, antibodies, etc.).

Roughly 9% of animals are used instead, specifically to satisfy regulatory requirements for toxicity and safety evaluation, this percentage corresponds to 1 million animals approximately (Figure 4A). Regulatory toxicology, which considers all toxicity endpoints (acute, chronic, irritation and skin sensitization, reproductive and developmental, ecotoxicity, mutagenicity and carcinogenicity) across all sectors, has shown a steady-state trend in the number of animals used in years 1999-2011 (Figure 4B).

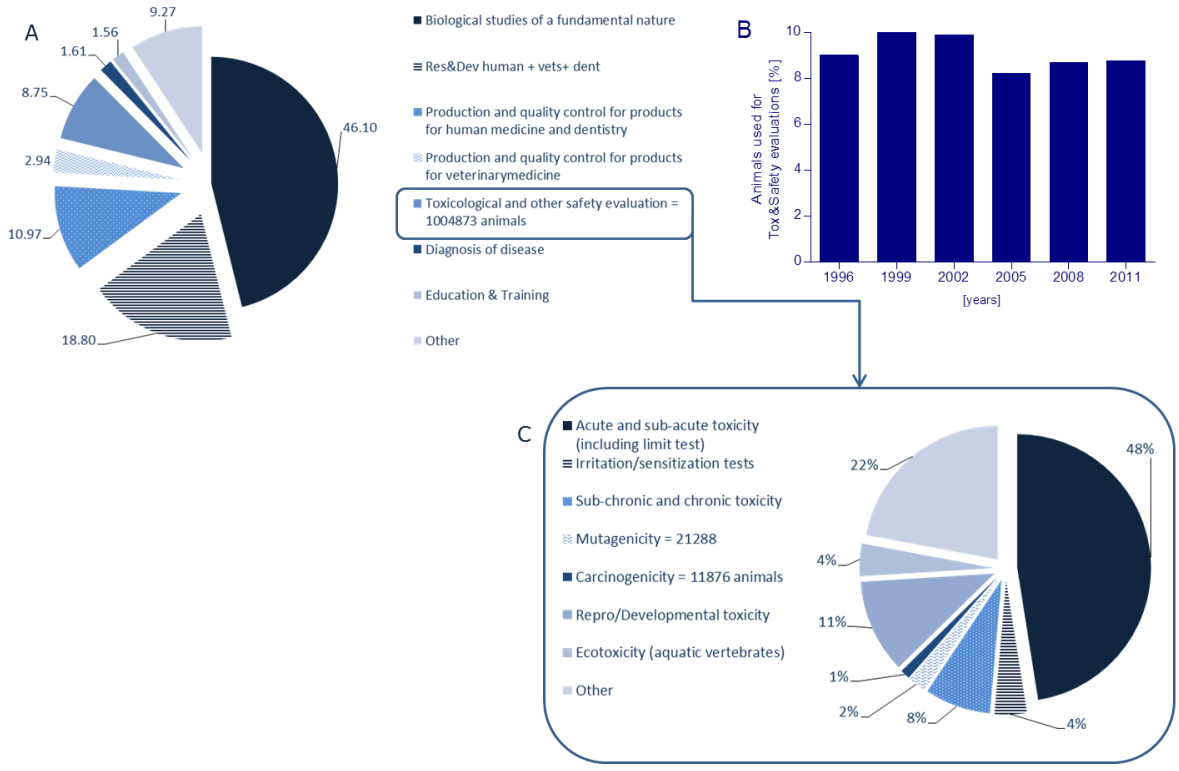


Figure 4 Animals used for experimental and scientific purposes. A) Number of animals used, as percent, on the basis of the different scientific purposes. In 2011, over 1 million animals were used for regulatory toxicology and other safety evaluation, representing 9% approximately of the total number of animals used in the EU. B) Trend of animals used for toxicology and safety evaluation in Europe, between 1996 and 2011. C) Percent number of animals used for toxicity testing of selected endpoints. Data were retrieved from the 7th DG ENV Report on animals used for experimental and scientific purposes in the EU as in 2011 [20].

In 2011, year of most recent data gathering, acute toxicity and reproduction and developmental studies employed the majority of animals, mainly rodents (ca. 50%, Figure 4C). Sub-chronic and chronic toxicity studies involved a substantial number of animals, as well (ca. 8%), then irritation and skin sensitization (ca. 4%) (Figure 4C).

The analysis of carcinogenicity testing and its impact on the use of animals cannot be performed without considering genotoxicity studies that represent the starting point for safety evaluation of carcinogenic potential. Thus, as mentioned previously, the impact of studies used for both endpoints were analysed throughout the report.

When compared with the above endpoints, a lower percent of animals has shown to be used for carcinogenicity testing. In 2011, 1% (12000 animals approx.) of the total number of animals has been employed to conduct cancer studies and 2% (21200 animals approx.) to conduct *in vivo* genotoxicity tests.

The number of animals employed for carcinogenicity and genotoxicity studies varied largely between 1999 and 2011, as reported by DG ENV (Figure 5A). An overall negative trend in terms of animal burden within both areas was observed (Figure 5A).

However, data from older DG ENV reports (from the first to the third report) need to be considered cautiously; as mentioned above, the methodology used for data collection changed over the years and the number of MS participating the collection exercise changed as well. The decreased impact on animals seems to align with that observed on the total number of experimental procedures carried out in the UK during the last decade (Figure 5B, analysis performed by UK Statistics Gov.).

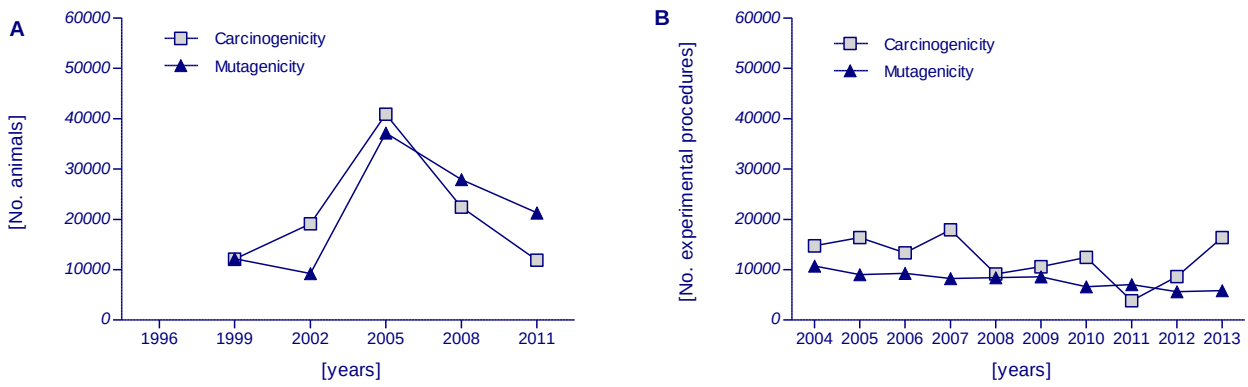


Figure 5. Animals used for carcinogenicity and mutagenicity in Europe. (A) Number of animals used for carcinogenicity and mutagenicity regulatory testing in Europe from 1996 to 2011, as reported in DG ENV Reports. Data are not normalized for the number of MSs participating to the survey. (B) Statistics in UK showing yearly-basis data on the number of scientific procedures involving animals for carcinogenicity and mutagenicity testing performed in UK in the last decade, 2004-2014.

Interestingly, while carcinogenicity and genotoxicity testing seem to exert a minimal impact on animal numbers when compared with other endpoints (Figure 4C), the animal burden varies significantly when considering different product-types, reflecting the differences lying within the rules guiding safety assessment across sectors (Figure 6).
In fact, the highest number of animals used for the safety assessment of the carcinogenic potential has been mainly ascribed to the testing of pesticides, biocides human and veterinary medicines for which, regulatory requirements are more conservative (Figure 6). In contrast, the full ban on *in vivo* testing for the safety assessment of cosmetics [25, 66] has zeroed the impact of the cancer study in terms of animals used for this sector, though in practice cancer studies have not been performed since 1999. Similarly, the introduction of REACH Regulation [38] within the industrial chemical sector has reduced substantially the requirement for carcinogenicity studies (Figure 6), limiting the conduct of two-year cancer studies to high tonnage band chemicals, previous submitter testing-proposal and approval by ECHA.

The overall number of animals and experimental procedures for *in vivo* genotoxicity has also lowered in the past decade (Figure 5A-B) though, with relevant differences depending on type of product (Figure 6).

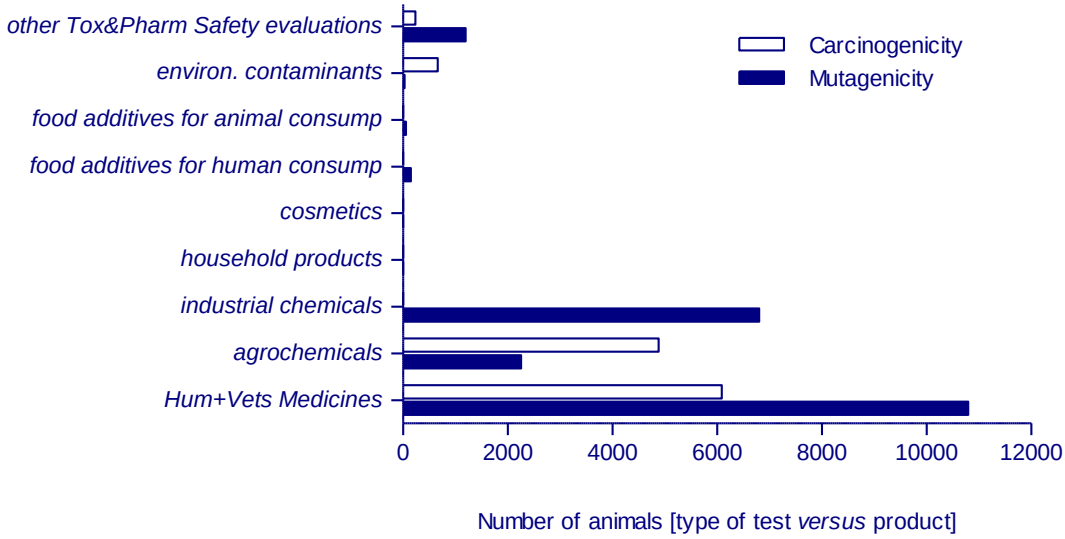


Figure 6. Number of animals used for carcinogenicity and mutagenicity testing. Number of animals used for carcinogenicity and mutagenicity testing across different product-sectors. Data were retrieved from the 7th DG ENV Report on animals used for experimental and scientific purposes in the EU as in 2011 [20]

Of worth, as shown from data of 2011 (Figure 6), are the striking differences in the number of animals used for carcinogenicity *versus* genotoxicity testing within pharmaceutical and agrochemical sectors.

In the case of human and veterinary medicines, the number of animals used for the genotoxicity endpoint doubles that for carcinogenicity. Reasons for the observed discrepancy can be several: a) data collected in the DG ENV report are from EU studies only, drug development is global (but this is valid for other sectors as well) thus, many *in vivo* studies, two-year bioassay especially, are performed outside Europe also for those products entering the European market; b) carcinogenicity studies are often conducted late in development, i.e. for those products with strong likelihood to get the marketing authorization whereas, genotoxicity studies are conducted at earlier stages of development on all products the majority of which will never get a marketing authorization and will die during development; c) there is no obvious temporal correlation between marketing authorization, conduct of genotoxicity studies and conduct of carcinogenicity studies per year, as in the case of DG ENV statistics report.

Differently, in the case of agrochemicals (Figure 6), a higher number of animals are used for carcinogenicity studies compared to those used for genotoxicity. As expected these data are in line with the substantial difference between number of animal per study per endpoint (50-60 per genotoxicity study; >600 per two-year bioassay). In addition, requirements for agrochemicals are extremely conservative and it is supposed that carcinogenicity studies are always performed, in two different species and are rarely waived.

3.3 Product authorizations in Europe

A third level of study related to the EU product marketing authorization dossiers in the decade 2004-2014. These, were investigated in order to sourcing out information on the actual demand of carcinogenicity and genotoxicity studies per year per sector in the EU.

Compounds from different product sectors were first grouped by date of authorization in the EU market (Figure 7).

Grouping of pesticides, human and veterinary medicines, and biocides was feasible, because of available databases. Data were available since 2004 on a year-base; while a systematic data collection for biocides begun in 2009 only. Overall, a constant amount of substances have been authorized per year per sector over time, except for human medicines, for which a steady-state increase is observed between 2004 and 2014.

Major variations in the amount of authorized products observed in years 2007, 2009 and 2011 correlate well with key modifications in regulatory testing requirements: introduction of REACH Regulation in 2006, CLP Regulation in 2008, PPPs Regulation in 2009 [10, 38, 63]. In this context, it is plausible that the extremely high number of authorizations of pesticides (approx. 160) in 2009 could be partly due to those newly-adopted regulatory requirements. Interestingly, out of those 160 authorizations for pesticides only one was for a new active ingredient, the remaining were re-submissions of pre-existing substances (Figure 7A).

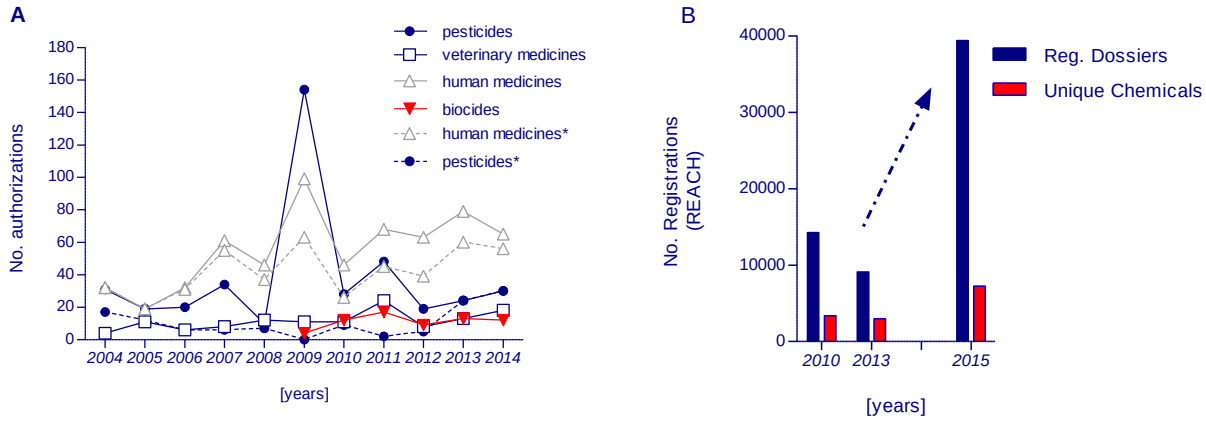


Figure 7. Number of authorizations in the EU between 2004 and 2014. (A) Data for pesticides, biocides and pharmaceutical products. Numbers were calculated on the basis of publicly available information gathered from the different databases (EFSA, EMA and ECHA) of authorized active subtances. *Number of new active substances. (B) Number of Registration dossiers and unique chemicals as reported for the different REACH deadlines: November 30 2010, May 31st, 2013 and most recent available update, August 21st 2015 (ECHA website).

Information for industrial chemicals instead, has been based for the time-being on the two available registration deadlines of 2010 and 2013 (Figure 7B), related to high tonnage level chemicals only (≥ 100 tonnes/yr and ≥1000 tonnes/yr).

It is worth noting that the registration applies to substances on their own, substances in mixtures and certain cases of substances in articles though, the analytical and spectral information provided shall be consistent and sufficient to confirm each substance identity.

Based on the above data, the average number of new products on the market per year in Europe could be estimated as follows: a) human medicines: 56 authorizations per

year, 35 of which being new entities; b) veterinary medicines: 11 authorizations per year, 10 of which being new entities; c) pesticides: 38 authorizations per year, 10 of which being new active substances; d) biocides: 12 authorizations per year, 5 of which being new active substances; e) cosmetic ingredients: 10 approximately; f) Industrial chemicals: n.d.. Pharmaceutical sector predominates the market.

This prompted to an in-depth analysis of the marketing authorization dossiers per each sector, as presented in the following results section for which year 2011 and year 2014 were chosen.

3.4 Industrial chemicals (ECHA, REACH Dossiers database)

The Regulation has set the following registration deadlines for phase-in[3] substances: *November 30, 2010*: for substances manufactured or imported at 1000 tonnes or more a year, substances that are carcinogenic, mutagenic or toxic to reproduction above 1 tonne a year and substances dangerous to aquatic organisms or the environment above 100 tonnes a year; *May 31, 2013*: for substances manufactured or imported at 100-1000 tonnes a year; *May 31, 2018*: for substances manufactured or imported at 1-100 tonnes a year.

Potential manufacturers and importers of the new substances, non-phase-in[4], have to submit an inquiry to ECHA and subsequently register the substances before they can manufacture or import them.

For the aim of the current analysis we have referred to the report published by ECHA in 2014 'The Use of Alternatives to Testing on Animals for the REACH Regulation' [24] where, a thorough investigation of the dossiers submitted by registrants for the 2010 and 2013 deadlines (with a cut-off for collecting data set at October 1st, 2013) has been performed by ECHA experts. Figure 8 shows the number of endpoint study records (ESR) relative to carcinogenicity which represent the number of study summaries provided in the ECHA website (as IUCLID format) at the moment of the analysis.

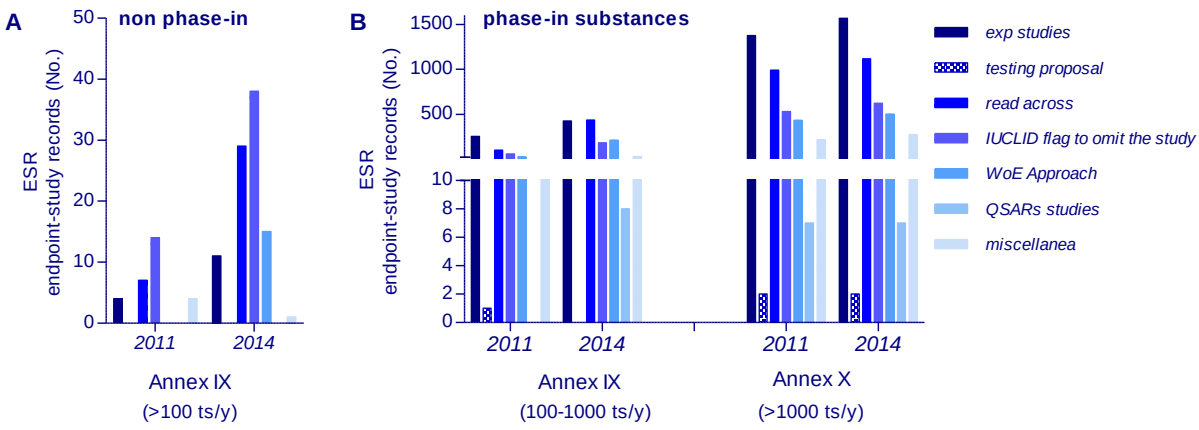


Figure 8.1 Carcinogenicity study records under REACH. Number of carcinogenicity study records submitted by registrants in the IUCLID database for non-phase-in (A) and phase-in substances (B) of dossiers subjected to analysis in 2011 and 2014. Study records for all substances between 100-1000 tonnes/y (Annex IX) and substances ≥1000 tonnes/y (Annex X) relative to deadlines 2010 and 2013 were analysed, including phase-in and non-phase-in as reported in [24].

[3] Substances that fulfil at least one of the following criteria may be considered as phase-in substances in accordance with REACH: 1) Substances listed in the European Inventory of Existing Commercial Chemical Substances (EINECS); 2) Substances that have been manufactured in the EU (including the countries that joined on 1 January 2007) but have not been placed on the EU market after 1 June 1992; 3) Substances that qualify as "no-longer polymer''.

[4] All substances that do not fulfil any of the criteria for phase-in substances are considered as non-phase-in substances. Normally, non-phase-in substances have not been manufactured, placed on the market or used in the EU before 1 June 2008, [unless they were notified under the Dangerous Substances Directive 67/548/EEC (70; replaced by CLP Regulation)].

Though highly represented for phase-in substances (Fig. 8.1B, Annex X especially), the majority of data on carcinogenicity studies were retrieved from published data, IARC reports or textbooks. On the other hand, the phase-in substances testing proposals for new carcinogenicity studies were reported to be 11 (Fig 8.1B), referring to 6 unique experimental studies (UES) and zero for non-phase-in substances (Fig. 8A).

Since the first deadline in 2010, carcinogenicity studies have been performed for two lead registration dossiers, referring to substances: 2-ethoxy-2-methyl propane EC 211-309-7 (CAS no. 637-92-3) and asphalt oxidized EC 265-196-4 (CAS no. 64742-93-2) (ECHA info, as of July 2015).

Standard requirements for high tonnage level have been mainly fulfilled with read-across information, weight of evidence approaches and partly with QSAR data (Fig. 8.1-8.2).

As expected, experimental studies to assess genotoxicity were more numerous than those performed in order to assess the carcinogenic potential of substances.

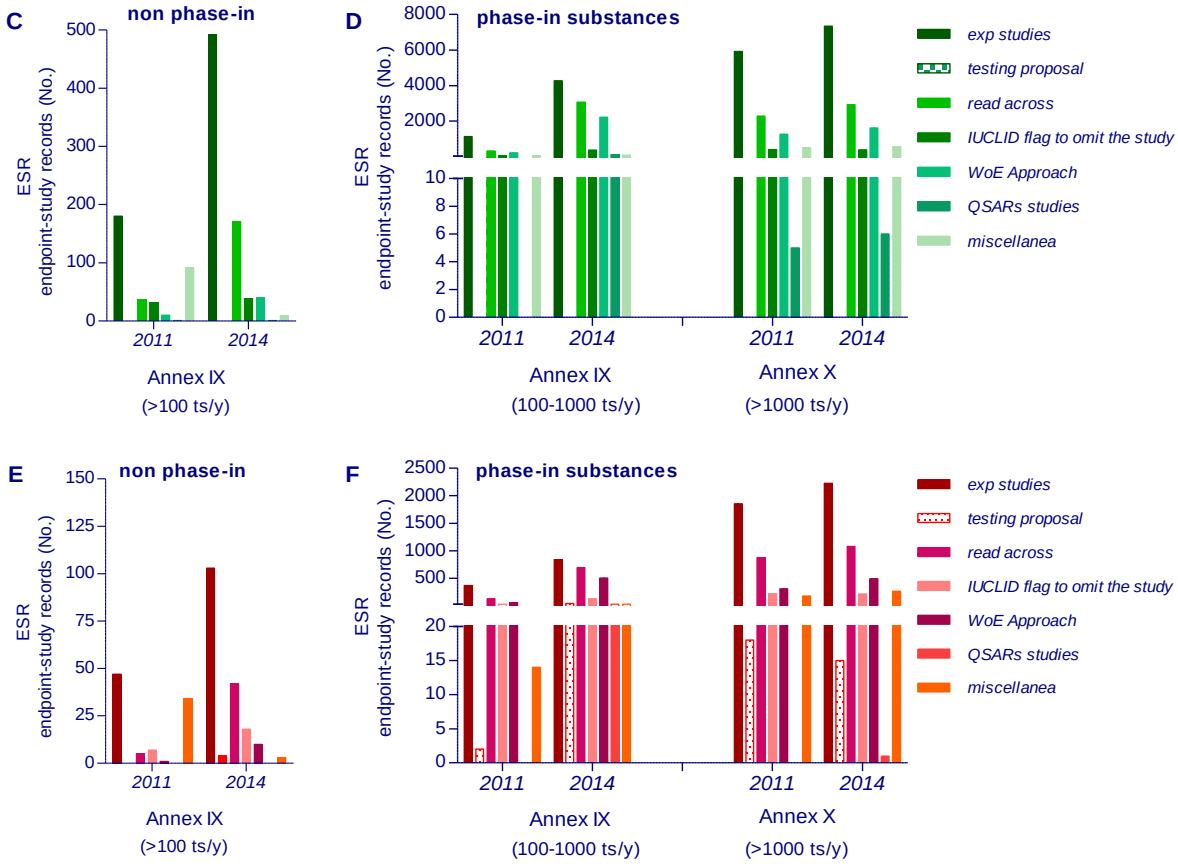


Figure 8.2 Genotoxicity study records under REACH. Number of study records submitted by registrants in the IUCLID database for non-phase-in and phase-in substances of dossiers subjected to analysis in 2011 and 2014. (C and D) Endpoint study records 'ESRs' for *in vitro* genotoxicity information. (E and F) ESRs for *in vivo* genotoxicity information. Study records for all substances between 100-1000 tonnes/y (Annex IX) and substances ≥1000 tonnes/y (Annex X) relative to deadlines 2010 and 2013 were analysed, including phase-in and non-phase-in as reported in [24].

3.5 Biocides (ECHA)

For almost 4420 biocidal products currently on the European market (those with released data, Article 95 List, published on February 27, 2015), 656 are unique active substances. Of those, 113 substances have been approved in accordance with Directive 98/8/EC (The Biocidal Products Directive concerning the placing of biocidal products on the market, 1998) [71] and EU Regulation 528/2012 [44]; while 521 are currently under review and 22 were not approved.

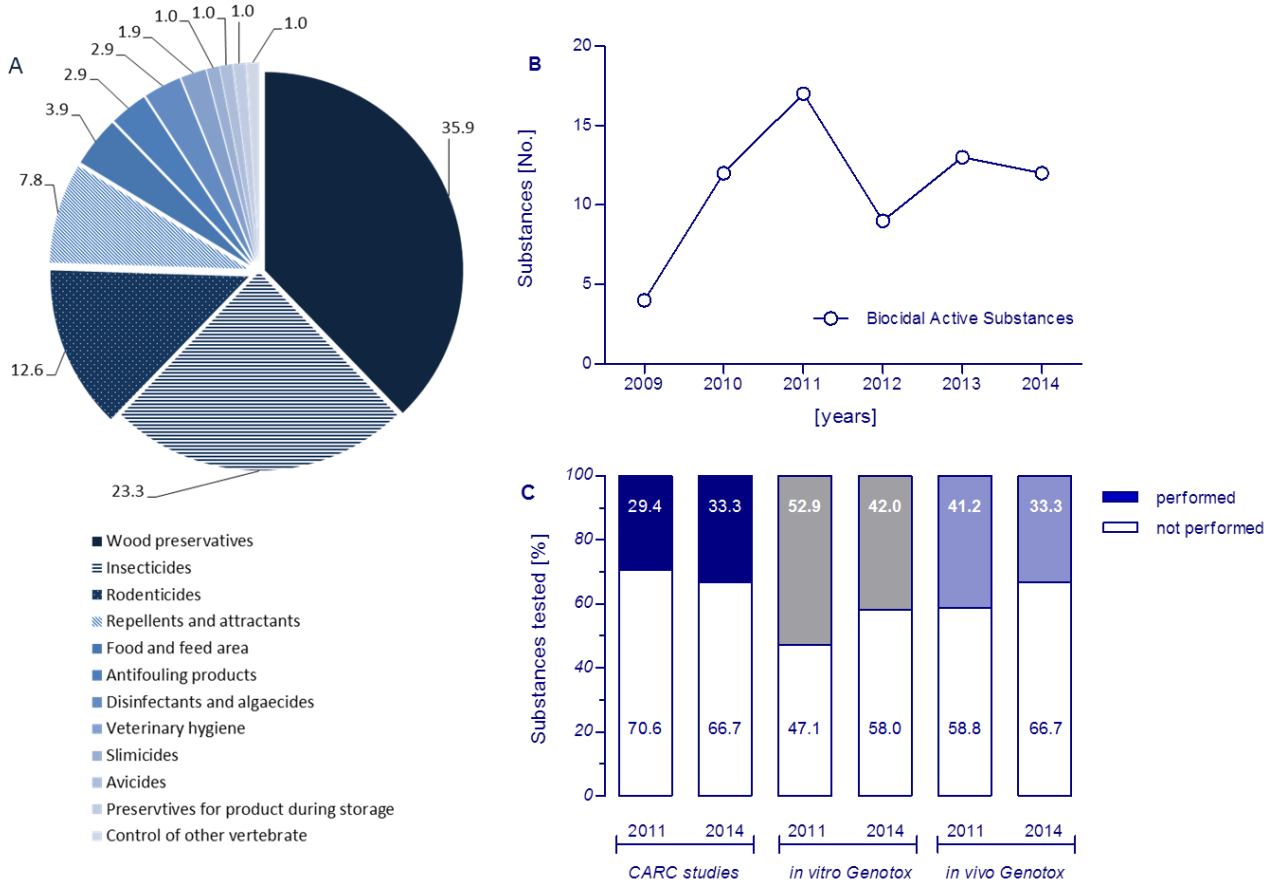


Figure 9. Information on biocides. A) Represented product types, as percent of total, within the authorized biocidal active substances list, available on ECHA website. B) Number of approved biocidal active substances per year between 2009 and 2014; data are available since 2009. C) Percent number of authorized biocidal active substances that has undergone carcinogenicity, *in vitro* and *in vivo* genotoxicity assessment in 2011 (n=17) and 2014 (n=12).

On average, 12 active substances approximately are authorized per year on the European market, the majority being represented by wood preservatives, insecticides, rodenticides and repellents and attractants (Fig. 9A-B). Not all of the authorizations though regard new active ingredients, as several substances were also assessed under PPPs or REACH legislations with respect of the specific human or environmental risk and/or the proposed use.

As other types of products, the genotoxic potential is assessed firstly on the majority of substances. In the two years considered, 2011 (total authorizations = 17) and 2014 (total authorizations = 12), genotoxicity tests *in vitro* were conducted in approximately 53% (8 out 17) and 42% (5 out of 12) of substances, respectively (Fig. 9C).

In vivo genotoxicity studies were integral part of 44% and 33% of assessment reports of substances authorized in 2011 and 2014, respectively. For those substances where opportunities for waiving genotoxicity *in vitro* and *in vivo* experiments were scientifically justifiable, supporting data from literature, IUCLID database or read-across were presented. In certain cases, based on the nature of the compound, the *in vivo* studies were not feasible (e.g. the compound is similar to warfarin: highly toxic and causing primary loss of blood coagulation).

Carcinogenicity studies were conducted in the 31% (5) and 33% (4) of substances in 2011 and 2014, respectively (Fig. 9C).

It is worth noting that in 2011 only 3 (out of 5) active substances underwent a thorough evaluation of carcinogenic hazard, as reported in the respective assessment reports. Two out of three compounds were tested by a combined chronic/carcinogenicity study in rats plus a second study in mice (chronic or two-year bioassay). A third compound was tested by a combined study in rats (TG 453; 16) and one study in dogs chronic/carcinogenicity (TG 452; 35) plus one 2yr-mouse study in B6CF and one two-year study in NMR1 mice (TG 451; 15). Two compounds (out of 5) were instead tested in two-year bioassay rats only (TG 451; 15).

In 2014, the two-year bioassay was performed in rats and in mice, for the safety assessment of two compounds. In a third case, the two-year bioassay was waived due to the non-genotoxic potential of the chemical assessed by a short-term (90-day) study in rats only. On a fourth compound, carcinogenicity was tested with a two-year bioassay in rats only. Justifications for waiving the carcinogenicity study required in the second species were mainly linked to the lack of genotoxic potential or the specificity to the animal response.

In one case, the waiving concept was refined also to avoid completely the carcinogenicity assessment on the basis of exposure pattern and toxicological profile. Hence, a negligible secondary human exposure, very low primary exposure, anticipated lack of substance-related non-genotoxic and genotoxic MOA and absence of structural alert were the main justifications.

In accordance with requirements set out in the *Annex II* of the Regulation [44], read-across data with respect of known chemicals and literature report documents were also used to waive carcinogenicity testing.

Detailed information from publicly available dossiers from ECHA website on biocides consulted for the analysis is reported in Annex I of this report.

3.6 Medicinal products for human and veterinary use (EMA)

3.6.1 Human medicines

Those medicines for human use authorized within the European market from 2004 to 2014 through the centralized procedure by the competent authority, the European Medicines Agency (EMA), for which there were publicly available assessment histories (EPARs), have been searched (Figure 10). This means that many other products, authorized through a decentralized procedure yet, without a traceable assessment profile history, were not considered for the analysis.

For a thorough analysis of carcinogenicity studies performed on human medicines, it was necessary to consider the type of submission and differences among pharmaceutical product classes. In fact, requirements specific to non-clinical as well as clinical aspects differ substantially on the basis of: A) the nature of the product (varying form a new active pharmaceutical ingredient (API), to a generic substance, a biosimilar, or orphan drug[5], B) the chemical identity, C) its formulation or D) the final therapeutic use.

Type of submissions/year	Average (2004-2014)	[%] of total
submissions	**65**	
authorizations	*56*	*86%*
withdrawn	*6*	*10%*
refused	*3*	*4%*
generic drugs	*12*	*18%*
biosimilars	*2*	*3%*
orphan drugs	*7*	*11%*
new entities*	*35*	*55%*

Table 3. Number (as average) and type of submissions at EMA for human medicines between years 2004-2014, as from EMA data.* new entities = new chemical entities. These include also vaccines or biotechnology-derived medicines.

[5] ***Generic designation*** A generic medicine is a medicine that is developed to be the same as a medicine that has already been authorised, called the 'reference medicine'. A generic medicine contains the same active substances as the reference medicine, and it is used at the same doses to treat the same diseases. However, a generic medicine's inactive ingredients, name, appearance and packaging can be different from the reference medicines. Generic medicines are manufactured according to the same quality standards as all other medicines. A company can only develop a generic medicine for marketing once the period of exclusivity on the reference medicine has expired. This is usually 10 years from the date of first authorisation.
Biosimilar designation A biosimilar is a biological medicinal product that contains a version of the active substance of an already authorised original biological medicinal product (reference medicinal product) in the European Economic Area (EEA). Similarity to the reference medicinal product in terms of quality characteristics, biological activity, safety and efficacy based on a comprehensive comparability exercise needs to be established [54].
Orphan designation To qualify for orphan designation, a medicine must meet a number of criteria: 1) it must be intended for the treatment, prevention or diagnosis of a disease that is life-threatening or chronically debilitating; 2) the prevalence of the condition in the EU must not be more than 5 in 10,000 or it must be unlikely that marketing of the medicine would generate sufficient returns to justify the investment needed for its development; 3) no satisfactory method of diagnosis, prevention or treatment of the condition concerned can be authorised, or, if such a method exists, the medicine must be of significant benefit to those affected by the condition. Applications for orphan designation are examined by the EMA's Committee for Orphan Medicinal Products (COMP), using the network of experts that the Committee has built up. The evaluation process takes a maximum of 90 days from validation.

The expectation that all medicines be tested in two-year rodent studies has been undergoing considerable discussion in the past decade. This is not only due to the limited human relevance of data, but also to the emerging field of biopharmaceuticals, to a more realistic benefit-risk analyses and to the availability of alternative test models. In addition, short-term repeat-dose toxicity studies, as well as other *in vitro* and *in vivo* mechanistic studies are currently used to better understand the human relevance of findings in rodents (e.g. toxicokinetic or species-specific effects) or the mechanism and associated dose-response for a chemical [72, 73]. Nevertheless, with some exceptions, two-year rodent carcinogenicity studies are still conducted for most of small molecule pharmaceuticals (SMs). Since genotoxic agents are generally screened out at the development phase, two-year carcinogenicity studies are primarily used to assess potential non-genotoxic carcinogenicity.

Approximately 50 to 70 assessment reports (EPARs) are published per year (Table 3). Based on data retrieved (as on March 20, 2015), roughly 86% of all submitted substances are positively evaluated and authorized to enter the European market every year; on average, 10% of drugs are withdrawn and 4% per year of all drugs are rejected.

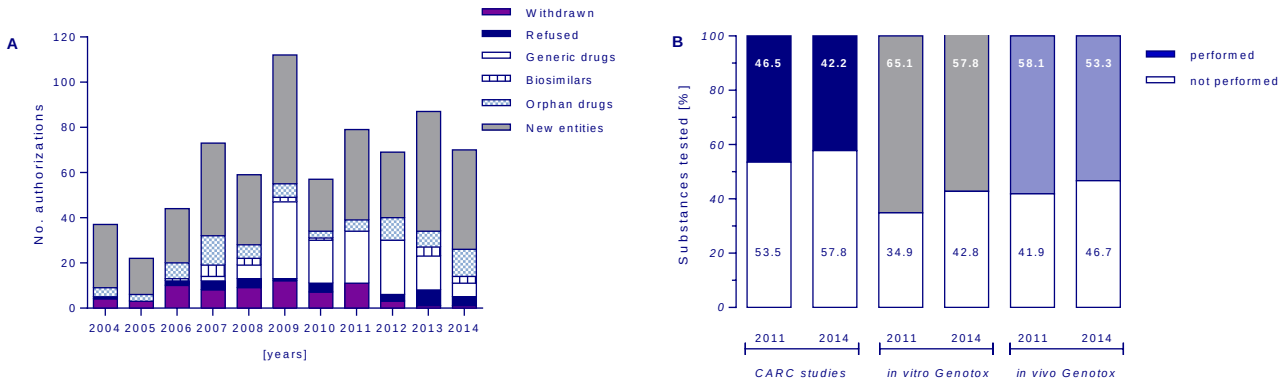


Figure 10. Information on human medicines. A) Total number of submissions and type of substances per year between 2004 and 2014. B) Percent number of authorized new substances (excluding generics, biotechnology-derived medicines and biosimilars) that has undergone carcinogenicity, *in vitro* and *in vivo* genotoxicity testing in 2011 (n=43) and 2014 (n=45), as from EPARs documents.

In Figure 10A, the 'new entities' portion of medicines showed a steady state increase over the years. New entities include both new small molecules and new biotechnology-derived medicines, the latter being gradually increasing.

Generic and biosimilar drugs introduced into the market between years 2006-2013, increased significantly instead, representing almost half of the whole market between years 2009 and 2012. The authorization of orphan drugs has increased, as well (Figure 10A). 34% of authorized products in 2011, for instance, had the 'generic' designation, 10% in 2014. None of them are normally tested either for carcinogenic potential or *in vivo* genotoxicity. Thus, they do not represent an animal welfare concern. In these cases, assessment reports justify the carcinogenicity testing waiving on the basis of general principles described in the Summary of Product Characteristics (SmPC) information [74, 75]. The non-clinical aspects of the SmPC are considered in line with the SmPC of each reference product, already tested. In addition, many generics products are also entering the market through a decentralized procedure of authorization, always without the need of carcinogenicity testing (two-year bioassay).

For this reason, when analysing the percentage of substances undergoing carcinogenicity or genotoxicity testing from years 2011 and 2014, as reported in Figure 10B, generics, biotechnology-derived medicines and biosimilars were not considered.

As shown in Figure 10B, when taking into account the dossiers submitted for authorization in the two years 2011 and 2014, approximately 47% and 42% of all new medicines have been tested for carcinogenicity by means of the two-year bioassay, respectively. The *in vitro* genotoxicity tests have been performed for approximately 65% and 58% of substances, in 2011 and 2014, respectively. *In vivo* genotoxicity tests have been performed in 58% and 53% of cases, respectively (Figure 10B).

Different types of justifications for waiving the carcinogenicity testing were reported in the dossiers, as in the case of medicines for very short-term treatments; life-threatening treatments; in the case of extremely short patient's life expectancy.
A new carcinogenicity testing was not required when information was already submitted and evaluated by other authorities (FDA or Japan). New products, as new combinations of known substances, could not be tested as well. In other cases information on carcinogenic potential could be also obtained from studies conducted post-authorization (summarized in Table 6).

Despite the number of alternative approaches to animal testing that are being explored, the percent of active substances undergoing carcinogenicity testing to comply with current regulatory requirements did not decrease accordingly in recent years. Detailed information from publicly available EPARs consulted for the analysis is reported in Annex II of this report.

3.6.2 Veterinary medicines

Differently from human medicines, a fewer number of dossiers are submitted to EMA and assessment reports (EPARs) published per year. Between 2004 and 2014, the average number of submissions in Europe has been approximately 12 per year: almost all the submitted dossiers resulted in approvals (90%), 10% instead were withdrawn. Among the authorized products, generic drugs account for 11% of total, but none has been authorized as biosimilar drug (Table 4 and Figure 11A).

As in the case of human medicines, for the purpose of this analysis, generic and biosimilar (none in the case of veterinary products within the period analysed) and biotechnology-derived medicines were not considered, since no requirement for carcinogenicity testing is generally in place.

Type of submissions/year	Average (2004-2014)	[%] of total
submissions	**12**	
authorizations	*11*	*89.6%*
withdrawn	*1*	*9.5%*
refused	*0*	*0.8%*
generic drugs (of those authorized)	*1.3*	*11.3%*
biosimilar drugs (of those authorized)	*0*	*0%*
new entities (of those authorized)	*10*	*88.7%*

Table 4 Number (as average) and type of submissions at EMA for veterinary medicines between years 2004-2014, as from EMA data.

As shown in Figure 11B, when taking into account the new substances authorized in 2011 and 2014, a lower impact on animal use for the testing of veterinary medicines compared to the human counterparts is evident.

In total, 24% approximately of 21 authorized substances in 2011 have been tested for carcinogenicity. Several were substances well characterized or known to be devoid of any genotoxic effect resulting in no carcinogenic potential risk. Instead, none of the 18 substances authorized in 2014 have been tested for carcinogenicity on the basis of different justifications.

Specifically, in 2014, 12 out 18 substances were vaccines, thus requiring nor carcinogenicity neither genotoxicity testing. Of the remaining 6 substances, 2 were combinations of known active ingredients; one substance was considered for minor use minor species/limited market (MUMS); 2 substances were considered clearly non-genotoxic, with no structural alert and devoid of any potential carcinogenic risk: as in the case of Iitraconazole, it was shown to be devoid of mutagenic/genotoxic potential in a suitable battery of tests and the molecule had no structural alerts. One substance was considered similar to another one, authorized in the same period. For all the above entities, the carcinogenicity testing was fully waived.

In vitro genotoxicity has been performed in approximately 38% of the substances in 2011 and 17% in 2014, while *in vivo* studies of genotoxicity have been performed in 33% and 11% of substances authorized in 2011 and 2014, respectively (Figure 11B).

Detailed information from publicly available EPARs consulted for the analysis is reported in Annex III of this report.

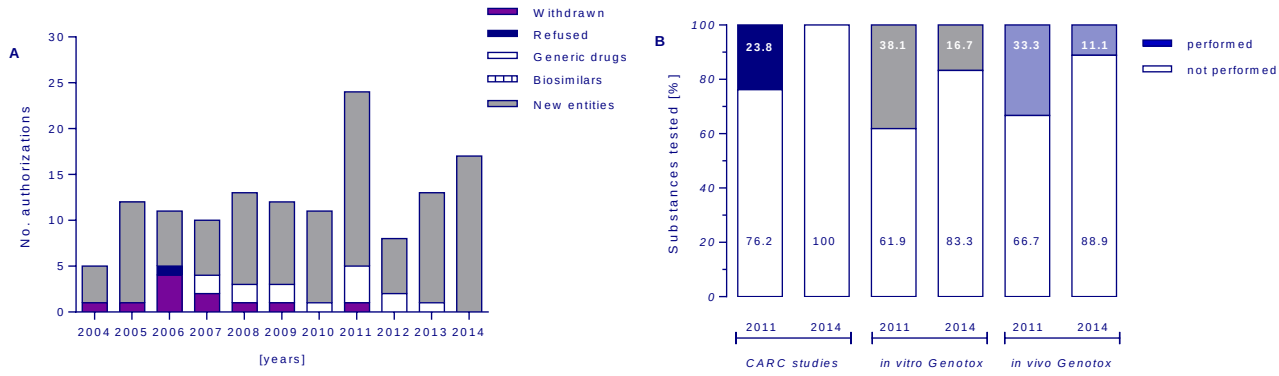


Figure 11. Information on veterinary medicines. A) Total number of submissions and type of substances per year between 2004 and 2014. B) Percent number of authorized new substances (excluding generics and biotechnology-derived medicines) that has undergone carcinogenicity, *in vitro* and *in vivo* genotoxicity testing in 2011 (n=21) and 2014 (n=18), as from EPARs documents.

3.7 Pesticides (EFSA)

Pesticides are intended for the following uses: 1) protecting plants or plant products against all harmful organisms or preventing the action of such organisms; 2) influencing the life processes of plants, such as substances influencing their growth, other than as a nutrient (e.g. plant growth regulators, rooting hormones); 3) preserving plant products, (e.g. extending the life of cut flowers); 4) destroying undesired plants or parts of plants (e.g. herbicides/weed-killers to kill actively growing weeds); 5) checking or preventing undesired growth of plants. Approved active substance included in pesticides products may consist also in micro-organisms, pheromones and botanical extracts (bio-pesticides). The most represented types of products are: fungicides, herbicides, insecticides, plant growth regulators, acaricides, etc. (Figure 12A).

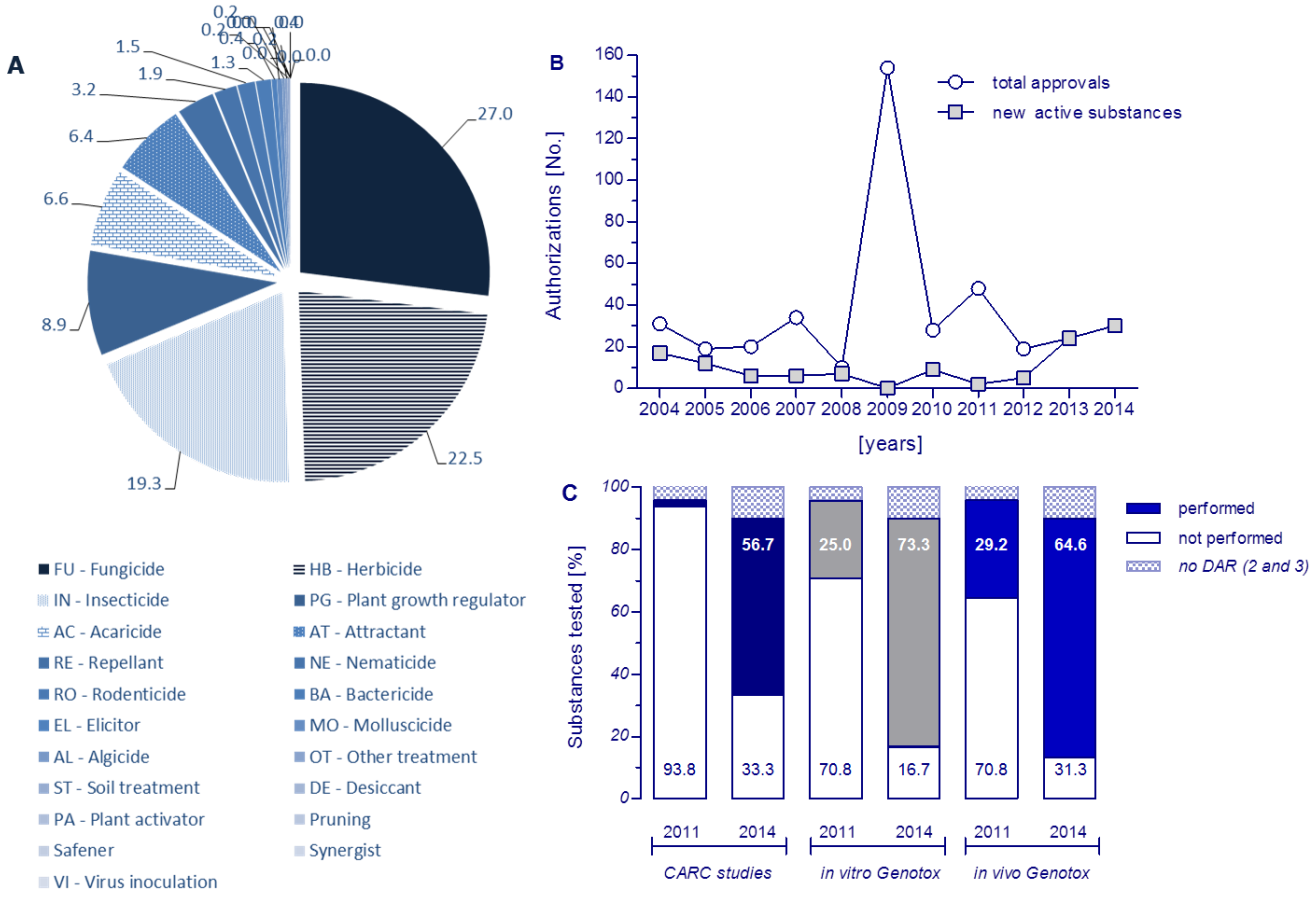


Figure 12. Information on pesticides. A) Represented product types, as percent of total, within the authorized substance list, available in DG SANTE PPPs DB. B) Total number of approvals and new active substances per year between 2004 and 2014. C) Percent of authorized active substances that has undergone carcinogenicity, *in vitro* and *in vivo* genotoxicity testing in 2011 (n=48; new active substances=2) and 2014 (n=30; all new) (as from available DARs documents and pesticides database).

The EU pesticide database, hosted on the DG SANTE website, collects information for 1315 active substances: 474 approved, 788 not approved, of 33 the authorization of which is still pending, 20 substances banned (as on July 21, 2015). Up to present, 77 active ingredients are on the list of candidates for substitution.

Within the last decade (2004-2014) there have been approximately 38 approvals per year on the European market, with an average of 10 new active substances each year (Figure 12B). It is worth noting that a significant increase of approvals, all related to renewals of existing substances, occurred in 2009 (n= 154). This probably reflects the introduction and updates of the 2009 Regulation on PPPs or CLP legislation earlier in 2008.

In 2011, exceptionally, 46 out 48 approvals regarded authorizations of pre-existent substances, undergoing re-evaluation. Only 2 new active substances were authorized and tested for carcinogenicity (2%) (Figure 12 B-C).

Testing in 2011		Substances tested for	Substances not tested for	DARs not available
Studies reported in the Dossier for 1^ submission	*Carcinogenicity*	**83.3**	**12.5**	**4.2**
	In vitro genotox	**91.7**	**4.2**	**4.2**
	In vivo genotox	**91.7**	**4.2**	**4.2**
Studies upon resubmission	*Carcinogenicity*	**2.1**	**93.8**	**4.2**
	In vitro genotox	**25.0**	**70.8**	**4.2**
	In vivo genotox	**31.3**	**64.6**	**4.2**

Table 5. Carcinogenicity, *in vitro* and *in vivo* genotoxicity assessment in 2011. Percent number [%] of substances that were previously tested for carcinogenicity, *in vitro* and *in vivo* genotoxicity endpoints, as reported in the original dossier and actual percent number [%] of substances tested upon resubmission. Far right column reports the percent number [%] of substances with no publicly available DAR.

Upon resubmission, several tests, mainly related to genotoxicity *in vitro* (25%) and *in vivo* (31%), have been requested for update or confirmation of data. The 46 substances undergoing re-evaluation had been previously tested for carcinogenicity (83%) and genotoxicity (92%), as reported in the original dossiers (Table 5).

In 2014, 57% of all new active substances have been tested for carcinogenicity (Figure 11C), 73% and 65% for *in vitro* and *in vivo* genotoxicity, respectively.

The opportunities for waiving the two-year bioassay study were limited to situations where, exposure was prevented or inexistent (treatments of ornamental plants) and clear negative results were obtained from sub-chronic or chronic toxicity tests and from tissues or organs in 90-days rat study.

Pesticides that have been derived from natural sources including, natural chemicals, pheromones, bacteria, fungi and insect predators[6] are not tested for carcinogenicity. Often data from tier 1 tests suggest that those substances are not compatible with the

[6] **Bio-pesticides** can be described as crop protection products that have been derived from natural sources. They include naturally occurring chemicals, pheromones, bacteria, fungi and insect predators. Whilst in many instances the environmental risks of these substances is considered to be less than that of the more traditional chemical pesticides this is not always the case. Data to support risk assessments is scarce and currently there is no single, reliable comprehensive data source. The University of Hertfordshire is a source of data for both the more traditional agricultural pesticides (PPDB) and veterinary substances (VSDB). The University offers the BioPesticide DataBase (BPDB) which is a relational database of data relating to pesticides derived from natural substances. [University of Hertfordshire (2013) The Bio-Pesticides Database (BPDB) developed by the Agriculture & Environment Research Unit (AERU); 76]

mammalian cell environment, thus short, chronic or carcinogenicity studies have been considered irrelevant.

In addition, for substances with extremely high toxicity, such as anticoagulants, chronic/carcinogenicity studies are not feasible (e.g. warfarin-like mode of action: high acute toxicity).

Detailed information from publicly available DARs consulted for the analysis is reported in Annex IV of this report.

3.8 Cosmetics (DG SANTE, DG GROW)

The two-year cancer bioassay had been rarely performed for testing cosmetic ingredients within the past 15 years. A combination of short term *in vitro* and *in vivo* studies has been used including *in vitro* and *in vivo* genotoxicity studies to assess genotoxic potential and repeat-dose toxicity studies (usually 90-day) to assess the risk of non-genotoxic chemicals, until 2009 [77].

For the purpose of the present investigation, the Scientific Opinions publicly available on the SCCS website [78] and adopted between 2004 and 2014 were consulted (as on October 27, 2015).

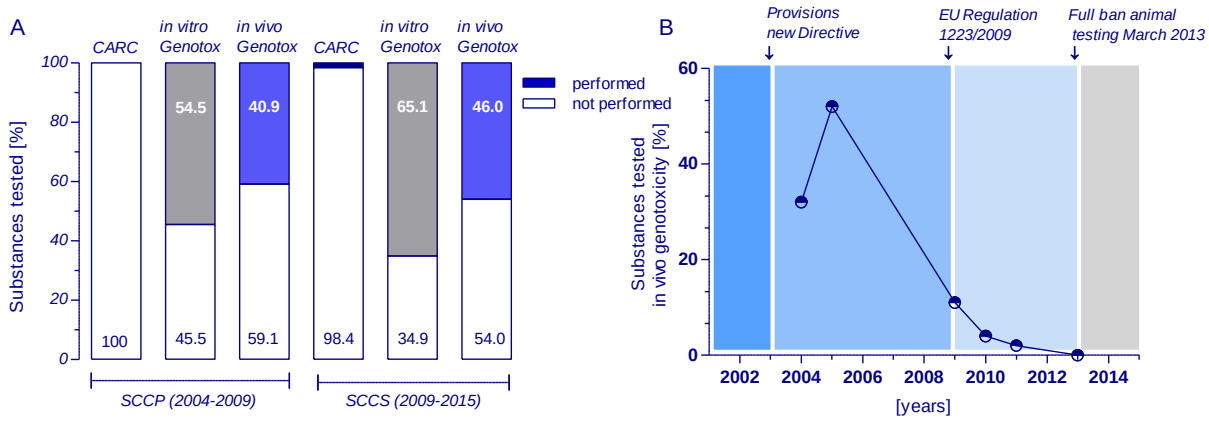


Figure 13. Information on cosmetics. A) Percent number of authorized cosmetic ingredients that has undergone carcinogenicity, *in vitro* and *in vivo* genotoxicity assessment between years 2004-2014. Data reported are based on Scientific Opinions published in 2004-2009 (n=63; revisions =23) and Scientific Opinions published within 2009-2015 (n=66; revisions 35) (as from publicly available SCCP and SCCS documents). B) Percent number of substances assessed for genotoxicity in *in vivo* tests between 2004 and 2014.

Cosmetic products consist of between 5 to 60 ingredients each. Overall, it can be estimated that there are more than 20000 cosmetic ingredients globally (see CosIng list reported below). A recent document published by the European Commission [79], has reported that only 10% out of total cosmetic ingredients are submitted to the SCCS for a Scientific Opinion. In about 90% of the cases cosmetic ingredients are used in other sectors and fall under different regulations, including cosmetic products outside the EU, food, pharmaceuticals, and detergents that are covered by REACH. It has been estimated that the number of new ingredients added per year to the market is around 70 for large suppliers and 22 for small medium enterprises (SMEs) between year 2000 and 2009, representing around 4% of their ingredient portfolio. Roughly, new cosmetic ingredients are estimated to be 5 per manufacturer from large suppliers and 2-3 from SMEs, respectively [79].

At present (as from 20 December 2015), active cosmetic ingredients registered within the European Commission Database CosIng [80] are:

- Ingredients & Fragrances: 24121
- Ingredients prohibited (Annex II): 1378
- Ingredients with restrictions (Annex III): 307
- List of allowed colourants (Annex IV): 153
- List of allowed preservatives (Annex V): 61
- List of allowed UV filters (Annex VI): 28

In the present analysis, those chemicals with different applications other than the cosmetics for which other regulations can warrant *in vivo* testing (e.g. industrial use) were not considered.

When consulting Safety Assessment Opinions from the last decade, the data on carcinogenicity were mainly references from literature or studies performed before 2004 (Figure 13A). Interestingly, the two-year bioassay has not been carried out since 2003 (two-year dermal in rat), ahead of full marketing ban on animal testing (March 2013). Nonetheless, safety assessment has included data from *in vivo* studies such as repeat-dose (90-day) or toxicokinetics, as well as, *in vivo* genotoxicity testing until March 2013. The last *in vivo* genotoxicity studies were performed in 2011 (Figure 13B).

Discussion and Conclusions

Marketed industrial chemicals, biocides, pesticides, human and veterinary medicines and cosmetic ingredients should be devoid, wherever possible, of toxic potential to humans or to the environment, including carcinogenic one and their toxicity should be well characterized in order to manage the risk and ensure an adequate degree of protection. Therefore, the carcinogenic hazard identification and characterisation represents an essential element of the safety assessment of any product manufactured, imported or sold in Europe and worldwide.

Since its introduction in the late 60s, the two-year cancer study in rodents has been a requirement for testing carcinogenicity for any product authorization dossier.

The objectives of carcinogenicity studies covered by test guidelines include:

- *The identification of the carcinogenic properties of a chemical, resulting in an increased incidence of neoplasms, increased proportion of malignant neoplasms or a reduction in the time to appearance of neoplasms, compared with concurrent control groups;*
- *The identification of target organ(s) of carcinogenicity;*
- *The identification of the time to appearance of neoplasms;*
- *Characterisation of the tumour dose-response relationship;*
- *Identification of a no-observed-adverse-effect level (NOAEL) or point of departure for*
- *establishment of a Benchmark Dose (BMD);*
- *Extrapolation of carcinogenic effects to low dose human exposure levels;*
- *Provision of data to test hypotheses regarding mode of action.*

[OECD TG 451, 2009 update; (15)]

Currently, the two-year bioassay represents the 'gold standard' and since the first OECD Test Guideline release in 1981, the study has remained almost unaltered.

Major modifications have applied, over the years, to the overall approach of carcinogenicity testing across the different product sectors, other than the study *per se*. The protocol, which specified the use of 3 dose groups of animals, the highest being a maximum tolerated dose (MTD)[7], with the focus of providing information on the possible health hazards likely to arise from repeated exposure for a period lasting up to the entire lifespan of the species used (objectives in the box) has been considered highly effective.

Hence, as mentioned in paragraph 3.1, the regulatory testing strategies for carcinogenicity have diversified significantly across the various legislations depending on the type of substance, the intended use and the level of exposure to humans and/or environment. However, two key elements have been maintained: testing for genotoxicity *in vitro*/*in vivo* and the two-year cancer study.

For industrial chemicals, for instance, requirements are based on a tier-approach testing method and on the annual amount of substance production, to which potential exposure and degrees of exposure are linked. Carcinogenicity testing is required only for the high tonnage level and mainly for mutagens category 3.
Extremely conservative requirements are in force and the testing of carcinogenicity is required for all the new pesticides and non-genotoxic new active biocides in two different species. For residues and metabolites the main concerns are also the exposure to very low doses that can persist for long periods of time and to occupational exposure. In those cases carcinogenicity is evaluated case-by-case.

[7] MTD was defined as the highest dose of a test substance used during the chronic study that can be predicted no to alter the animals normal longevity from effects other than carcinogenicity [81].

Of high concern are residues of veterinary drugs in food for human consumption (paragraph 3.1). It is a priority of this sector to rely on genotoxicity testing firstly and structural similarities, so positive results from those studies are further tested. Only when results from genotoxicity are clearly negative tests, no structure alerts are detected and the human exposure is negligible, carcinogenicity testing can be waived [57].

Human medicines are commonly administered at high doses to reach the effective pharmacological dose, with short or chronic exposures. Usually carcinogenicity testing is performed mainly for all drugs for which a chronic administration is foreseen. In this case a test-battery approach is used starting always with genotoxicity *in vitro* followed by a thorough *in vivo* testing of genotoxicity and carcinogenicity.

In contrast, no *in vivo* testing is allowed since March 2013 for cosmetic products [25] and carcinogenicity is predicted on the basis of alternative approaches only, relying mainly on *in vitro* genotoxicity studies.

Diversification across regulations can be considered as a consequence of diversity in the human health risk in regard of each product use, of new products development, of raising economic issues and partly of 3Rs initiatives. The latter have promoted several changes within regulatory toxicology testing since their first legal embedment in the first EU Directive (1986) on animal welfare. According to the latest figures, there has been a minimal decrease in the animal testing burden used for cancer studies (at least until 2011; Figure 4). In terms of absolute numbers this reduction could be regarded as negligible, as safety assessment of carcinogenicity *per se* is making use of fewer animals overall in comparison with other regulatory toxicity areas (e.g. acute, repro-, chronic, etc.), representing 1% of all toxicity testing (Figure 3C). However, in terms of animal welfare, a single two-year bioassay involves a large number of rodents, >600/study, it induces extended suffering, it implies a long-lasting period of data analysis and it has become extremely resource-consuming [77].

A significant number of carcinogenicity studies are performed in the area of basic research, mainly within Academia [20], though it is hardly referred to the standard two-year bioassay used for toxicological purposes. The ZEBET, the "Centre for Documentation and Evaluation of Alternative Methods to Animal Experiments", part of the German Federal Institute for Risk Assessment (BfR) has released a database on the number of experimental procedures and relative animals proposed to be used in submitted projects in the last two years. In 2014, for instance, the use of 215000 rodents approximately and 103 primates was estimated only for basic science projects related to cancer.

In addition to animal welfare concerns, the relevance to humans of the two-year cancer study has been also questioned at length [30-32, 82]. Several investigations on medicinal products, for instance, have shown that the majority of tumour findings observed in rodent carcinogenicity studies are considered not to be relevant for several reasons: a) a rodent-specific mechanism of carcinogenicity; b) a high safety margin between exposures at the NOAEL (No Observed Adverse Effect Level) in rodents and recommended therapeutic doses in humans; c) based on historical control data; d) differences in metabolism/local concentrations between rodents and humans [83, 84]. The authors have shown that in total 65% of medicines tested for carcinogenicity in rodents result positive and among these findings, the majority are not relevant for humans (69/94, 73%). Similarly, Alden et al (2011) [17] found 80% of positive rodent cancer tests involving commercially available drugs (searched in the Physicians' Desk Reference Network, PDR) without concern for human risk. The above data were in line with previous reviews [85, 86].

The resistance to the development and/or use of alternative (animal-free) approaches (*in vitro* tests, QSARs, structural alerts, mechanistic studies, read across, etc.) in this field has been mainly linked to technical difficulties of translating *in vitro* the whole process of carcinogenesis. Even in the presence of more elaborated *in vitro* tests, such as the cell transformation assay (CTA) or toxicogenomic-based tests [87-89]; the use of alternatives has been limited by concerns regarding the predictive capacity of these methods or the interpretation of data within the overall carcinogenicity assessment. Other concerns related to limited metabolic competences of the test systems, difficulty to discriminate genotoxic from non-genotoxic carcinogens or lack of mechanistic understanding [90]. Thus, carcinogenicity is far from being tested by only means of animal-free tests. It should be noted that several of the limitations explicitly highlighted for *in vitro* methods apply also to the animal carcinogenicity study.

Data reviewed in this report have shown that the two-year bioassay is most frequently conducted within the pesticides sector (DAR reports information), even if a decrease of the number of substances tested likewise has been observed between year 2011[8] and 2014. For most of the new active substances, a two-year rat bioassay study plus a combined chronic/carcinogenic rat study, often in combination with a second study in a second rodent species are conducted, even though the relevance of the latter has been questioned (Figure 11, Table 6) [91]. The PPPr Regulation foresees the use of alternative models to waive the second species though, this is rarely implemented. The carcinogenicity study is waived mainly on the basis of known limited human exposure risk, when it is technically not feasible, as in the case of some natural products or microorganisms, or on the basis of lack of genotoxicity effect of the substance (Table 6). In this regard, a conspicuous amount of substances are tested in *in vivo* genotoxicity studies. Each substance undergoes several genotoxicity tests: micronucleus, UDS study and COMET assay.

Differently from pesticides sector, the use of alternative approaches has been observed more frequently in the biocides sector. The use of read-across data has been reported in several authorization dossiers for either the testing of carcinogenic potential or of the genotoxic one. Opportunities for waiving the carcinogenicity testing of biocidal products are similar to those described above for pesticides (Table 6). Overall, the two-year bioassay has been performed on 30% of products (Table 6).

Within the pharmaceuticals sector, the introduction of specific shorter-term carcinogenicity studies as the transgenic mouse model agreed to be included in the ICHS1B guidance in 1997 [47], seemed at first to impact positively on the 3Rs. The use of P53+/- and the Tg.AC model, then the inclusion of the TgHras2, the XPA and XPAP53+/- murine genotypes [92] have shown promises for more technical specificity and impact on animal number. However, the transgenic model has resulted not to be a real reduction model, because of the amount of animals needed for the breeding of the specific knockout. It is worth noting that the use of transgenic animals has increased drastically in the recent years within the pharmaceuticals sector but especially in basic research [93].

Some modifications to the testing approach of human medicines have been included within the ICH guidance documents revisions [48-50] though, the latest versions are as for 1996 (S1A), 1997 (S1B), 2012 (S6 and revised S1 Concept Paper and Business Plan). Some 'opportunities/justifications' for waiving the cancer study include intrinsic product hazard, human health risk, risk/benefits ratios, the final use of medicine or the patient health status. However, in 2014, a substantial portion of authorized human medicines have undergone carcinogenicity testing and the use of only alternative approaches has been rarely considered.

[8] Data collected from 2011 authorizations were mainly re-submissions (see paragraph 3.5)

Within this sector, the two-year bioassay is not conducted for specific classes of therapeutic/diagnostic agents when it is not scientifically relevant or technically feasible (Table 6). For instance, none of the products authorized for veterinary use in 2014 have been tested for carcinogenicity, only because they were vaccines and biotechnology-derived proteins. Only few of them had known non-genotoxic non-carcinogenic potential (Table 6).

Sector	*Substances tested for carcinogenicity in 2014*	*Substances authorized or registered in 2014*	*Waiving of carcinogenicity testing is justified when:*
Pesticides	**66.7%**	**30**	natural products, highly toxic compounds: warfarin-like, short-exposure to humans (products for ornamental plants), microorganisms (incompatible with mammalian cell environment; the study is not feasible), short effect, not genotoxic, inexistent risk of exposure target (plant) not for human consumption
Biocides	**33.3%**	**12**	negligible secondary human exposure, very low primary exposure, lack of substance related non-genotoxic and genotoxic effect, satisfactory MOE and absence of structural alert, availability of read-across data
Human medicines	**42.2%**	**65** (69 % new entities =45)	*antibodies,* *recombinant proteins, vaccines,* *generics, biosimilars,* anti-cancer drugs, very short-term treatments, life-threatening treatments, extremely short patient's life expectancy, dossiers were presented to other authorities (FDA or Japan) Some of the studies will be performed post-authorization.
Veterinary medicines	**0.0%**	**18** (67% vaccines)	justifications similar to human drugs
Chemicals (REACH)	**0.0% (2 only)**	**>3000** 1600 exp. studies (>1000 tons/yr)	[data refer to the 2013 deadline registration, mainly old chemicals; up to date, only 2 cancer studies have been performed]
Cosmetics	**0.0%**	**7-10 approx.**[9] (10-15 SCCS Opinions)	n.a.

Table 6. Carcinogenicity testing across sectors in 2014. Percent number of authorized substances tested for carcinogenicity and type of justifications considered for waiving the testing: snapshot of 2014 situation.

[9] Estimate from EC Impact Assessment (2013) [79].

Of interest is the proposal to change the actual testing approach for rodent carcinogenicity (ICHS1 guideline) which is summarized in the latest Status Report of Regulatory Notice Document [52]. The new concept, formulated by the ICHS1 Expert Working Group, is based on the hypothesis that: *'a weight of evidence evaluation can, in certain cases provide sufficient information to conclude that a given pharmaceutical presents a negligible risk or, conversely, a likely risk of human carcinogenicity without conducting a two-year rat carcinogenicity study'*. The weight of evidence includes but is not limited to, data from chronic toxicity studies, genotoxicity and pharmacology. The rationale behind this proposal has been supported by a retrospective evaluation of several datasets from Industry and Drug Regulatory Agencies (DRAs) which suggests that up to 40% rat cancer studies might be avoided by integrating data from (6-month) chronic rat studies, evidence of hormonal perturbation and genotoxic profile in the standard battery [84, 94]. In addition, the knowledge on the pharmacological properties of the compounds, together with the histopathology findings from the chronic toxicity study in rodents may allow to define conditions under which the two-year rat carcinogenicity studies will or will not add value to the toxicology profile [95]. A prospective evaluation study to confirm the above hypothesis is undergoing [52]. The sponsors are invited, on a voluntary base, to submit to DRAs a Carcinogenicity Assessment Document (CAD) to address the carcinogenic potential of an investigational pharmaceutical and predict the outcome and value of the planned two-year rat carcinogenicity study and eventually claim a virtual waiver. A decisional analysis based on the outcomes from this exercise is expected by the end of 2019. Positive outcome from this exercise could infer the classic way of approaching the testing of carcinogenicity and might yield a significant reduction in the two-year cancer study conduct.

Since the first registration deadline of the REACH Regulation [38] in 2009, only two industrial chemicals have been tested for carcinogenicity, following acceptance of the testing proposal by ECHA. These chemicals were among those of high tonnage level registered within 2013 deadline for which, the majority of the dossiers reported information from references and old literature. New carcinogenicity studies are not expected to be conducted for the 2018 deadline registration due to reduced testing requirements for lower tonnage band chemicals. Though, new phase-in substances may be registered for which carcinogenicity assessment should be considered.

Finally, the impact of carcinogenicity testing on the authorization of substances has been null within the cosmetics sector. Yet, no cancer study has been performed since 2003, ten years ahead the full ban of *in vivo* testing of March 2013, (Figure 12, Table 6). In fact, a combination of shorter-term *in vitro* and *in vivo* studies has been used including *in vitro* and *in vivo* genotoxicity assays, to assess genotoxic potential and repeat-dose (typically 90-day) toxicity studies to assess the risk of non-genotoxic chemicals [77].

What can we learn from waiving opportunities? Can they be applied across sectors or are they sector-specific due to the specific use of each substance? The data reported here have shown differences across sectors in testing approaches but also in the demand of carcinogenicity studies, the number of animals used and the opportunities for waiving the two-year study. As summarised in Table 6, some waivers are put in place for certain types of substances. Waivers are often supported by the absence of structural alerts and lack of genotoxic potential and/or specific properties. Though, risk-based information regarding destination of use, target population and foreseen exposure levels are also considered and these vary substantially across sectors. Moreover, different Regulations with specific testing requirements and levels of concerns are in place. For instance, the EU pesticides law forbids the marketing of active substances which can cause cancer, while for certain types of medicines instead, an intrinsic carcinogenic potential may be tolerated. At present, it is rather difficult to apply similar waiving conditions across sectors. It is thus recommended to explore whether existing waiving opportunities can

be further exploited and applied cross-sectorial. Where possible, this might imply the need for a better harmonization of regulatory requirements and testing strategies in different areas.

The recourse to approaches alternative to animal testing is also quite variable across sectors. Unfortunately, their application is far to be systematic, even though several testing approaches are being explored in recent years, as in the case of human medicines or of some regulations which are calling for their use.

The analysis highlights also that a substantial portion of new substances, such as bio-pesticides and new drugs (biotechnology-derived ones) entering on the market, bear specific features that make their testing not technically feasible with the current available methods. This makes their safety characterisation rather difficult. The introduction of these new types of products is bringing regulatory and technical challenges for a correct safety evaluation that needs to be addressed accordingly with new tools. Several groups have recently highlighted the need for improvements of carcinogenicity assessment with the scope of harmonize strategies and advancing the 3Rs across sectors [90, 92, and 96]. Results from our study suggest a similar direction of efforts.

It might be worthwhile to look to different toxicity areas as to systemic toxicity, immunotoxicity and the area of sensitization. Many of the same mechanisms are involved in either endpoint [77, 97]. This can also help building accurate pathways as adverse outcome pathways (AOP) to recapitulate the mechanism of cancer toxicity at different levels.

Of note is also the recent exercise from an IARC Working group of experts on the identification of key characteristics which can provide the basis for an objective evaluation of hazard from exposure to carcinogens [98]. Ten key characteristics have been identified so far and they imply indeed abilities of agents that can: act as an electrophile; act as genotoxic; alter DNA repair mechanisms; induce epigenetic alterations, chronic inflammation; oxidative stress; immune-suppression; modulate receptor-mediated effects; cause immortalization; alter cell proliferation, death or nutrient supply [98]. The above characteristics might serve for the design of more accurate and predictive test methods, which would also cover non-genotoxic mechanisms.

In this direction is the recent activity regarding non-genotoxic carcinogens carried at international level by The Organisation for Economic Co-operation and Development (OECD) expert group on non-genotoxic carcinogenicity on the development of Integrated Approaches to Testing and Assessment (IATA). There is a wide gap in the area of non-genotoxic carcinogens identification due to the differences in regulatory requirements across sectors and lack of specific validated/authorized test methods; especially, in the area of cosmetics and industrial chemicals of low tonnage band. Mechanistic understanding needs to be built upon as soon as possible [77]. In fact, the aim of the initiative is to identify relevant appropriate carcinogenicity mechanisms, MoAs, and assays which would be appropriate for the development of an IATA. Aspects/blocks of the signalling pathways that are not covered and would require further development will also be identified.

Promising modelling applications of *toxicogenomics*, have been recently developed to capture specific gene expression changes observed for non-genotoxic carcinogens [99]. Together with *toxicogenomics, exposomics, metabolomics* and *epigenomics* areas represent emerging tools that may contribute in the future to the improved assessment of safety [100]. According to that, it might be worthwhile to exploit the advances in the characterization of the human genome and whole genome sequencing, which can inform on the distinct pattern of mutations that can hint at the causative origins of each type of cancer. Consequently, the study of various mutations spectra induced by different

environmental mutagens can serve as a platform to better characterize those agents responsible for specific human tumours [101].

Interestingly, new approaches are currently being tested as new way of identifying chemicals that may increase specific cancer risk. For instance the Hazard Identification Approach for Breast Carcinogens (HIA-BC) project which has been developed to detect those chemicals that may increase specifically breast-cancer risk [102]. The project identifies those assays capable of detecting alterations to biological processes relevant to breast cancer, including, molecular, cellular events, tissues changes and factors altering the susceptibility to the disease.

It is foreseen that the above tools and approaches could provide opportunities to add knowledge to the characterization of cancer and safety evaluation of environmental carcinogens and could also result more relevant to humans.

Overall, the analysis conducted here suggests that the following objectives should be pursued with the aim of further improving carcinogenicity assessment and reducing animal testing:

- *sharing experiences and approaches across sectors and explore if they are applicable to different sectors;*
- *developing alternative approaches to ensure an accurate carcinogenicity hazard assessment where it is technically limited or in areas where in vivo testing is limited (e.g. cosmetics or industrial chemicals of lower tonnage band) or where information gaps are identified within legislations;*
- *integrating information provided by other toxicity endpoints into carcinogenicity assessment.*

References

1. EU Cancer Registries, ENCR Network.
   Available at: http://www.encr.eu/index.php/activities/2015-call-for-data.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr, accessed on day/month/year.
3. Bray F, Jian-Song Ren, Eric Masuyer and Jacques Ferlay, 2013. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. International journal of cancer. International Journal of Cancer Volume 132, Issue 5, pages 1133–1145, 1 March 2013. Available at: http://doi.wiley.com/10.1002/ijc.27711.
4. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray (2014). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer doi:10.1002/ijc.29210 PMID: 25220842 published online 9 October 2014.
5. Ferlay, J, Steliarova-Foucher, E, et al., 2013. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. European Journal of Cancer, 49(6), pp.1374–1403.
   Available at: http://dx.doi.org/10.1016/j.ejca.2012.12.027.
6. Madia F, Phrakonkham P, Corvi R. "Current and emerging *in vitro* methods for genotoxicity and carcinogenicity." In: Anna K. Bal-Price and Paul Jennings, editor. *In vitro* Toxicology Systems. New York, NY 10013-1578, Humana Press Springer Science + Business Media, LLC; JRC84619: Chapter 14, 307-334 (2014).
7. IARC website http://www.iarc.fr/
8. Stewart BW, Wild CP, editors (2014).World Cancer Report 2014. Lyon, France: International Agency for Research on Cancer.
9. de Martel C, Ferlay F, Franceschi S, Vignat J, Bray F, Forman F, Plummer M, 2012. Global burden of cancers attributable to infections in 2008: A review and synthetic analysis. The Lancet Oncology, 13(6), pp.607–615. Available at: http://dx.doi.org/10.1016/S1470-2045 (12)70137-7.
10. EC Regulation 1272, 2008. Regulation (EC) No. 1272/2008 of the European Parliament and of the Council on Classification, Labelling and Packaging of Substances and Mixtures, Amending and Repealing Directives 67/548/EEC and 1999/45/EC, and Amending Regulation (EC) No 1907/2006. OJ L 353 of 16.12.2008, pp.1–1355.
11. CMR substances from Annex VI of the CLP Regulation registered under REACH and/or notified under CLP - A first screening Report 2012. ECHA-12-R-01-EN (2012) pp. 1-79.
12. Deandrea S, Lerda D, Lopez Alcalde J, Neamtiu L, Saz Parkinson Z, Uluturk A. Review and analysis of external quality assessment of breast cancer services in Europe: Supporting information for the development of a European Quality Assurance scheme for Breast Cancer Services. EUR 27413. Luxembourg (Luxembourg): Publications Office of the European Union; 2015. JRC96105
13. Martos Jimenez M, Crocetti E, Visser O, Rous B. A proposal on cancer data quality checks: one common procedure for European cancer registries. EUR 27008. Luxembourg (Luxembourg): Publications Office of the European Union; 2014. JRC93456

14. Lerda D, Deandrea S, Lopez Alcalde J, Neamtiu L, Saz Parkinson Z, Uluturk A. European Commission Initiative on Breast Cancer: Concept document . Luxembourg (Luxembourg): Publications Office of the European Union; 2015. JRC96308
15. OECD (2009), Test No. 451: Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/9789264071186-en
16. OECD (2009), Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/9789264071223-en
17. Alden CL, Lynn A, Bourdeau A, Morton D, Sistare FD, Kadambi VJ, Silverman L (2011) A critical review of the effectiveness of rodent pharmaceutical carcinogenesis testing in predicting human risk. Vet Pathol 48:772–784.
18. Gottmann E, Stefan Kramer, Bernhard Pfahringer, and Christoph Helma, 2001. Data quality in predictive toxicology: Reproducibility of rodent carcinogenicity experiments. Environmental Health Perspectives, 109(5), pp.509–514.
19. European Commission website, http://ec.europa.eu/index
20. DG ENV Report, 2013. Seventh Report from the Commission to the Council and the European Parliament on the Statistics on the number of animals used for experimental and other scientific purposes in the member states of the European Union COM (2013)859/final. , 859.
21. UK Home Office, 2012. Statistics of Scientific Procedures on Living Animals Great Britain 2011.
22. Home Office, 2014. Annual Statistics of Scientific Procedures on Living Animals, Great Britain 2013. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/327854/spanimals13.pdf
23. Regulation, H.O.A. in S.U. (Asru) & Department for Business, I. and S. (Bis), 2014. Working to reduce the use of animals in scientific research
24. ECHA, 2014. The Use of Alternatives to Testing on Animals for the REACH Regulation Second report under Article 117(3) of the REACH Regulation, ECHA-14-A-07-EN. Helsinki, Finland (2014), pp.1-131.
25. EC Regulation 1223/2009. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Official Journal of the European Union L 342, 52, pp.59–209.
26. Ashby J, P.I., 1993. Will all chemicals be carcinogenic to rodents when adequately evaluated? Mutagenesis, 8(6), pp.489–493.
27. Gold, LS, Slone, TH & Ames, BN, 1998. What do animal cancer tests tell us about human cancer risk? : Overview of analyses of the carcinogenic potency database. Drug metabolism reviews, 30(2), pp.359–404. Available at: http://www.tandfonline.com/doi/abs/10.3109/03602539808996318#.VjHSFxI5Yws.mendeley [Accessed October 29, 2015].
28. Rall, DP, 2000. LABORATORY ANIMAL TESTS AND HUMAN CANCER. Drug Metabolism Reviews, 32(2), pp.119–128.
29. Haseman J, Melnick R , Tomatis L , Huff J, 2001. Carcinogenesis bioassays: study duration and biological relevance. Food Chem Toxicol, 39(7), pp.739–744.
30. Knight A, Bailey J, & Balcombe, J, 2006a. Animal carcinogenicity studies: 1. Poor human predictivity. Alternatives to laboratory animals : ATLA, 34(1), pp.19–27.

31. Knight, A, Bailey, J & Balcombe J, 2006b. Animal carcinogenicity studies: 2. Obstacles to extrapolation of data to humans. Alternatives to laboratory animals : ATLA, 34(1), pp.29–38.
Available at: http://www.ncbi.nlm.nih.gov/pubmed/16522148.

32. Knight, A, Bailey, J & Balcombe J, 2006c. Animal carcinogenicity studies: 3. Alternatives to the bioassay. Alternatives to laboratory animals : ATLA, 34(1), pp.39–48. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16522149.

33. Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J, Vickers C. 2008. IPCS Framework for Analyzing the Relevance of a Non cancer Mode of Action for Humans. Critical Reviews in Toxicology. Available at: doi/abs/10.1080/10408440701749421.

34. Paules RS, Aubrecht J, Corvi R, Garthoff B, Kleinjans JC, 2011. Moving forward in human cancer risk assessment. Environmental Health Perspectives, 119(6), pp.739–743.

35. OECD (2009), Test No. 452: Chronic Toxicity Studies, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/9789264071209-en

36. EU Directive 63, 2010. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Official Journal of European Union, (0), p.47. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32010L0063:EN:NOT.

37. Corvi R, Madia F, Worth A, and M Whelan 2013. EURL ECVAM Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing European Commission ed., JRC86616 http://publications.jrc.ec.europa.eu/repository/handle/JRC86616

38. EC Regulation 1907, 2006. REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/4. Official Journal of the European Union, 396(793), pp.1–849.

39. ECHA, 2011. ECHA (2008), Guidance on information requirements and chemical safety assessment Part B: Hazard assessment. Version 1 May 2008, Version 2.1 December 2011. , part B (Version 2.1).

40. ECHA, 2012. Guidance on information requirements and chemical safety assessment Chapter R.7a: Endpoint specific Guidance Version 2.0 November 2012, R.7.7. Mutagenicity and Carcinogenicity. http://echa.europa.eu/documents/10162/13632/information_requirements_r7a_en.p.

41. ECHA, 2010a. Practical Guide How to report read-across and categories. European Chemicals Agency, 6 (Practical Guide). Available at: http://echa.europa.eu/doc/publications/practical_guides/pg_report_readacross_categ.pdf.

42. ECHA, 2010b. Practical Guide on how to report (Q)SARs. European Chemicals Agency, 5 (Practical Guide). Available at: http://echa.europa.eu/doc/publications/practical_guides/pg_report_qsars.pdf.

43. ECHA, 2015a. Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance. European Chemicals Agency, (v. 4.1), pp.1–496.

44. EU Regulation 528, 2012. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. Official Journal of the European Union, L

167, pp.1–123. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:167:FULL:EN:PDF.

45. ICH S1A, 1995. S1A Guideline on the need for carcinogenicity studies. Guidance for Industry, Step 4(November), pp.1–8. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1A/Step4/S1A_Guideline.pdf.

46. EMA CPMP 997, 1996. Note for guidance for the pre-clinical evaluation of anticancer medicinal products. CPMP/SWP/997/96, (23 July 1998), pp.1–5.

47. ICH S1B, 1997. S1B Guidance on testing for Carcinogenicity of pharmaceuticals. Guidance for Industry, Step 5(July), pp.1–11. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1B/Step4/S1B_Guideline.pdf.

48. ICH S1C(R2), 2008. S1C(R2) Dose selection for carcinogenicity studies of pharmaceuticals. Guidance for Industry, Step 4(March), pp.1–12. Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1C_R2/Step4/S1C_R2__Guideline.pdf.

49. ICH S1, 2012. S1 Rodent Carcinogenicity Studies for Human Pharmaceuticals. Guidance for Industry, (September 2013). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S1/S1_Regulatory_Notice_Document_10Mar2015.pdf.

50. ICH S9, 2013. ICH S9 - Non-Clinical evaluation for anti-Cancer pharmaceuticals. Guidance for Industry, Step 5(December 2008), pp.1–10.

51. ICH S2(R1), 2012. S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use. Guidance for Industry, 2(June), pp.1–28. Available at: http://www.fda.gov/downloads/Drugs/Guidances/ucm074931.pdf.

52. Proposed Change to Rodent Carcinogenicity Testing of Pharmaceuticals - Regulatory Notice Document (2016); Revised 22 January 2016, pp 1-14 (dated 2 March 2016).

53. ICH S6(R1), 2011. S6 (R1) Preclinical safety evaluation of biotechnology-derived pharmaceuticals. ICH harmonized tripartite guidance, Step 4 ver(12 June), pp.1–23.

54. EMEA, 1995. Note of Guidance on preclinical pharmacological and toxicological testing of vaccines. CPMP/SWP/465/95, (17 December 1997), pp.1–7.

55. EMEA, 2004. Guidance on Adjuvants in vaccines for human use. EMEA/CHMP/VEG/134716/2004, (20 January 2005), pp.1–18.

56. CHMP 437, 2014. Guideline on similar biological medicinal products. CHMP/437/04, Rev 1(23 October), pp.1–7.

57. VICH GL28, 2005. Studies to evaluate the safety of residues of veterinary drugs in human food: carcinogenicity testing. Guidance for Industry, Revision (Step 9), pp.1–5.

58. EC Regulation 470, 2009. Regulation (EC) NO. 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation Official Journal of European Union, (470), pp.11–22. Available at: \nhttp://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:152:0011:0011:EN:PDF.

59. EC Regulation 37, 2010. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Official Journal of

the European Union, L15, pp.1–72. Available at: \nhttp://ec.europa.eu/health/files/eudralex/vol5/reg_2010_37/reg_2010_37_en.pdf.

60. Woodward, KN, 2008. Assessment of user safety, exposure and risk to veterinary medicinal products in the European Union. Regulatory toxicology and pharmacology : RTP, 50(1), pp.114–28. Available at http://www.sciencedirect.com/science/article/pii/S0273230007001584 [Accessed October 29, 2015].
61. EC Directive 82, 2001. DIRECTIVE 2001/82/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 6 November 2001 on the Community code relating to veterinary medicinal products. Official Journal of European Union, L 311(28/11/2001), pp.1–66.
62. EMA 388694, 2014. Guidance on the classification of veterinary medicinal products indicated for minor use minor species (MUMS) / limited market. EMA/CVMP/388694/2014, (15 September), pp.1–15.
63. EC Regulation 1107, 2009. Regulation (EC) No 1107/2009 of the european parliament and of the council of 21 october 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. Official Journal of the European Union, L 309(24/11/2009), pp.1–50.
64. EU Regulation 283, 2013. Regulation (EU) No 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market. OJ L 93 of 01.03.2013, (3), pp.1–84.
65. EU Regulation 284, 2013. COMMISSION REGULATION (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protectio. OJ L 93, 3.4.2013, (3), pp.p. 85–152.
66. SCCS, 2015. Notes of Guidance for the testing of cosmetics substances and their safety evaluation, 9th Revision, SCCS/1501/. Available at: http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_190.pdf
67. ECHA, 2015b. Factsheet Interface between REACH and Cosmetics regulations. European Chemicals Agency, (ECHA-14-FS-04-EN), pp.1–4. Available at: http://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf.
68. EU Directive 63, 2010b. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. Official Journal of European Union, (0), p.47. Available at: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32010L0063:EN:NOT.
69. Louhimies, S, 2002. Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. Alternatives to laboratory animals : ATLA, 30 Suppl 2, pp.217–9.
70. Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal 196 , 16/08/1967 P. 0001 – 0098 (replaced by EU Regulation 1272/2008)
71. Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market. OJ L 123, 24.4.1998, p. 1–63

72. OECD, 2012. OECD Guidance Document (2012), no. 116. On the conduct and Design of Chronic Toxicity and Carcinogenicity Studies, Support test Guidelines 451, 452 AND 453. ENV/JM/MONO (2011) 47, 2nd Edition (13 April).

73. Luijten, M, Muller, JJA, Hernández, LG, van der Ven, LTM, van Benthem, J., 2012. Prediction of carcinogenic potential of substances using repeated dose toxicity data. RIVM Report, 340700006, pp.1–18.

74. EMA 627621, 2012. QRD general principles regarding the SmPC information for a generic/hybrid/biosimilar product. EMA/627621/2011 Patient Health Protection, (3 May), pp.1–2.

75. EMEA CHMP 225411, 2015. EMA Procedural advice for users of the centralised procedure for generic/hybrid applications. EMEA/CHMP/225411/2006, (September), pp.1–34.

76. The Bio-Pesticides Database (BPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2011-2013. http://sitem.herts.ac.uk/aeru/bpdb/

77. Adler S, Basketter D, Creton S, Pelkonen O, van Benthem J, Zuang V, Andersen KE, Angers-Loustau A, Aptula A, Bal-Price A, Benfenati E, Bernauer U, Bessems J, Bois FY, Boobis A, Brandon E, Bremer S, Broschard T, Casati S, Coecke S, Corvi R, Cronin M, Daston G, Dekant W, Felter S, Grignard E, Gundert-Remy U, Heinonen T, Kimber I, Kleinjans J, Komulainen H, Kreiling R, Kreysa J, Leite SB, Loizou G, Maxwell G, Mazzatorta P, Munn S, Pfuhler S, Phrakonkham P, Piersma A, Poth A, Prieto P, Repetto G, Rogiers V, Schoeters G, Schwarz M, Serafimova R, Tähti H, Testai E, van Delft J, van Loveren H, Vinken M, Worth A, Zaldivar JM. Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. Arch Toxicol. 2011 May; 85 (5):367-485.

78. SCCS website, http://ec.europa.eu/health/scientific_committees/all_opinions/index_en.htm

79. IMPACT ASSESSMENT ON THE ANIMAL TESTING PROVISIONS IN REGULATION (EC) 1223/2009 ON COSMETICS, COM(2013) 135 final http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52013SC0066&from=EN

80. CosIng database, http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.simple

81. NIH guideline, GUIDELINES FOR CARCINOGEN BIOASSAY IN SMALL RODENTS NCI-CG-TR-1. February 1 1976, pp. 1-76

82. Contrera JF, Jacobs AC, DeGeorge JJ. Carcinogenicity testing and the evaluation of regulatory requirements for pharmaceuticals. Regul Toxicol Pharmacol. 1997 Apr; 25 (2):130-45.

83. Friedrich A, Olejniczak K. Evaluation of carcinogenicity studies of medicinal products for human use authorised via the European centralised procedure (1995-2009). Regul Toxicol Pharmacol. 2011 Jul; 60 (2):225-48.

84. Sistare, F.D., Morton, D., Alden, C., Christensen, J., Keller, D., De Jongue, S., Storer, R.D., Reddy, M.V., Kraynac, A., Trela, B., Bienvenu, J.-G., Bjurström, S., Bosmans, V., Brewster, D., Colman, K., Dominick, M., Evans, J., Hailey, J.R., Kinter, L., Liu, M., Mahrt, C., Marien, D., Myer, J., Perry, R., Potenta, D., Roth, A., Sherratt, P., Singer, T., Slim, R., Soper, K., Fransson-Steen, R., Stolz, J., Turner, O., Turnquist, S., van Heerden, M., Woicke, J., DeGeorge, J.J., 2011. An analysis of pharmaceutical experience with decades of rat carcinogenicity testing: support for a proposal to modify current guidelines. Toxicol. Pathol. 39, 716–744.

85. Monro, A., 1996. Are lifespan rodent carcinogenicity studies defensible for pharmaceutical agents? Exp. Toxicol. Pathol. 48, 155–166.

86. Van Ousterhout, J.P.J., van der Laan, J.W., de Waal, E.J., Olenjniczak, K., Hilgenfeld, M., Schmidt, V., Bass, R., 1997. The utility of two rodent species in carcinogenic risk assessment of pharmaceuticals in Europe. Regul. Toxicol. Pharmacol. 25, 6–17.

87. Guidance Document on the *in vitro Syrian hamster* embryo cell transformation assay. OECD No. 214 Series on Testing & Assessment. ENV/JM/MONO(2015)18.

88. Guidance Document on the *in vitro* Bhas 42 cell transformation assay. OECD No. 231 Series on Testing & Assessment. ENV/JM/MONO(2016)1.

89. Herwig R, Gmuender H, Corvi R, Bloch KM, Brandenburg A, Castell J, Ceelen L, Chesne C, Doktorova TY, Jennen D, Jennings P, Limonciel A, Lock EA, McMorrow T, Phrakonkham P, Radford R, Slattery C, Stierum R, Vilardell M, Wittenberger T, Yildirimman R, Ryan M, Rogiers V, Kleinjans J, 2015. Inter-laboratory study of human *in vitro* toxicogenomics-based tests as alternative methods for evaluating chemical carcinogenicity: a bioinformatics perspective. Arch Toxicol. Nov 2. [Epub ahead of print] PubMed PMID: 26525393.

90. Creton S, Aardema MJ, Carmichael PL, Harvey JS, Martin FL, Newbold RF, et al., Cell transformation assays for prediction of carcinogenic potential: state of the science and future research needs, Mutagenesis. 27 (2012) 93–101. doi:10.1093/mutage/ger053.

91. Annys E, Billington R, Clayton R, Bremm KD, Graziano M, McKelvie J, Ragan I, Schwarz M, van der Laan JW, Wood C, Öberg M, Wester P, Woodward KN. Advancing the 3Rs in regulatory toxicology - Carcinogenicity testing: Scope for harmonisation and advancing the 3Rs in regulated sectors of the European Union. Regul Toxicol Pharmacol. 2014 Jul; 69 (2):234-42.

92. Jacobs AC and Hatfield KP. History of Chronic Toxicity and Animal Carcinogenicity Studies for Pharmaceuticals. Veterinary Pathology (2012) 00(0) 1-10.

93. Daneshian M, Busquet F, Hartung T, Leist M. Animal use for science in Europe. ALTEX. 2015; 32 (4):261-74.

94. Morton D, Bourcier T, Alden CL. Improving carcinogenicity assessment. Toxicol Pathol. 2013 Feb; 41 (2):263-70.

95. van der Laan JW, Kasper P, Silva Lima B, Jones DR, Pasanen M., 2016. Critical analysis of carcinogenicity study outcomes. Relationship with pharmacological properties. Crit Rev Toxicol. Apr 26:1-28.

96. Heinonen T., Louekari K., Tahti H. Need for Harmonized strategies and improved assessment of carcinogenic and genotoxic potencies of chemical substances. J. Translational Toxicol. (2014) 1: 76-87.

97. Basketter D.A., H. Clewell, I. Kimber, A. Rossi, B. Blaauboer, R. Burrier, et al., A roadmap for the development of alternative (non-animal) methods for systemic toxicity testing - t4 report*, ALTEX. 29 (2012) 3–91.

98. Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert P, Hecht SS, Bucher JR, Stewart BW, Baan R, Cogliano VJ, Straif K. Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environ Health Perspect. 2015 Nov 24.

99. Eichner J, Wrzodek C, Römer M, Ellinger-Ziegelbauer H, Zell A. Evaluation of toxicogenomics approaches for assessing the risk of nongenotoxic carcinogenicity in rat liver. PLoS One. 2014 May 14; 9 (5):e97678.

100. Langley G, Austin CP, Balapure AK, Birnbaum LS, Bucher JR, Fentem J, Fitzpatrick SC, Fowle JR 3rd, Kavlock RJ, Kitano H, Lidbury BA, Muotri AR, Peng SQ, Sakharov D, Seidle T, Trez T, Tonevitsky A, van de Stolpe A, Whelan M, Willett C. Lessons from Toxicology: Developing a 21st-Century Paradigm for Medical Research. Environ Health Perspect. 2015 Nov; 123 (11):A268-72.

101. Nik-Zainal S, Kucab J.E., Morganella S., Glodzik D., Alexandrov L.B., Arlt V.M., Weninger A., Hollstein M, Stratton M.R., and Phillips D.H. The genome as a record of environmental exposure. Mutagenesis (2015) 30 (6): 763-770.

102. Schwarzman MR, Ackerman JM, Dairkee SH, Fenton SE, Johnson D, Navarro KM, Osborne G, Rudel RA, Solomon GM, Zeise L, Janssen S. Screening for Chemical Contributions to Breast Cancer Risk: A Case Study for Chemical Safety Evaluation. Environ Health Perspect. 2015 Dec; 123 (12):1255-64.

List of abbreviations and definitions

CAD	Carcinogenicity Assessment Document
CARC	Carcinogenicity
CHMP	The Committee for Medicinal Products for Human Use
CLP	Classification, Labelling and Packaging
CVMP	The Committee for Medicinal Products for Veterinary Use
DAR	Draft Assessment Report
DG ENV	Directorate General for the Environment
DRAs	Drug Regulatory Agencies
EC	European Commission
ECHA	European Chemicals Agency
EFSA	European Food and Safety Authority
EINECS	Inventory of Existing Commercial Chemical Substances
EMA	European Medicines Agency
EPAR	European public assessment report
ESR	Experimental study record
EU	Europe Union
EURL ECVAM	European Union Reference Laboratory for alternatives to animal testing
GENOTOX	Genotoxicity
GHS	Globally Harmonized System Classification
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
MDT	Maximum Tolerated Dose
MOE	Margin of Exposure
MS	Member State
OECD	The Organisation for Economic Co-operation and Development
PDR	Physicians' Desk Reference Network
PPPs	Plant protection products
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCS	Scientific Committee on Consumer Safety
SmPC	Summary of product characteristics
SMs	Small molecules
TG	Test guidelines
UDS	Unscheduled DNA synthesis
VICH	International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WoE	Weight of Evidence

List of figures

List of tables

Annexes

The following Tables report relevant information on carcinogenicity, *in vitro* and *in vivo* genotoxicity studies collected for pesticides, biocides, human and veterinary medicines from publicly available EFSA DAR Reports, ECHA website documents on biocides and EPARs documents from EMA website. Data refer to year 2011 and year 2014.

Annex I. Biocides list of authorized substances in 2011 and 2014

	Substance	Category	Carcinogenicity Studies		*In vitro* genotox studies		*In vivo* genotox studies	Date of approval
1	Metofluthrin	Insecticides, acaricides and products to control other arthropods	yes	combined chronic/CARC study in rats, and chronic/CARC study in mice	yes	AMES, CA in CH ling cells, HGPRT in CHO cells,	yes	MN vivo mice PCEs, 2^ study *in vivo* germ cells waived	01/05/2011
2	Alphachloralose	Rodenticides	no	waived	yes	AMES, CA, MLA, HPRT	no	waived	01/07/2011
3	Bromadiolone	Rodenticides	no	waived	yes	AMES, CA in CHO, HPRT in CHO	no	waived	01/07/2011
4	Chlorophacinone	Rodenticides	no	The closely related molecule warfarin is not carcinogenic to humans. Study on chlorophacinone is not available. Carcinogenicity and long-term toxicity studies are not submitted and Applicant present a justification on the basis of the knowledge of mechanism of toxicity and technical difficulties to test so low dose needed for a long term exposure without lethality.	yes	AMES, CA, MLA	yes	MN vivo in mice	01/07/2011
5	Coumatetralyl	Rodenticides	no	waived, because non genotoxic, no metabolites, similar to warfarin	yes	AMES, Rec –assay in *S. cerevisiae*, HPRT in V79cells, CA waived	yes	MN vivo in mice BM, 2^ *in vivo* waived	01/07/2011
6	Fenpropimorph	Wood preservatives	yes	1-combined chronic/CARC study in rats, one 2-yrs in mice, 2-yr study mice	yes		yes		01/07/2011
7	Sulfuryl fluoride*	Insecticides, acaricides and products to control other arthropods		2-yr study per inhalation in rats; 18-M and 12-M in mice;1 study with various species		AMES (E.coli, S. typhimurium.), CA vitro, UDS vitro, MLA vitro		MN vivo; 2^ study *in vivo* waived	01/07/2011
8	Aluminium phosphide releasing phosphine	Rodenticides	yes	2-yr study per inhalation in rats; waived 2^ study in mice	yes		yes		01/09/2011
9	Boric acid	Wood preservatives	yes	2-yrstudy, feeding, in rats; waived 2^ species study	yes	AMES, CA in CHO, MLA all negatives	no	waived because negative *in vitro* and IUCLID reports -ve *in vivo* study	01/09/2011
10	Boric oxide	Wood preservatives				read across with boric acid			01/09/2011
11	Disodium octaborate tetrahydrate	Wood preservatives				read across with boric acid			01/09/2011

[continue]

	Substance	Category	Carcinogenicity Studies		*In vitro* genotox studies		*In vivo* genotox studies		Date of approval
12	Disodium tetraborate	Wood preservatives			read across with boric acid				01/09/2011
13	Disodium tetraborate decahydrate	Wood preservatives			read across with boric acid				01/09/2011
14	Disodium tetraborate pentahydrate	Wood preservatives			read across with boric acid				01/09/2011
15	Nitrogen	Insecticides, acaricides and products to control other arthropods			not applicable				01/09/2011
16	Flocoumafen	Rodenticides	no	Waived; refine waiving concept based on exposure pattern and toxicological profile. Negligible secondary human exposure, very low primary exposure, anticipated lack of substance related non-genotoxic and genotoxic effect anticipated satisfactory MOE and absence of structural alert.	yes	AMES, CA in rats liver cells, HPRT in V79 cells, CTA supporting study	yes	CA *in vivo* BM cells, 2^ *in vivo* study waived because negative in other studies, waived also genotoxicity study in germ cells	01/10/2011
17	Tolylfluanid	Wood preservatives	yes	2-yr combined chronic/CARC study in rats TG 453 and TG 452 study in dogs; 2-yr mouse study in B6CF and 2-yr study in NMR1 mice TG 451	yes	AMES and HPTR in CHO); CA in CHL cells; MLA tk mouse; UDS in rat liver primary cells : Negative results	yes	MN, CA, Mouse Spot test, mouse spermatogonia test TG 483: negative result	01/10/2011

Legend

CA: chromosomal aberrations assays
MLA: mouse lymphoma assay mammalian cell gene mutation using *Tk* gene
HPRT/HGPRT: mammalian Cell gene mutation using the *Hprt* and *Xprt* genes
MN: micronucleus test
UDS: Unscheduled DNA Synthesis
CHO: Chinese hamster ovary cells
CHL: Chinese hamster lung cells
PCEs: polychromatic erythrocytes
BM: bone marrow
COMET: DNA damage (COMET assay)
DML: Dominant lethal assay in rodent
The information reported was retrieved from ECHA biocides website and related documents where available.
*sulfuryl fluoride was tested for a different authorization (as wood preservative) right in 2009.

[continue]

	Substance	Category	Carcinogenicity Studies		*In vitro* genotox studies		*In vivo* genotox studies	Date of approval
1	Basic Copper carbonate	Wood preservatives	no	short-term study only, waived the 2-yr studies for non genotoxic potential	yes	AMES study	yes	MN vivo, UDS vivo	01/02/2014
2	Bendiocarb	Insecticides, acaricides and products to control other arthropods	yes	2-yr studies in rats and mice	yes	AMES, CA in human lymphocytes, UDS in HeLa cells, MLA	yes	MN in CD-1 mice PCEs cells, CA in BM, rodent DML	01/02/2014
3	Copper (II) oxide	Wood preservatives	read across with copper (II) sulphate pentahydrate						01/02/2014
4	Copper hydroxide	Wood preservatives	yes	Short-term study only, waived the 2-yr studies for non genotoxic potential	yes	AMES, UDS	yes	MN in PCEs of BM mice, UDS in rat hepatocytes	01/02/2014
5	Flufenoxuron	Wood preservatives	yes	2-yr rat study; two 2-yr mouse studies	yes	AMES, CA, MLA	yes	CA in rat BM, MN vivo in mouse BM, UDS in rat liver	01/02/2014
6	Hydrochloric acid	Disinfectants and algaecides not intended for direct application to humans or animals	no	Supporting information from IARC reported study	yes	AMES Salmonella only, recombination test in Yeast, E. coli pol-A repair test, CA in CHO, MLA	no	waived - because scientifically unjustified	01/05/2014
7	Margosa extract	Insecticides, acaricides and products to control other arthropods	yes	2-yr study in rats only, waived the 2^ study in mice	yes	AMES, CA in human lymphocytes, HPRT in CHO cells	yes	MN in BM mice, 2^ test waived	01/05/2014
8	Methyl nonyl ketone	Repellents and attractants	no	read across with other aliphatic ketons	yes	AMES, CA, MLA	no	waived - because scientifically unjustified	01/05/2014
9	cis-Tricos-9-ene (Muscalure)	Repellents and attractants	no	waived	yes	AMES, CA, QSARs from OECD toolbox	no	waived, some data from literature but used as informative only	01/10/2014
10	Hydrogen cyanide	Wood preservatives	no	literature reported documents	no	literature reported documents	no	literature only	01/10/2014
11	Hydrogen cyanide	Rodenticides	similar	literature reported documents	no	literature reported documents	no	literature only	01/10/2014
12	Hydrogen cyanide	Insecticides, acaricides and products to control other arthropods	similar	literature reported documents	no	literature reported documents	no	literature only	01/10/2014

Annex II. Human medicines list of authorized substances in 2011 and 2014

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
1	Ameluz	(5-aminolevulinic acid HCl)	*unique entity*	4	No CARC studies Topical therapy with Ameluz 10% is limited to a single application of the gel to lesional skin and ALA-PDT is an established treatment for AK. Therefore, the lack of carcinogenicity studies is considered to be acceptable	No studies were conducted by the applicant on the mutagenic and clastogenic potential of ALA or PpIX as part of the development program for Ameluz. Based on published data, the likelihood of sustained genetic damage in surviving cells after ALA-PDT is considered to be low (Fuchs et al., 2000).		14/12/2011
2	Benlysta	belimumab	*unique entity*	10	No CARC studies have been conducted. A traditional rodent carcinogenicity study would be limited due to rapid formation of anti-drug-antibodies to both belimumab and a homologous hamster anti-mouse BLyS antibody. A study in BLyS ko mice for pre-neoplastic changes would not be a representative model as mice deficient in BLyS or the main BLyS receptor B3, have severely depleted numbers of peripheral B cells while belimumab treatment in humans reduces peripheral B cell populations by 50% but does not deplete them. No proliferative or pre-neoplastic changes were reported in any of the monkeys in a 6 month repeat dose toxicity study.	No genotoxicity studies were conducted. Belimumab is a monoclonal antibody and is not expected to interact directly with DNA or other chromosomal material. Regulatory guidance is consistent with studies on genotoxicity not being necessary for this type of product.		13/07/2011
3	Buccolam	midazolam	*unique entity*	4	2-yr CARC studies in mice and rats	Tested *in vitro* and *in vivo*		05/09/2011
4	Bydureon	exenatide	*unique entity*	5	2-yr CARC study in rats with exenatide QW, previously done on a different formulation in mice and rats	Tested *in vitro* and *in vivo*		17/06/2011
5	Cinryze	C1 inhibitor – human	*unique entity*	8	Only human study A highly purified, viral-inactivated, nano filtered concentrate of C1 esterase inhibitor (C1 INH) produced from human plasma	No genotoxicity testing		15/06/2011
6	Clopidogrel Teva Pharma B.V.	clopidogrel HBr	*generic*	3	On the basis of the CHMP Guidance for users of the centralised procedure for generic application (EMEA/CHMP/225411/2006), no studies were performed			16/06/2011 No longer authorized
7	Daliresp	roflumilast	*unique entity*	5	Since this application is an informed consent of the Daxas marketing authorisation, the non-clinical data in support of the Daliresp application are identical to the up-to-date non-clinical data of the Daxas dossier, which have been assessed and approved (including all post-marketing procedures).			28/02/2011
8	Dasselta	desloratidine	*generic*	3	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. The impurity profile has been discussed and was considered acceptable.			28/11/2011
9	Desloratadine Teva	desloratiden	*generic*	3	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. The impurity profile has been discussed and was considered acceptable.			24/11/2011
10	Dexdor	dexmedetomide HCl	*unique entity*	3	No CARC studies were performed and this can be considered acceptable in view of the proposed and anticipated maximum duration of treatment (14 days).	Tested *in vitro* and *in vivo*		16/09/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
11	Edurant	rilpivirine HCl	*unique entity*	6	2-yr CARC studies in mice and rats	Tested *in vitro* and *in vivo*		28/11/2011
12	Eliquis	apixaban	*unique entity*	4	2-yr CARC studies in mice and rats	Tested *in vitro* and *in vivo*		18/05/2011
13	Entacapone Orion	entacapone	*unique entity*	3	Informed consent of the Comtess application, the non-clinical data in support of the Entacapone Orion application are identical to the up-to-date non-clinical data of the Comtess dossier,	No genotoxicity testing		18/08/2011
14	Entacapone Teva	entacapone	*generic*	5	Toxicology is based on literature searches and adequate scientific literature has been provided	No genotoxicity testing		18/02/2011
15	Esbriet	pirfenidone	*orphan*	10	2-yr CARC study rats and mice and mechanistic hepatic assays in rats and mice	Tested *in vitro* and *in vivo*		28/02/2011
16	Eurartesim	piperaquine tetraphosphate /dihydroartemisinin	*unique entity*	3	No data is available in the literature on the carcinogenicity of DHA or PQP or the combination. As treatment is only intended to be given for three days, carcinogenicity tests are not required.	Tested *in vitro* only		27/10/2011
17	Eviplera	emtricitabine / rilpivirine hydrochloride /tenofovir disoproxil fumarate	*unique entity*	9	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		28/11/2011
18	Fampyra	fampridine	*unique entity*	5	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		20/07/2011
19	Gilenya	fingolimod hydrochloride	*unique entity*	9	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		17/03/2011
20	Halaven	eribulin	*unique entity*	7	As anti-cancer drug, no carcinogenicity study was performed	Tested *in vitro* and *in vivo*		17/03/2011
21	Hizentra	human normal immunoglobulin (SCIg)	*unique entity*	10	Human immunoglobulins are naturally occurring proteins with well-established safety and tolerability not required toxicity studies			14/04/2011
22	Iasibon	ibandronic acid	*generic*	3	Toxicological properties of ibandronic acid are well characterised. An overview based on the literature was considered appropriate			21/01/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
23	Dificlir	fidaxomicin	*unique entity*	6	No CARC studies Considering the short (10 days) planned duration of dosing, carcinogenicity studies were not conducted with fidaxomicin.	Tested *in vitro* and *in vivo*		05/12/2011
24	Edarbi	azilsartan medoxomil	*unique entity*	3	2-yr CARC studies were performed with TAK-491 in rats and with TAK-536 in mice and rats A 26-week carcinogenicity study was performed with TAK-491 in transgenic mice	Tested *in vitro* and *in vivo*		07/12/2011
25	Ibandronic Acid Sandoz	ibandronic acid irbesartan / hydrochlorothiazide	*generic*	2	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			26/07/2011
26	Ifirmacombi	ibandronic acid irbesartan / hydrochlorothiazide	*generic*	3	Generic application referring to the originator product CoAprovel, no new non-clinical studies on the pharmacology, pharmacokinetics and toxicology of irbesartan and hydrochlorothiazide have been undertaken			04/03/2011
27	Incivo	telaprevir	*unique entity*	16	Since the duration of treatment is limited to 12 weeks and no concern for carcinogenicity have been observed during toxicity testing, carcinogenicity studies for Telaprevir were deemed unnecessary. As well as genotoxicity studies			19/09/2011
28	Jevtana	cabazitaxel	*unique entity*	9	According to the ICH Topic S9, carcinogenicity study is not required to support marketing for therapeutics intended to treat patients with advanced cancer, but cabazitaxel can be considered as a carcinogenic agent due to the genotoxic properties	Tested *in vitro* and *in vivo*		17/03/2011
29	Komboglyze	metformin hydrochloride /saxagliptin hydrochloride	*unique entity*	6	Extensive clinical experience does not indicate a relevance for humans. Consequently, further studies on the combination product were not necessary and the combination saxagliptin/metformin has not been tested for carcinogenicity			24/11/2011
30	Lamivudine/Zidovudine Teva	lamivudine / zidovudine	*generic*	5	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. There is thus no need for conducting tests on animals.			28/02/2011
31	Leganto	rotigotine	*unique entity*	6	It is an informed consent of the Neupro application, the non-clinical data in support of the Leganto application are identical to the up-to-date non-clinical data of the Neupro dossier			16/06/2011
32	Levetiracetam Accord	levetiracetam	*generic*	4	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. No toxicity testing			03/10/2011
33	Levetiracetam Actavis	levetiracetam	*generic*	5	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. No toxicity testing			03/10/2011
34	Levetiracetam Actavis Group	levetiracetam	*generic*	3	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. No toxicity testing			05/12/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
35	Levetiracetam ratiopharm	levetiracetam	*generic*	4	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		26/08/2011
36	Levetiracetam Sun	levetiracetam	*generic*	5	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		14/12/2011
37	Levetiracetam Teva	levetiracetam	*generic*	7	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		26/08/2011
38	Levodopa/Carbidopa/Entacapone Orion	levodopa / carbidopa /entacapone	*unique entity*	4	Informed consent of the Stalevo application, the non-clinical data in support of the Levodopa/Carbidopa/Entacapone application are identical to the up-to-date non-clinical data of the Stalevo dossier,	No genotoxicity testing		24/08/2011
39	Libertek	roflumilast	*unique entity*	5	Informed consent of the Daxas marketing authorisation, the non-clinical data in support of the Libertek application are identical to the up-to-date non-clinical data of the Daxas dossier,	No genotoxicity testing		28/02/2011
40	Matever	levetiracetam	*generic*	11	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		03/10/2011
41	Methylthioninium chloride Proveblue	methylthioninium chloride	*generic*	7	Literature only	AMES test only		06/05/2011
42	Nulojix	belatacept	*unique entity*	3	No carcinogenicity testing. A lifetime study (study nr 97610) in mice was conducted with abatacept to determine the carcinogenic potential of CD28 blockade	Genotoxicity testing is generally not required for protein therapeutics. No genotoxicity studies were conducted with belatacept. However, a battery of validated *in vitro* genotoxicity assays was conducted with similar abatacept.		17/06/2011
43	Plenadren	hydrocortisone	*orphan*	3	No testing			03/11/2011
44	Potactasol	topotecan	*generic*	0	Topotecan was described as genotoxic to mammalian cells. Long-term carcinogenicity studies with topotecan were not submitted. According to CPMP/SWP/997/96, “Note for Guidance on the pre-clinical evaluation of anticancer medicinal products” this is acceptable, because carcinogenicity studies are not usually required due to the intended therapeutic indications. However, topotecan is known to be genotoxic to mammalian cells and is probable carcinogen.			06/01/2011
45	Pramipexole Accord	pramipexole dihydrochloride monohydrate	*generic*	2	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			30/09/2011
46	Pravafenix	fenofibrate / pravastatin	*unique entity*	2	No new studies have been performed, which is acceptable according to the current guideline: The Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/2005).			14/04/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
47	Pumarix	pandemic influenza vaccine (H5N1) (split virion, inactivated)	*Exceptional Circumstance*	4	No CARC studies were performed according to the Note for Guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95 guidance) and the Guideline on adjuvants in vaccines for human use (EMEA/CHMP/ VEG/134716/2004).	Genotoxicity of the adjuvant alone was assessed in two *in vitro* tests (reverse mutation (Ames) test in bacteria; gene mutation in mouse cells) and one *in vivo* test (micronucleus test in the rat after intravenous administration). The vaccine was not tested. No indication of genotoxicity was evident.		04/03/2011
48	Rasilamlo	aliskiren / amlodipine	*unique entity*	4	No CARC studies were conducted with Rasilamlo since this area has been adequately investigated for the monocomponents	Tested *in vitro* only		14/04/2011
49	Repaglinide Accord	repaglinide	*generic*	1	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		22/12/2011
50	Repso	leflunomide	*generic*	7	Relevant non-clinical safety data for Repso have been summarized in a non-clinical overview and included in section 5.3 of the SmPC which is identical to the texts of the reference medicinal product Arava.	No genotoxicity testing		14/03/2011
51	Rivastigmine Actavis	rivastigmine hydrogen tartrate	*generic*	7	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.	No genotoxicity testing		16/06/2011
52	Telmisartan Teva Pharma	telmisartan	*generic*	3	Because Telmisartan Teva Pharma is a generic medicine, studies in patients have been limited to tests to determine that it is bioequivalent to the reference medicine, Micardis. Two medicines are bioequivalent when they produce the same levels of the active substance in the body	No genotoxicity testing		03/10/2011
53	Temozolomide Sun	temozolomide	*generic*	6	Six cycle in rats only	Tested *in vitro* only		13/07/2011
54	Teysuno	tegafur / gimeracil / oteracil	*unique entity*	8	The 2-yr CARC studies in both mice and rats were both preceded by dose range finding studies of 4 week duration in the mouse and 13-week duration in both the mouse and rat	Tested *in vitro* and *in vivo*		14/03/2011
55	Tobi Podhaler	tobramycin	*orphan*	4	No CARC study with TIP was performed. However in a 95-week carcinogenicity study in rats with the TOBI inhalation formulation, no evidence of a carcinogenic potential was seen	No genotoxicity testing		20/07/2011
56	Trajenta	linagliptin	*unique entity*	4	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		24/08/2011
57	Trobalt	pandemic influenza vaccine (H5N1) (split virion, inactivated)	*unique entity*	8	2-yr CARC study in rats + 1 study neonatal carc in mice pnd8-pnd15 gavage treatment	Tested *in vitro* and *in vivo*		28/03/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
58	Vibativ	telavancin	*unique entity*	5	Treatment of telavancin is expected to be up to 21 days and the need for carcinogenicity testing is only required for drugs administered for at least 6 months.	Tested *in vitro* and *in vivo*		02/09/2011
59	Victrelis	boceprevir	*unique entity*	16	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		18/07/2011
60	Votubia	everolimus	*orphan*	11	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		02/09/2011
61	Vyndaqel	tafamidis	*orphan - exceptional circumstances*	5	A 26-week CARC study in transgenic rasH2 mice was conducted by the oral (gavage) route of administration	Tested *in vitro* and *in vivo*		16/11/2011
62	Xeplion	paliperidone palmitate	*unique entity*	5	2-yr CARC study in rats only	Tested *in vitro* only		04/03/2011
63	Xgeva	denosumab	*unique entity*	4	No specific studies were conducted. Denosumab is a recombinant protein and contains no inorganic or synthetic organic linkages or other non-protein portions. Regulatory guidance is consistent with studies on genotoxicity not being necessary for this type of product	No genotoxicity testing		13/07/2011
64	Xiapex	collagenase Clostridium histolyticum	*unique entity*	10	In accordance with ICH guidance (ICH, 1995 and ICH, 1997) carcinogenicity studies with AA4500 were not conducted. ICH (1997) indicates that standard carcinogenicity bioassays are generally not required for biotechnology derived pharmaceuticals, particularly proteins with no known growth factor activity.	Tested *in vitro* and *in vivo*		28/02/2011
65	Yellox	bromfenac sodium sesquihydrate	*unique entity*	3	In line with ICH S1A, CARC studies are not warranted for Yellox, since it will be indicated for a treatment period of only two weeks, and there is no special concern regarding carcinogenic potential	Tested *in vitro* and *in vivo*		18/05/2011
66	Yervoy	ipilimumab	*unique entity*	5	In accordance with ICH S6 (R1) guideline and ICH S1A Guideline on the need for carcinogenicity studies of pharmaceuticals (CPMP/ICH/140/95) no studies on genotoxicity and carcinogenicity were conducted, which was acceptable. In addition, considering the lack of relevance of the rodent species for ipilimumab, the limited value of short-term carcinogenicity studies with homologous products (as reflected in the draft reviewed			13/07/2011
67	Zoely	nomegestrol acetate / estradiol	*unique entity*	6	No test on combination: single ingredients already tested	Tested *in vitro* and *in vivo*		27/07/2011
68	Zytiga	abiraterone acetate	*unique entity*	7	rat and monkey repeated dose studies, 2-yr rat CARC study, a 6-month CARC study in Tg.rasH2 mouse model will be performed.	Tested *in vitro* and *in vivo*		05/09/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
1	Adempas	riociguat	*orphan*	1	2-yr CARC study in rats and mice oral administration	Tested *in vitro* and *in vivo*		27/03/2014
2	Anoro	umeclidinium bromide / vilanterol trifenatate	*unique entity*	0	Two 13-week mouse oral studies; 2-year CARC rat 2-year CARC mouse inhalation studies. Tested single ingredients.	Tested *in vitro* and *in vivo*		08/05/2014
3	BiResp Spiromax	budesonide / formoterol fumarate dihydrate	*unique entity*	0	The toxicology studies were taken from the FDA Pharmacology Reviews (FDA, 2001; FDA 2006) cited unless otherwise specified. The doses of drugs and species used in a number of the toxicology studies reported were not specified.	No genotoxicity testing		28/04/2014
4	Brimica Genuair	aclidinium / formoterol fumarate dihydrate	*unique entity*	0	No CARC studies have been conducted with aclidinium/formoterol since both individual active substances have been comprehensively investigated. The lack of carcinogenicity studies with the proposed combination is accepted by the CHMP.	Tested impurities *in vitro* only		19/11/2014
5	Budesonide/Formoterol Teva	budesonide / formoterol fumarate dihydrate	*unique entity*	0	No new toxicity studies have been performed for this MAA; the nonclinical toxicology summary is largely based on the information available for the Symbicort Turbohaler reference product. No original study reports are available. Considering this is a hybrid application this approach is acceptable			19/11/2014
6	Budesonide/Formoterol Teva Pharma B.V.	budesonide / formoterol	*unique entity*	0	No new toxicity studies have been performed for this MAA; the nonclinical toxicology summary is largely based on the information available for the Symbicort Turbohaler reference product. No original study reports are available. Considering this is a hybrid application this approach is acceptable			19/11/2014
7	Clopidogrel/Acetylsalicylic acid Teva	clopidogrel / acetylsalicylic acid	*unique entity*	0	2-yr CARC study in rats; 78-weeks in mice on single compound			01/09/2014
8	Cometriq	cabozantinib	*orphan-conditional approval*	2	Carcinogenicity evaluations of XL184 have not been conducted based on the absence of genotoxicity in both *in vitro* and *in vivo* bioassays, the lack of preneoplastic lesions in chronic repeat-dose toxicity studies in rats and dogs, the lack of demonstrated carcinogenic potential in the RTKi product class, and the intended treatment population of subjects with advanced, progressive MTC with limited treatment options and a relatively short life expectancy (which is supported by the interim overall survival results from the pivotal study XL184-301), in accordance with ICH S1A and ICH S9.			21/03/2014
9	Daklinza	daclatasvir dihydrochloride	*unique entity*	1	2-yr CARC study in SD rats 26-week study in Tg-rasH2 transgenic mice	Tested *in vitro* and *in vivo*		22/08/2014
10	Deltyba	delamanid	*orphan-conditional approval*	2	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		28/04/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
11	Duaklir Genuair	aclidinium bromide / formoterol fumarate dihydrate	*unique entity*	0	No CARC studies have been conducted with aclidinium/formoterol since both individual active substances have been comprehensively investigated. The lack of carcinogenicity studies with the proposed combination is accepted by the CHMP.	No genotoxicity testing		19/11/2014
12	DuoResp Spiromax	budesonide / formoterol fumarate dihydrate	*unique entity*	1	5 studies; + 91 weeks in mice. The toxicology studies were taken from the FDA Pharmacology Reviews (FDA, 2001; FDA 2006) cited unless otherwise specified. The doses of drugs and species used in a number of the toxicology studies reported were not specified.	No genotoxicity testing		28/04/2014
13	Entyvio	vedolizumab	*unique entity*	0	Conventional carcinogenicity risk assessment studies (ie, rodent bioassays) have not been conducted with vedolizumab as rodents are not pharmacologically responsive to this mAb. This is consistent with ICH Guideline S6(R1). Only unconventional *in vitro*	Genotoxicity studies were not conducted as vedolizumab is a humanized monoclonal antibody and so are not required in-line with ICH Guideline S6(R1).		22/05/2014
14	Envarsus	tacrolimus	*unique entity*	0	The toxicity of tacrolimus has been previously well described in the literature. Therefore, no new toxicity studies were conducted by the applicant with the prolonged release formulation of tacrolimus, which is accepted by the CHMP.	No genotoxicity testing		18/07/2014
15	Eperzan	albiglutide	*unique entity*	1	No CARC studies have been conducted. Because of the emergence of clearing anti-albiglutide antibodies by 14 days in rodents, meaningful 2-year studies in rats or mice are not feasible.	No genotoxicity testing		21/03/2014
16	Gazyvaro	obinutuzumab	*orphan*	0	The lack of genotoxicity studies with obinutuzumab is acceptable because antibodies do not have the potential to cross the cell membrane.No studies have been performed to establish the carcinogenic potential of obinutuzumab (SmPC, section 5.3). The lack of carcinogenicity studies is considered acceptable based ICH S6 guidance. It should be noted that the present application concerns obinutuzumab co-treatment with chlorambucil, which is a human carcinogen.			23/07/2014
17	Granupas (previously Para-aminosalicylic acid Lucane)	para-aminosalicylic acid	*orphan*	1	performed previously; literature data reported	The applicant has suggested that a review of the literature identified no general toxicity studies of PAS that were relevant to the application. Some studies will be performed post-authorization.		07/04/2014
18	Harvoni	sofosbuvir / ledipasvir	*unique entity*	0	A 6-month rasH2 transgenic mouse study and a 2-year rat oral gavage carcinogenicity study with ledipasvir are being conducted. This is acceptable in view of the proposed short term treatment duration. The applicant will submit these studies in December 2015.	Tested *in vitro* and *in vivo*		17/11/2014
19	Hemangiol	propranolol HCl	*unique entity*	2	No carcinogenicity testing	The applicant detailed the published genotoxicity data with propranolol - known substance		23/04/2014
20	Imbruvica	ibrutinib	*orphan*	0	The carcinogenicity will be tested in a Transgenic (Tg) mouse range-finder study (to be submitted by 3Q 2015) followed by a Tg ras H2 6 month mouse carcinogenicity study as a non-clinical post authorisation measure. Advance cancer treatment no need for testing.	No genotoxicity testing		21/10/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
21	Incruse	umeclidinium bromide	*unique entity*	0	2-yr CARC study in rats and mice	**Tested *in vitro* and *in vivo***		28/04/2014
22	Izba	travoprost	*unique entity*	0	Old data evaluated already for previous formulation	Data from literature		20/02/2014
23	Jardiance	empagliflozin	*unique entity*	0	2-yr CARC study in rats and mice + additional *in vivo* in mice	Tested *in vitro* and *in vivo*		22/05/2014
24	Kolbam (previously Cholic Acid FGK)	cholic acid	*orphan - exceptional circumstances*	2	Long-term monitoring of the risk for carcinogenicity is included as an element in the Risk Management Plan.	Data from literature		04/04/2014
25	Latuda	lurasidone	*unique entity*	1	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		21/03/2014
26	Laventair	umeclidinium bromide / vilanterol	*unique entity*	0	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		08/05/2014
27	Lynparza	olaparib	*orphan*	0	No CARC studies were provided which is acceptable according to ICH S9 and in line with the protocol assistance received. Possible occurrence of new malignancies due to the pharmacological action of olaparib will be closely monitored	Tested *in vitro* and *in vivo*		16/12/2014
28	Mekinist	trametinib	*unique entity*	0	No carcinogenicity testing. In accordance with ICH S9, carcinogenicity studies are not necessary for the approved anti-cancer indication.	Tested *in vitro* and *in vivo*		30/06/2014
29	Mirvaso	brimonidine tartrate	*unique entity*	1	In support of the application, a dermal photo (co)carcinogenicity study in mice and a 2-year dermal carcinogenicity study in rats supported by toxicokinetic data have been performed.	No genotoxicity testing		21/02/2014
30	Moventig	naloxegol oxalate	*unique entity*	0	2-yr CARC studies in CD1 mice and SD rats.	Tested *in vitro* and *in vivo*		08/12/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
31	Neuraceq	florbetaben (18F)	*unique entity*	3	Considering the absence of a genotoxic risk for florbetaben HCl and the intended clinical use of florbetaben, and according to the Guideline on the need of carcinogenicity studies of pharmaceuticals (CPMP/ICH/140/95 S1A), no carcinogenicity studies are required for florbetaben.	No genotoxicity testing		20/02/2014
32	Nuwiq	simoctocog alfa	*unique entity*	0	Nuwiq is a recombinant B-domain-deleted (BDD) rFVIII human FVIII concentrate that is produced in genetically modified human embryonic kidney (HEK) 293F cells.	No genotoxicity testing		24/07/2014
33	Olysio	simeprevir	*unique entity*	0	The lack of carcinogenicity studies was justified by the Applicant in line with ICH S1A and based on the proposed treatment duration of 12 weeks for TMC435.	tested *in vitro* and *in vivo*		14/05/2014
34	Plegridy	peginterferon beta-1a	*unique entity*	2	Consistent with the ICHS6 Addendum, a weight of evidence approach was taken in the evaluation of the potential carcinogenicity risk of BIIB017 for humans.	Tested *in vitro* only		18/07/2014
35	Pregabalin Pfizer	pregabalin	*unique entity*	1	Since this application is an informed consent of the Lyrica application, the non-clinical data in support of the Pregabalin Pfizer application are identical to the up-to-date non-clinical data of the Lyrica dossier, which has been assessed and approved	No genotoxicity testing		10/04/2014
36	Revinty Ellipta	fluticasone furoate / vilanterol trifenatate	*unique entity*	0	No non-clinical data have been submitted in the Revinty Ellipta dossier, since this application is an informed consent of the Relvar Ellipta application: the non-clinical data in support of the Revinty Ellipta application are identical to the up-to-date non-clinical data of the Relvar Ellipta dossier, which have been assessed and approved.	No genotoxicity testing		02/05/2014
37	Rezolsta	darunavir / cobicistat	*unique entity*	0	No non-clinical studies were performed with the combination of darunavir and cobicistat. This was considered acceptable by the CHMP. The two active ingredients were previously authorized	No genotoxicity testing		19/11/2014
38	Simbrinza	brinzolamide / brimonidine tartrate	*unique entity*	0	This application concerns a fixed combination medicinal product including both brinzolamide and brimonidine in the same strengths as approved for the mono-component products The non-clinical assessment of the Simbrinza is mainly based upon the established non-clinical profiles of the individual approved active drug substances	No genotoxicity testing		18/07/2014
39	Sirturo	bedaquiline fumarate	*orphan – condition approval*	1	On-going study in rats, only. Due to poor tolerability of bedaquiline in mouse, a carcinogenicity study in mouse is not being conducted	Tested *in vitro* and *in vivo* single substances only		05/03/2014
40	Sovaldi	sofosbuvir	*unique entity*	1	2-yr CARC study in rats and mice.	Tested *in vitro* and *in vivo*		16/01/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
41	Sylvant	siltuximab	*orphan*	1	No carcinogenicity testing. Evidence from studies conducted with siltuximab and other IL-6 inhibitors suggest that the potential for siltuximab to cause carcinogenicity is low.	No genotoxicity testing		22/05/2014
42	Tecfidera	dimethyl fumarate	*unique entity*	2	2-yr CARC study in rats and mice, oral	Standard test battery according to ICHS2A and B guidelines		30/01/2014
43	Tivicay	dolutegravir	*unique entity*	2	101-104-week study in rats, oral 88-95-week study in mice, oral	Tested *in vitro* and *in vivo*		16/01/2014
44	Translarna	ataluren	*orphan – condtion approval*	1	26-week CARC study in Tg.rasH2 mice 24-month CARC study in rats	Tested *in vitro* and *in vivo*		31/07/2014
45	Triumeq	abacavir sulfate / dolutegravir sodium / lamivudine	*unique entity*	0	The applicant applied for registration of Triumeq film-coated tablets, a fixed dose combination (FDC) product containing dolutegravir, abacavir and lamivudine. No new studies were performed with the combination dolutegravir, abacavir and lamivudine UNDER MONITORING			01/09/2014
46	Trulicity	dulaglutide	*unique entity*	0	The human relevance of thyroid C-cell tumours from the GLP-1 receptor agonist class is unknown and at this time a potential to cause carcinogenicity in man cannot be completely ruled out. The findings have been included in SmPC.	No genotoxicity testing		21/11/2014
47	Ulunar Breezhaler	glycopyrronium bromide / indacaterol maleate	*unique entity*	0	This application is an informed consent of the Ultibro Breezhaler application: the non-clinical data in support of the Ulunar Breezhaler application are identical to the up-to-date non-clinical data of the Ultibro Breezhaler dossier, which have been assessed and approved.	No genotoxicity testing		23/04/2014
48	Vargatef	nintedanib	*unique entity*	0	Vargatef is indicated in combination with docetaxel for the treatment of adult patients with locally advanced, metastatic or locally recurrent non-small cell lung cancer (NSCLC) of adenocarcinoma tumour histology after first-line chemotherapy.	Genotoxicity studies indicated no mutagenic potential for nintedanib.According to the ICH S9 carcinogenicity studies are not warranted in the context of the proposed indication for (second line and in combination with docetaxel).		21/11/2014
49	Velphoro	mixture of polynuclear iron(III)-oxyhydroxide, sucrose and starches	*unique entity*	0	2-yr CARC studies in mice and rats	Tested *in vitro* and *in vivo*		26/08/2014
50	Vimizim	recombinant human n-acetylgalactosamine-6-sulfatase (rhgalns)	*orphan*	2	No carcinogenicity testing. The enzymatic activity of elosulfase alfa is restricted to the lysosomal compartment where it specifically degrades KS and this mode of action does not raise concern of a potential for neoplasm induction or tumour promotion	No genotoxicity studies were performed and the conduct of such studies is not considered necessary due to the protein structure and the enzymatic activity of the drug substance, which is acceptable to the CHMP.		28/04/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
51	Vizamyl	flutemetamol (18F)	*unique entity*	1	Carcinogenicity studies have not been conducted, because Flutemetamol (18F) Injection is a diagnostic imaging agent intended for infrequent administration with significant intervals between treatments	Tested *in vitro* and *in vivo*		22/08/2014
52	Vokanamet	canagliflozin / metformin hydrochloride	*unique entity*	1	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		23/04/2014
53	Vylaer Spiromax	budesonide / formoterol fumarate dihydrate	*unique entity*	0	No carcinogenicity testing. The nonclinical toxicology summary is largely based on the information available for the Symbicort Turbohaler reference product. No original study reports are available. Considering this is a hybrid application this approach is acceptable.	No genotoxicity testing		19/11/2014
54	Xigduo	metformin hydrochloride / dapagliflozin propanediol monohydrate	*unique entity*	1	No carcinogenicity testing. The individual toxicities of dapagliflozin and metformin were previously established in a comprehensive investigational program	No genotoxicity testing		16/01/2014
55	Xultophy	insulin degludec / liraglutide	*unique entity*	0	This is a fixed combination of the two approved active substances insulin degludec and liraglutide. The applicant has submitted summaries on the pharmacology, pharmacokinetics and toxicology of the individual components. No data reported	No genotoxicity testing		18/09/2014
56	Zydelig	idelalisib	*unique entity*	0	No CARC studies with idelalisib have been conducted. Carcinogenicity studies are in general not required to support marketing for therapeutics intended to treat patients with advanced cancer.	Tested *in vitro* and *in vivo*		18/09/2014
57	Busulfan Fresenius Kabi	busulfan	*generic*	0	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			22/09/2014
58	Ebilfumin	oseltamivir	*generic*	1	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			22/05/2014
59	Levetiracetam Hospira	levetiracetam	*generic*	1	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justified why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC were in line with the SmPC of the reference product.			08/01/2014
60	Tadalafil Mylan	tadalafil	*generic*	0	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			21/11/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
61	Zoledronic Acid Accord	zoledronic acid monohydrate	*generic*	0	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. The impurity profile has been discussed and was considered acceptable.			16/01/2014
62	Zoledronic acid Teva Generics	zoledronic acid monohydrate	*generic*	2	A non-clinical overview on the pharmacology, pharmacokinetics and toxicology has been provided, which is based on up-to-date and adequate scientific literature. The overview justifies why there is no need to generate additional non-clinical pharmacology, pharmacokinetics and toxicology data. The non-clinical aspects of the SmPC are in line with the SmPC of the reference product. The impurity profile has been discussed and was considered acceptable.			27/03/2014
63	Abasaglar (previously Abasria)	insulin glargine	*biosimilar*	1	In line with current guidelines on the development of similar biological medicinal products, no carcinogenicity studies have been performed; more data were provided to confirm biosimilarity	In line with current guidelines on the development of similar biological medicinal products, no genotoxicity studies have been performed		09/09/2014
64	Accofil	filgrastim	*biosimilar*	0	According to the Guidance on similar medicinal products containing recombinant granulocyte-colony stimulating factor (EMEA/CHMP/BMWP/31329/2005), safety pharmacology, reproduction toxicology, mutagenicity and carcinogenicity studies are not routine requirements for non-clinical testing of similar biological medicinal products containing recombinant G-CSF as active substance.			18/09/2014
65	Bemfola	follitropin alfa	*biosimilar*	1	No carcinogenicity testing. In accordance with both the ICH S6 guideline on the development of biotechnology-derived pharmaceuticals	Genotoxicity studies are not required for a biosimilar medicinal product. This is in accordance with both the ICH S6 guideline and the CHMPguideline on the development of biosimilar products		27/03/2014
66	Rivastigmine 3M Health Care Ltd	rivastigmine	*generic*	-	The non-clinical aspects of the SmPC are in line with the SmPC of the reference product.			withdrawn
67	Masican	masitinib	*orphan*	-	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		refused
68	Masiviera	masitinib	*orphan*	-	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		refused
69	Nerventra	laquinimod	*unique entity*	-	104-week study in rats 26-week in p53 mice	Tested *in vitro* and *in vivo*		refused
70	Reasanz	serelaxin	*unique entity*	-	No carcinogenicity testing. Relaxin (H2) is a naturally occurring peptide hormone (molecular weight of 5963 Daltons)	No genotoxicity testing		refused

Legend
Rev.: Revision number
Cat.: Category of medicines as: unique entity, orphan, generic, biosimilar etc.
The information reported in the table was retrieved from EMA website (http://www.ema.europa.eu/ema/) and EPARs publicly available documents.

Annex III. Veterinary medicines list of authorized substances in 2011 and 2014

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
1	Activyl	indoxacarb	*unique entity*	0	Tested	Tested *in vitro* and *in vivo*		18/02/2011
2	Bluevac BTV8	bluetongue virus inactivated, serotype 8	*Accelerated procedure – condition approval*	0	No carcinogenicity testing - vaccine	No genotoxicity testing		14/04/2011
3	CaniLeish	Leishmania infantum excreted secreted proteins	*unique entity*	0	No carcinogenicity testing - vaccine	No genotoxicity testing		14/03/2011
4	Certifect	fipronil /amitraz /(S)-methoprene	*unique entity*	0	The omission of reproductive, genotoxicity and carcinogenicity studies is justified by the demonstration of the non-interaction and the use of well-established substances.	No genotoxicity testing		06/05/2011
5	Cimalgex	cimicoxib	*unique entity*	0	No carcinogenicity studies were performed. Cimicoxib is not genotoxic, no structural alerts have been identified, and there was no signal indicating carcinogenic potential in the repeated dose toxicity studies.	Tested *in vitro* and *in vivo*		18/02/2011
6	Comfortis	spinosad	*unique entity*	0	18- and 24-moths studies in mice; A combined chronic toxicity/neurotoxicity/oncogenicity study was conducted in rats.	Tested *in vitro* and *in vivo*		11/02/2011
7	Emdocam	meloxicam	*generic*	0	Given that bioequivalence of the test and reference products was accepted, the Committee agreed that no data in respect of pharmacology or toxicology are required.	No genotoxicity testing		18/08/2011
8	Hiprabovis IBR Marker Live	live gE- tk- double-gene-deleted bovine herpes virus type 1, strain	*unique entity*	0	No need for carcinogenicity testing. The active substance of Hiprabovis IBR Marker Live is a live double-gene deleted (deleted glycoprotein E (gE-) and deleted thymidine kinase (tk-/+) Bovine Herpes Virus type 1, strain	No genotoxicity testing		27/01/2011
9	Inflacam	meloxicam	*generic*	0	As essential similarity to the reference product was confirmed, the results of toxicological and pharmacological tests and clinical trials were not required in accordance with Article 13 of Directive 2001/82/EC, as amended.	No genotoxicity testing		09/12/2011
10	Melosus	meloxicam	*generic*	0	The applicant has claimed bioequivalence with Metacam 1.5mg/ml oral solution and that data in support of the pharmacology and toxicology of meloxicam are not required	No genotoxicity testing		21/02/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
11	MS-H Vaccine	Mycoplasma synoviae strain MS-H	*unique entity*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		14/06/2011
12	Nobivac Myxo-RHD	live myxoma-vectoredrabbit-haemorrhagic-disease virus strain 009	*unique entity*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		07/09/2011
13	Panacur AquaSol	fenbendazole	*unique entity*	0	Fenbendazole, the active substance contained in Panacur AquaSol, is a well-established substance that has been widely and safely used for more than 30 years. Therefore, the applicant refers to published, valid safety assessment of fenbendazole including the CVMP MRL summary report EMEA/MRL/193/97-FINAL.	No genotoxicity testing		09/12/2011
14	Procox	emodepside/ toltrazuril	*unique entity*	0	Tested for carcinogenicity - combination	Tested *in vitro* and *in vivo*		20/04/2011
15	Proteq West Nile	vCP2017 virus	*unique entity*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		05/08/2011
16	Purevax Rabies	vCP65 virus	*unique entity*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		18/02/2011
17	Recocam	meloxicam	*generic*	0	No carcinogenicity testing	No genotoxicity testing		13/09/2011
18	Recuvyra	Fentanyl	*unique entity*	0	No carcinogenicity testing	Tested *in vitro* and *in vivo*		06/10/2011
19	TruScient	dibotermin alfa	*unique entity*	0	*In vitro* studies to assess the potential effects of dibotermin alfa on tumour cell growth using various tumour cell lines and primary tumour isolates. Additionally, studies in mice to assess whether surgical implantation of dibotermin alfa/ACS augmented the growth of subcutaneously injected human tumour cell lines (xenografts) suggest minimal potential for dibotermin alfa to stimulate tumour cell growth.	Tested *in vitro* (AMES) only		withdrawn
20	Veraflox	pradofloxacin	*unique entity*	0	2-yr CARC study in rats and mice	Tested *in vitro* and *in vivo*		12/04/2011

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
21	Zulvac 1 Bovis	inactivated bluetongue virus, serotype 1	*accelerated procedure*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		05/08/2011
22	Zulvac 1 Ovis	inactivated bluetongue virus, serotype-1	*accelerated procedure*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		05/08/2011
23	Zulvac 1+8 Ovis	inactivated bluetongue virus, serotype 1/ inactivated bluetongue virus, serotype 8	*Condition approval*	0	Vaccine – No carcinogenicity testing	No genotoxicity testing		14/03/2011
24	Zuprevo	tildipirosin	*unique entity*	0	No carcinogenicity testing Due to the absence of a chemical relationship to known carcinogens, the negative results of genotoxicity assays and the lack of carcinogenic potential of other macrolide antibiotics, it is assumed that tildipirosin is devoid of a carcinogenic risk.	Tested *in vitro* and *in vivo*		06/05/2011

Legend
Rev.: Revision number
Cat.: Category of medicines as: unique entity, orphan, generic, biosimilar etc.
The information reported in the table was retrieved from EMA website (http://www.ema.europa.eu/ema/) and EPARs publicly available documents.

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
1	Bravecto	fluralaner	*unique entity*	0	Studies on fluralaner for carcinogenic potential were not submitted. This is justified by the negative results in all mutagenicity assays and the absence of pre-neoplastic lesions in repeated dose toxicity studies	Tested *in vitro* and *in vivo*		11/02/2014
2	Equisolon	prednisolone	*unique entity*	0	Toxicology data for prednisolone have previously been assessed by the CVMP and the toxicological profile is presented in the European Public MRL Assessment Report (EPMAR) of prednisolone (EMEA/MRL/629/99-FINAL, July 1999). No new data on prednisolone toxicity were provided in this application. The applicant requested classification for minor use minor species (MUMS)/limited market status this product by the CVMP.			12/03/2014
3	Eryseng	*Erysipelothrix rhusiopathiae*, strain R32E11 (inactivated)	*unique entity*	0	Vaccine similar to a combined one for which several studies were performed – no carcinogenicity test needed	No genotoxicity testing		04/07/2014
4	Eryseng Parvo	porcine parvovirus, strain NADL-2 and *Erysipelothrix rhusiopathiae*, strain R32E11 (inactivated)	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		08/07/2014
5	Fungitraxx	itraconazole	*unique entity*	0	As itraconazole was shown to be devoid of mutagenic/genotoxic potential in a suitable battery of tests, the absence of any further carcinogenicity data/studies is acceptable. In addition the molecule has no structural alerts related to any potential carcinogenic risk. Itraconazole should therefore be considered as devoid of mutagenic/genotoxic and carcinogenic potential.			12/03/2014
6	NexGard	afoxolaner	*unique entity*	0	Similar product authorized in 2015 combination – no testing	Tested *in vitro* and *in vivo*		11/02/2014
7	Nobilis IB Primo QX	live avian infectious bronchitis virus, strain D388	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		04/09/2014
8	Osurnia	terbinafine /florfenicol /betamethasone acetate	*unique entity*	0	3 active substances combined data reported in previous MRL studies reports no genotoxic no carcinogenic potential. No further testing needed			31/07/2014
9	Porcilis PCV M Hyo	porcine circovirus type 2 ORF2 subunit antigen, *Mycoplasma hyopneumoniae* strain ATCC 25934 (inactivated)	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		07/11/2014
10	Vectra Felis	pyriproxyfen / dinotefuran	*unique entity*	0	Data already presented for a previous product (VECTRA 3D)			06/06/2014

[continue]

	Name	Active ingredient	Cat.	Rev.	Carcinogenicity Studies	*in vitro* Genotox studies	*in vivo* Genotox studies	Date of approval
11	Versican Plus DHPPi	Canine distemper adenovrus…	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		04/07/2014
12	Versican Plus DHPPi/L4	Canine distemper adenovrus…	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		07/05/2014
13	Versican Plus DHPPi/L4R	Canine distemper adenovrus…	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		07/05/2014
14	Versican Plus L4	Leptospira interrogans serogroup Australis serovar Bratislava,	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		31/07/2014
15	Versican Plus Pi	Canine parainfluenza type-2 virus, strain CPiV-2 Bio 15 (live attenuated)	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		04/07/2014
16	Versican Plus Pi/L4	Canine parainfluenza type 2 virus	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		31/07/2014
17	Versican Plus Pi/L4R	Canine parainfluenza virus (live attenuated), canine leptospirosis and rabies (inactivated) vaccine	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		31/07/2014
18	Bovela	modified live bovine viral diarrhoea virus type 1,	*unique entity*	0	Vaccine - No carcinogenicity testing	No genotoxicity testing		22/12/2014

Annex IV. Plant protection products list of authorized substances in 2011 and 2014

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Studies requested upon submission	Date of approval
1	1-Decanol	Plant growth regulator	A4	no	Waived: is a natural product found in environment and body so no need to test carcinogenicity; similar conclusion from US EPA , it is used as plant growth regulators on tobacco so not for human consumption and operators exposed for short periods	yes	Ames, MLA, HPRT, COMET, MN	yes	MN in mouse bone marrow		01/06/2011
2	6-Benzyladenine	Plant growth regulator	A4	yes	Partially waived: one study only in rats with limited info; further studies have been waived because not needed	yes	6 studies: Ames, UDS, CA, 3 MLA studies	yes	MN *in vivo*		01/06/2011
3	Aluminium sulphate	Bactericide	A4	no	Data from literature	yes	Data from literature	yes	Data from literature		01/06/2011
4	Azadirachtin	Insecticide	A4	no	No reliable study submitted	yes	Data from literature	yes	Studies performed with extracts not all of them;	Data GAPS, though already in annex 1 as insecticide now as acaracide for ornamentals plants and greenhouse	01/06/2011
5	Bispyribac	Herbicide	C	yes	2-yr CARC study in rats and in mice; 52-week study in dog	yes	Reverse mutation in Ames test, forward mutations in V79 cells, of chromosomal aberrations in Chinese hamster ovary cells, and of DNA damage (UDS in rat hepatocytes).	yes	MN *in vivo*		01/08/2011
6	Bromadiolone	Rodenticide	A4	no	waived as: study is not feasible, anticoagulant similar to warfarin extremely toxic	yes	Ames, CA, MLA	yes	MN *in vivo* mouse		01/06/2011
7	Bromuconazole	Fungicide	A3	yes	2-yr CARC study in rats and mice	yes	Ames, 2 CA, 2 gene mutation assays (MLA, and V79), UDS ex vivo	yes	2 studies: MN and UDS	Some tests repeated with metabolite and impurities	01/02/2011
8	Bupirimate	Fungicide	A3	yes	2-yr CARC study in rats and mice; 80-week study in mice; 105-week in dogs	yes	Ames, CA,	yes	MN, DML		01/06/2011
9	Buprofezin	Insecticide	A3	yes	2-yr CARC study in rats and mice; 105- week study in dogs	yes	Ames (2 exp. one study insufficient) , UDS, MLA, CA, Rec assay(I)	yes	MN (insufficient)	Ames test repeated with parent and metabolites	01/02/2011
10	Carbetamide	Herbicide	A3	yes	combined chronic/CARC in rats, 2-yr study in mice; 52-week in dogs	yes	Several old studies with no reliable data or not following TG	yes	UDS in rat liver and MN in BM cells, revised	Category 3 R40	01/06/2011

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Studies requested upon submission	Date of approval
11	Carboxin	Fungicide	A3	yes	combined chronic/CARC study in rats; 19-month CARC study in mice	yes	Ames, CA, UDS, HGPRT	yes	2 CA studies	*in vivo* UDS, *in vitro* UDS	01/06/2011
12	Clethodim	Herbicide	A3	yes	2-yr CARC study in rats	yes	Ames, CA, MLA with different metabolites	yes	MN in mice	No original DAR; experiments performed with clethodim sulfone and clethodim oxazole	01/06/2011
13	Cycloxydim	Herbicde	A3	no	Only historical data: 18-monthstudy,drinking, in rats; 2-yr CARC study in rats and in mice	no	Ames, MLA, CA, UDS	no	MN and CA		01/06/2011
14	Cyproconazole	Fungicide	A3	yes	Revised studies in rats and mice; added few more metabolic studies	yes	Ames , CA (3), HGPRT in v79cells, mitotic non-Dis-junct.in yeast, UDS, CTA in SHE	yes	MN, CA, DML	Change of cat from Cat 3 to Cat 2	01/06/2011
15	Dazomet	Nematicide, Fungicide, Herbicide, Soil treatment	A3	yes	2-yr combined chronic/CARC in rats; 78-week combined chronic/CARC in mice; 2-yr chronic study in rats	yes	Ames, REC, MLA, HPRT, CA-SHE, CA lymph + CTA in Balb cells	yes	UDS, MN bone marrow, CH spermatogonia	Asked for measuring [conc] of metabolites for exposure limits	01/06/2011
16	Diclofop	Herbicide	A3	yes	one combined chronic/CARC and 2-yr CARC study in rats; 2-yr CARC study in mice	yes	Ames, CA, HGPRT, UDS	yes	CA in CHO, CA in BM	Asked for further discussion	01/06/2011
17	Diethofencarb	Fungicide	A3	yes	3 combined chronic/CARC in rats (3 batches); two 2-yr studies in mice; mechanistic study in rat follicle tumour formation. Thyroid tumour in rats due to secondary damage to the hormone pituitary axis	yes	Ames, V79 conversion, CA (low sensitivity) , SCE, UDS (not acceptable)	yes	CA	UDS *in vivo*	01/06/2011
18	Dithianon	Fungicide	A3	yes	2-yr CARC study in rats; 80-week CARC study in mice, 7-d and 28-d oral female rats	yes	Ames, MLA (2), CA, UDS (indicative only short exposure)	yes	CA and MN	Re-evaluation of 2-yr study in rats; mechanistic studies; *in vivo* : MN, Comet, UDS. COMET assay was considered as indicative because not fully validated	01/06/2011
19	Dodine	Fungicide	A3	yes	2-yr CARC study in rats; 18-month in mice	yes	Ames (2 batches), HGPRT, CA	yes	2 MN studies		01/06/2011
20	Etridiazole	Fungicide	A3	yes	2-yr CARC study in rats; 18-month in mice; plus historical data and older studies	yes	Ames, CA, SCE (2), MLA	yes	MN, CA, UDS		01/06/2011

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Studies requested upon submission	Date of approval
21	Fenazaquin	Acaricide	A3	yes	2-yr combined chronic/CARC study in rats; 18-month in S. hamster	yes	Ames, CA, MLA, UDS	yes	MN, liver rat DNA repair, *in vivo* SCE in BM cells	*in vivo* MN with appropriate staining procedure FISH, and *in vitro* MLA and CA for the metabolites	01/06/2011
22	Fenbuconazole	Fungicide	A3	yes	2-yr CARC study in rats; 18-month CARC study in mice	yes	Not specified	yes	Not specified		01/05/2011
23	Fenoxycarb	Insecticide	A3	yes	Combined chronic/CARC study in rats; 78-week study in albino mice; 80-week study in CD-1 mice; plus several mechanistic studies in rats and mice	yes	Ames, yeast REC, HPRT,CA (3)	yes	MN (2)	performed re-evaluation of 2 year CARC study in rats; mechanistic studies	01/06/2011
24	Fluometuron	Herbicide	A3	yes	52-week chronic study in rats, 2-yr CARC study diet in rats, 2-yr CARC study in mice	yes	Ames (2), MLA, CA,DNA repair test (3) Clastogenic	yes	nucleus anomaly in BM, MN, negative *in vivo*	explanations on impurities; re-evaluation of CARC data in mice was not accepted	01/06/2011
25	Flurochloridone	Herbicide	A3	yes	Not specified in the addendum	yes	Ames (3) + 1new, MLA (2), CA, SCE, CTA in Balb cells, DNA repair and UDS	yes	CA in bone marrow cells	Additional data for impurities (toluene) Ames test, acute toxicity	01/06/2011
26	Flutriafol	Fungicide	A3	yes	2-yr CARC study in rats and in mice;	yes	Ames, MLA, CA	yes	CA, MN, UDS and germ cells, DML	3 more *in vitro* genotoxicity studies with new batch with impurities in line with EC guidance on technical equivalences, based on results perhaps further studies could be requested	01/06/2011
27	Haloxyfop-P (Haloxyfop-R)	Herbicide	A3	yes	2-yr CARC study in rats and in mice; plus QSAR data	yes	Ames (3), HGPRT, UDS (2), CA (3), in the first submission plus QSAR data	yes	MN	Asked for metabolites relevance	01/01/2011
28	Hexythiazox	Acaricide, Insecticide	A3	yes	2-yr combined chronic/CARC feeding in rats, 2-yr CARC study, feeding in mice	yes	Ames, yeast REC, HPRT in v79,CA in CHO (2), rec in *B. subtilis*, UDS	yes	CA, MN (2)	Ames test and *in vitro* MLA and CA and *in vivo* MN genotox,	01/06/2011
29	Hymexazol	Fungicide	A3	yes	2-yr combined chronic/CARC rats; 91-week CARC study in mice	yes	Ames (2), CA, MLA, genmutat in v79, UDS in HeLa cells	yes	MN, sex recessive Drosophyla,	*in vitro* CA and *in vivo* MN or CA	01/06/2011
30	Indolylbutyric acid	Plant growth regulator	A4	no	Waived also in the first submission because use for ornamental plants	yes	Ames (2), HGPRT,CA	yes	MN	ornamental plants, no exposure for operators, fastly secreted >85% in 24hrs in rats, no histopathol changes in 90-days, 1-generation study in rats, no Derek alerts; 1 Ames test and *in vivo* MN performed	01/06/2011

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Studies requested upon submission	Date of approval
31	Isoxaben	Herbicide	A3	yes	1-yr chronic study rats; 2-yr combined study in rats, 2-yr combined study in mice, 1-yr study in dog	yes	Ames (2), UDS, CA, *B. subtilis* Rec, MLA	yes	CA,MN (3), DML	New data genotox were presented for metabolites; pec grow on metabolite	01/06/2011
32	Lime sulphur (calcium polysulphid)	Fungicide, Insecticide, Acaricide	A4	no	No data presented because no references are on its possible carcinogenicity effect	yes	Ames, CA, MLA (in submission 2008)	yes	MN	No further test on carc or chronic toxicity is required but, a UDS *in vivo*	01/06/2011
33	Metaldehyde	Molluscicide	A3	yes	2-yr combined chronic/CARC in rats; 2-yr CARC study in rats (limited validity), 78-week CARC in mice	yes	Ames, CA, MLA, DML in *E.coli*	yes	MN		01/06/2011
34	Metosulam	Herbicide	A3	yes	2-yr chronic/CARC in rats; 18-month chronic/CARC in mice; plus exploratory studies on kidney tumour	yes	Ames (2), gene mutation (2), CA (2), UDS	yes	MN, and then new COMET	Ames test; cat CARC 3 R40 no genotoxic mechanism	01/05/2011
35	Myclobutanil	Fungicide	A3	yes	2-yr chronic/CARC rats; 2-yr CARC study in mice, 18-month study repeated in female mice	yes	Ames, HGPRT, CA, UDS	yes	CA (2) , DML	Literature	01/06/2011
36	Napropamide	Herbicide	A3	yes	2-yr combined chronic/CARC studies in rats; 18-month CARC in mice	yes	Ames 2, MLA, HPRT,HPRT in v79, CA 2, DNA damage (2), Rec assay, UDS	yes	MN (2), UDS	Additional data on the metabolite NOPA: Ames, MLA, CA *in vitro*, CA *in vivo*	01/01/2011
37	Oryzalin	Herbicide	A3	yes	12-month study and two 2-yr CARC studies in rats; 12-month and two 2-yr CARC studies in mice	yes	Ames, MLA, CA and UDS (not accepted),	yes	CA (3, but one not accepted), DML (2, but supportive only)	Further data and discussion for carcinogenicity in mice and genotox *in vivo* CA one more Ames sudy was performed and data discussed; after submitting additional discussion , more info of non-relevance of thyroid follicular tumour in rats was asked; Xn harmful and R40	01/06/2011
38	Paclobutrazol	Plant growth regulator	A3	yes	2-yr CARC studies in rats and mice	yes	Ames, MLA, CA,	yes	2 CA, 2 MN in mice, UDS, DML	only discussion and historical data	01/06/2011
39	Pencycuron	Fungicide	A3	yes	2-yr CARC study in rats; 2-yr CARC study in mice	yes	Ames (3 studies, one performed for resubmission), rec assays (4), yeast mit-rec (2), CA (2), UDS, HGPRT,	yes	2 MN, 2 DML	no further data requested; Ames performed, re-evaluation of carc study in rats;	01/06/2011
40	Profoxydim	Herbicide	C		in the list of new database for genotoxicity profiling launched in July 2014		no DAR available				01/08/2011

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Studies requested upon submission	Date of approval
41	Pyridaben	Acaricide, Insecticide	A3	yes	2-yr CARC study in rats; 18-month CARC study in mice	yes	Ames, HGPRT, CA, DNA repair	yes	MN	Further discussion on CARC data in rats was presented for resubmission	01/05/2011
42	Quinmerac	Herbicide	A3	yes	12-month in rats; 2-yr combined chronic/CARC study in rats; 78-week CARC study in mice; Plus supplementary 78-weeks in mice	yes	Ames (2), CA, HPRT, UDS, MLA	yes	MN	Asked for more data especially in regard of metabolites; Ames test, MLA with parent and MLA with metabolites MN *in vivo* were additionally performed for submission	01/05/2011
43	Sintofen (aka Cintofen)	Plant growth regulator	A3	yes	52-weeks dietary rats interim, 2-yr combined chronic/CARC study in rats; 78-week CARC study in mice	yes	Ames (3, one performed in 2008),HPRT in v79 cells, CA in CHO,UDS	yes	MN in BM cells,	No further studies requested presented additional information on CARC and historical data	01/06/2011
44	tau-Fluvalinate	Insecticide	A3	yes	2-yr combined chronic/CARC study in rats and mice	yes	Ames, MLA, CA, SCE (2), UDS	yes	CA	Compliance of the batches tested in the mammalian toxicology data package to the proposed specification cannot be demonstrated, in particular for the genotoxicity and carcinogenicity studies	01/06/2011
45	Tebufenozide	Insectide	A3	yes	2-yr combined chronic/CARC study in rats; 18-month study CARC in mice	yes	Ames, CA, HGPRT, UDS	yes	CA		01/06/2011
46	Triazoxide	Insectide	A3	yes	2-yrr CARC study rats; 21-month study in mice	yes	Ames, polA assay, HGPRT, CA, UDS	yes	DML, CA (2), MN	Re-evaluation of cancer study 2-yr rats for dark spleen colouring; LOAEL and ADI reconsidered in May 2010	01/10/2011
47	Triflumuron	Insecticide	A3	yes	2-yr CARC study rats and in mice	yes	Ames (2), HGPRT, UDS,SCE, CA	yes	MN		01/04/2011
48	Zinc phosphide	Rodenticide	A4	yes	Performed only in rats combined chronic/CARC; Two 2-yr CARC studies in rats	yes	No data with zinc phosphide; only with phosphine PH3: Ames (4), CA, HGPRT	yes	CA, MN, UDS, DML, SCE (2)	Tested only with phosphine no other test required	01/05/2011

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Date of approval
1	1,4-Dimethyl-naphthalene	Plant growth regulator	C	yes	2-yr combined chronic/CARC in rats; 2-yr combined chronic/CARC study in mice	yes	2 AMES studies, MLA, UDS	yes	MN in mouse MPEs and UDS in rat liver	01/07/2014
2	Acequinocyl	Acaricide	C	yes	2-yr combined chronic/CARC study in rats; 80-week CARC study mouse	yes	AMES, CA in CHL cells MLA - on metabolites also	yes	MN - on metabolites also	01/09/2014
3	Amisulbrom	Fungicide	C	yes	2-yr combined chronic/CARC study in rats; 78-week CARC study in mice	yes	AMES, MLA, CA in human lymphocytes studies	yes	MN in mouse BM oral, UDS, MN in liver rats oral, Rat liver COMET, Mouse liver COMET, Rat stomach/forestomach COMET, MN in mouse BM by injection	01/07/2014
4	Ascorbic acid	Fungicide	C	no		no	Not necessary Data from literature reported only	no	GRAS product =generally recognized as safe. Essential factor in physiology of plants and animal, evaluated as safe by cosmetic ingredient review panel 2005 (CIR)	01/07/2014
5	*Aureobasidium pullulans* (strains DSM 14940 and DSM 14941)	Fungicide, Bactericide	C	no	Waived as it is a microorganism and pathogenicity/infectivity studies by oral or inhalation or subcutaneous demonstrate that the microorganism does not survive at condition prevailing in mammalian cells (temp, pH), short term toxicity study not necessary as well as CARC study	no	waived as: *in vitro* standard test are not appropriate to test genotoxicity potential of fungal cells	yes	*in vivo* micronucleus on erythrocytes using dry granulate cells of *Aureobasidium*	01/02/2014
6	*Bacillus pumilus* QST 2808	Fungicide	C	no	Waived as it is a microorganism only tier I test performed	no		no	No infective species which are not able to enter mammalian cells. Do not exhibit mutagenic effect. (OECD guidance no 43, 2008) existing TG are not applicable to microbial products) genotoxicity is not necessary	01/09/2014
7	Benalaxyl-M	Fungicide	C	yes	Historical data performed as combined chronic/CARC study in rats and one 2-yr CARC study in mice, plus pathology peer review	yes	2 AMES studies, yeast gene mutation (2), MLA, gene mutation in V79 cells CA in CHO cells, CA in human cells	yes	MN *in vivo* in BM cells	01/05/2014
8	Chitosan hydrochloride		C				no DAR available			01/07/2014
9	Chlorantraniliprole	Insecticide	C	yes	combined chronic CARC 2-yrs-study in rats; 18-month CARC study in mice	yes	2 AMES studies, 2 CA in human lymphocytes, HGPRT in CHO cells and metabolites	yes	MN *in vivo* in mouse BM	01/05/2014
10	Disodium phosphonate	Fungicide	C	yes	4 studies: two studies in rats: one combined chronic/CARC study in rats; one CARC study in rats; one 2-yr CARC study in dog, one in mice; all performed in 1981.	yes	AMES, CA in V79 cells studies	yes	MN *in vivo* in mouse BM	01/02/2014

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Date of approval
11	Emamectin	Insecticide	C	yes	104-week combined chronic/CARC study in rats; 79-week CARC study in mice; 13-week study in mice for range-finding study, semi chronic studies: 13-week in rats; 52-week in rats; 14-week in dogs; 52–week- in dogs.	yes	AMES, CA in CHO cells, HPRT in V79 cells, alkaline/elution in rat hepatocytes	yes	CA in mouse BM cells	01/05/2014
12	*Equisetum arvense L.*		C	no	Waived as a plant extract, no assessment performed	no		no	The available data are insufficient both qualitatively and quantitatively, and are not specifically relevant for *Equisetum* to allow a specific assessment to be performed.	01/07/2014
13	Flubendiamide	Insecticide	C		no DAR available					01/09/2014
14	Fluopyram	Fungicide	C	yes	104-week combined chronic/ CARC in rats; 78-week CARC study in mice	yes	Studies: 2 Ames, HPRT and CA in V79 cells	yes	MN study in BM cells	01/02/2014
15	Ipconazole		C	yes	104- week combined chronic/CARC study in rats; 78-week CARC study in mice	yes	AMES, Bacterial DNA repair, CA, HGPRT in CHO cells studies	yes	MN study in BM cells	01/09/2014
16	Orange oil (D-limonene)	Insecticide	C	yes	2-yr bioassay reported from NTP studies in rats and mice	yes	5 AMES studies, 2 MLA studies, CA, SCE	yes	Mammalian Spot test, transgenic BigBlue mouse induction of *Lacz mut*, COMET assay in kidney,	01/05/2014
17	Penflufen	Fungicide	C	yes	2-yr combined chronic/CARC in rats; 80-week CARC study in mice; plus mechanistic non-standard study DNA synthesis and enzyme activity in cultured rat hepatocytes then in human hepatocytes	yes	2 AMES studies, CA in V79 cells, HPRT in V79 cells	yes	MN study in BM cells	01/02/2014
18	Penthiopyrad	Fungicide	C	yes	52-week chronic in rats; 2-yr CARC study in rats; 7-week in mice; 2-week hepatic enzyme induction in rats and one in mice; 2-weeksin rats thyroid function					yes
19	*Pseudomonas sp.* Strain DSMZ 13134		C	no	Waived as: it is a microorganism only tier I test performed	no		no		01/02/2014
20	Pyridalyl	Insecticide	C		no DAR available					01/07/2014

[continue]

	Substance	Category	Submission		Carcinogenicity Studies		*in vitro* Genotox studies		*in vivo* Genotox studies	Date of approval
7	Pyriofenone	Fungicide	C	yes	90-day in rats (range finding); 13-weeks mice range finding); 1-yr chronic rat, 2-yr CARC study in rats, 18-month CARC study in mice	yes	3 studies: Ames, CA in CHL cells; MLA	yes	3 studies: MN in BM mouse, UDS rat hepatocytes and COMET mouse liver	01/02/2014
22	Pyroxsulam	Herbicide	C	yes	2-yr combined chronic/CARC in rats; 18-month CARC study in mice	yes	Ames, CA in rat lymphocytes, HPRT in CHO	yes	2 studies MN in mouse BM and UDS in mouse liver	01/05/2014
23	S-Abscisic acid	Plant growth regulator	C	no	waived as no effect from short and chronic toxicity, low order of toxicity it does not increase with exposure) natural regulator no genotoxicity effect no effect on pregnancy no histopathology in tissues and organs in 90-days rat study	yes	Ames, CA in CHO and MLA tk	yes	MN in BM cells mice	01/07/2014
24	Sedaxane	Fungicide	C	yes	2-yr combined chronic/CARC in rats; 80-week CARC study in mice	yes	Ames, CA, MLA	yes	MN mouse BM and UDS rat liver	01/02/2014
25	Sodium silver thiosulphate	Plant growth regulator	C	no	Waived: not performed because inexistent exposure and used for ornamental flower	yes	Ames, CA, MLA	yes	UDS in rat liver	01/05/2014
26	Spinetoram	Insecticide	C	yes	2-yr combined chronic/CARC in rats; 18-month CARC study in mice	yes	Ames, CA in rat lymphocytes, HGPRT in CHO cells x2 (two mixtures)	yes	MN *in vivo* in BM cells (x1)	01/07/2014
27	Spirotetramat	Insecticide	C	yes	1-yr study in rats; 2-yr CARC study in rats; 18-month study in mice	yes	Ames, CA, MLA	yes	2 CA *in vivo*, 1 UDS	01/05/2014
28	Tembotrione	Herbicide	C	yes	2-yr CARC study in rats (stopped for males at 43 weeks); 2-yr study in male rats; 18-month study in mice	yes	Ames, CA, MLA	yes	MN and UDS	01/05/2014
29	Thiencarbazone	Herbicide	C	yes	2-yr combined chronic/CARC in rats; 18-month CARC study in mice	yes	Ames, CA in V79 cells, HPRT in V79 cells	yes	MN *in vivo* in mice PCEs; no study in germ cells because of negative results in other genotox tests	01/07/2014
30	Valifenalate (formerly Valiphenal)	Fungicide	C	yes	2-yr combined chronic/CARC in rats; 78-weeks CARC study in mice	yes	Ames, CA, MLA	yes	MN *in vivo* mice BM cells; study UDS and in germ cells not performed because of the results of previous studies	01/07/2014

Legend

Submission type (A1 to A4): existing active substances divide into four phased evaluations. Stage 1. Substances were reviewed by the Commission and the Member States without the participation of EFSA. This stage was completed by the Commission in 2007. Stage 2. For stage 2 EFSA was given the responsibility to organise the peer review of the initial risk assessments of the substances. EFSA completed its work in July 2006 publishing 'conclusions' on 50 substances. Stage 3 and 4. EFSA completed the evaluation of the substances of stage 3 and stage 4 in 2008. C: New active substances.

CA: chromosomal aberrations assays

MLA: mouse lymphoma assay mammalian cell gene mutation using *Tk* gene
HPRT/HGPRT: mammalian Cell gene mutation using the *Hprt* and *Xprt* genes
MN: micronucleus test
UDS: Unscheduled DNA Synthesis
CHO: *Chinese hamster* ovary cells
CHL: *Chinese hamster* lung cells
PCEs: polychromatic erythrocytes
BM: bone marrow
COMET: DNA damage (COMET assay)
The information reported was retrieved from EU pesticide Database and available EFSA DAR reports.

How to obtain EU publications

Our publications are available from EU Bookshop (http://bookshop.europa.eu),
where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents.
You can obtain their contact details by sending a fax to (352) 29 29-42758.

LB-NA-27765-EN-N

JRC Mission

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle.

Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Serving society
Stimulating innovation
Supporting legislation

Publications Office

doi:10.2788/547846

ISBN 978-92-79-56876-3