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Dermal Toxicity: From Lab Bench to Veterinary Clinic – A Review

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Abstract: Dermal exposure represents the most common route of the chemical application especially in pet animals. The study of environmental pollutants, industrial chemicals and therapeutics is mainly focusing on the detection of potential dermal toxicity of these xenobiotics. The aim of this review is to summarize the experimental and clinical toxic responses of the animal skin when exposed to different toxicants. Moreover, the dermal responses include chemical burns, dermatitis, photosensitization (primary and hepatogenous), skin fibrosis, dermal hyperplasia and carcinogenesis. Electronic searches were carried out using PubMed MEDLINE®, CABDirect and CONSULTANT database without date or language restrictions. The searches identified 119 including articles, reviews and book chapters. Careful analysis of these articles allowed us to: i) identify the different dermal responses to chemical; ii) investigate both *in vitro* and *in vivo* studies on dermal toxicity testing; iii) analyze the cellular and molecular steps of the chemically induced-wound or injury and the possible regenerative events; iv) evaluate the various updated technologies used in dermatology research such as SKINOMICS. This work enabled us to shed light on the dermal toxicity in veterinary clinics with a comprehensive integration of different biotechnologies in the experimental dermatology field. Furthermore, there will be a continuing need for careful translation of experimental data to the veterinary clinical application.

Keywords: dermatotoxicity; photosensitization; wound healing; dermal toxicity testing; skinomics.

1 Microanatomy of The Dermal System

The dermal system - commonly referred as “skin” covers the animal body. The skin acts as a barrier to protect the body from different external insults. In addition, the dermal system plays an important role in fluid and electrolyte balances. The dermal system consists of several parts: the epidermis, dermis, hypodermis and other components include sebaceous gland, hair, hair follicles, sweat glands, hooves, claws, and nails. At the histological level, the epidermis consists of several layers of keratinized stratified squamous epithelium mainly keratinocyte, Langerhans (tissue macrophage) and melanocytes while the dermis -the thicker portion- is composed of connective tissue and plays pivotal roles in protection, sensation and thermoregulation (Osweiler, 1996; Hascheck et al., 2010).

2 Dermal responses to toxicant

The dermal exposure represents the most common route of application of several compounds. The intact dermal system is not permeable to several compounds, while the moistened mucous membranes in the abraded skin offer good opportunities for xenobiotic absorption (Tiwari and Sinha, 2010). However, the oily solutions or emulsions are readily absorbed from the intact skin. The epidermis layers are able to metabolize some chemicals while the dermis has no metabolizing capabilities (Osweiler, 1996).

In veterinary field, animals can dermally be exposed to the chemicals in form of acids, alkalis, house hold products, pesticides and through topical administration of drugs. While, the spilling of a cleaning product or disinfectant on a companion animal body represents the most common route of cutaneous exposure. Several dermal alterations (Figure 1) are induced by direct and/or systemic exposure to toxicants such as erythema, irritation, corrosion, degeneration, necrosis, alopecia, primary photosensitization, hepatogeneous photosensitization, allergic contact dermatitis, trauma, hyperkeratosis, epidermal hyperplasia and carcinogenesis (Williams et al., 2000; Hascheck et al., 2010). As an example, hair loss (alopecia) is induced by arsenic, thallium and selenium exposure and some cytostatic drugs in dogs (Osweiler, 1996).

Following skin damage, a tightly-controlled regenerative

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process restores and repairs the wound and forms structurally intact skin. In conclusion, several responses take place due to chemical exposure. These responses are followed by regenerative events of the surviving cells to restore the skin tissue.

Figure 1

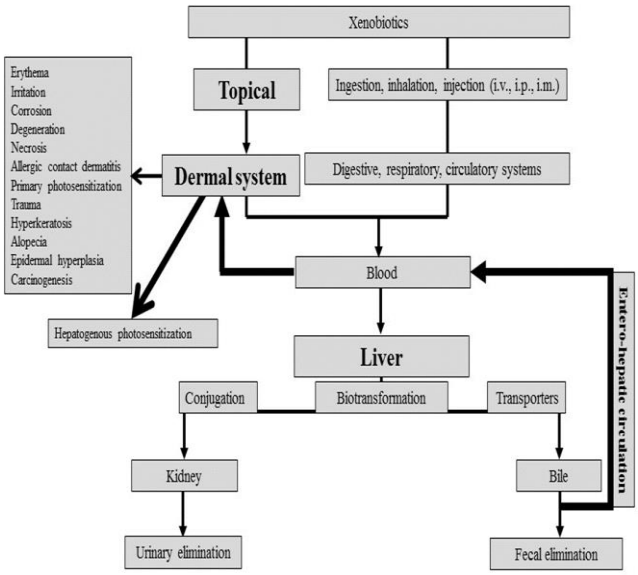

Figure 1. Direct and/or systemic exposure to toxicants.

2.1 Chemical burns

Thousands of chemicals have a direct destructive effect on the dermal system. This destruction is ranged from mild erythema, irritation and necrosis to corrosion. These chemicals (some chemicals are summarized in Table 1 and 2) include acids, alkalis, powerful oxidizing and reducing agents, phenols, arsenical compounds, mercuric salts, bromine, thallium, detergents and formaldehyde (Hardwicke et al., 2012).

In general, the pH value of the chemical more than 2 and less than 12 are considered safe (Osweiler, 1996). The most susceptible animals are pets and companion animals. Pets are exposed through the direct contact with house-hold products, accidental ingestion of potentially toxic chemicals, overdose of the prescribed medications and ingestion of toxic beetles (Stocks and Lindsey, 2008). Also, the livestock can be exposed to mild corrosives and irritant compounds in toxic plants, environmental pollutants and industrial contaminants (English et al., 2003; Collett et al., 2011; Souza et al., 2012).

Generally, the substance is able to damage the integrity of the stratum corneum and attacking the skin cells directly. The adverse effects of chemical on the skin depend mainly upon the concentration of the compound and the exposure time (English et al., 2003; Welss et al., 2004). A group of chemicals can produce immediate skin damage in form of necrosis, ulceration and tissue sloughing and referred as a third degree chemical burn (Klaassen, 2008). This group is known as “corrosives”. The continuation of dermal exposure leads to further skin damage and systemic intoxication. Corrosives include strong acids e.g., sulfuric , hydrochloric, hydrofluoric, carbolic, oxalic and glacial acetic acids (Dunn et al., 1996; Yoo et al., 2010; Goertz et al., 2013). Basics e.g., sodium, potassium and calcium hydroxides and hydroxidyl ions, ammonium salts as well as some metallic salts e.g., barium chloride and antimony trichloride are categorized as corrosives. Another example, sodium and potassium ortho-phenylphenols - used as fungicides and disinfectants - are considered as corrosive agents (Goertz et al., 2013; Qattan and Pitkanen, 2001).

The local corrosive action is induced through a proton donation in case of acids e.g., free H+ (Klaassen, 2008), thus the epidermal cellular and intercellular proteins are coagulated. This coagulated tissue forms eschar and inhibits further penetration (Hascheck et al., 2010). However, alkali agents lead to lipid saponification and protein denaturation through a proton recipient. Subsequently, liquefactive necrosis is produced and enhances the deeper penetration of the damaging hydroxyl ions (Hascheck et al., 2010).

Clinically, the chemical burns are manifested by severe skin damage in form of necrosis, ulceration, eschar formation and tissue sloughing. These symptoms are proceeded by the cardinal signs of inflammation e.g., swelling, erythema, heat and pain (Osweiler, 1996). We conclude that, several types of chemical burn are reported depending upon the chemical pH value and exposure time. Strong acids and alkalis have a local corrosive effect.

2.2 Dermatitis

Dermatitis is an inflammatory process of the dermal system and produces lesions are characterized by mild erythema, vesiculation and ulceration depending on the pH concentration of the agent and the frequency of application. Topical application of several agents induces dermatitis lesions in animals (see Table 1 and 2) e.g., plants (Jung et al., 2010). The exposure of skin to mustard gas induces erythema, edema, blister formation as well as irritation. Nitrogen and sulfur mustard have a direct effect on the basement membrane zone (Smith et al., 1998).

Indirectly, dermatitis can be induced by repeated oral administration of oxazolone in a mouse model (Yeom et al., 2012). Irritant contact dermatitis is a special type of dermatitis and resulted from activation and recruitment of innate inflammatory cells to the skin due to the direct injury. No good correlation exists between biochemical changes, chemical reactivity and irritation potential of one chemical with another. On the clinical level, erythema, oedema and itching represent the cardinal signs of chemical dermatitis (Osweiler, 1996).

Table 1. Dermal toxic responses in experimental animals

Xenobiotics	Animals	Topical application	**Toxic responses**		References
			Dermal	Others	
1-chloro-2,4-dinitrobenzene 2-phenyl-4-ethoxymethylene-5-oxazolone Toluene 2,4-diisocyanate	Female CBA/N Mice (8 weeks)	3 days	Ear thickness, skin inflammation dermal edema and epidermal hyperplasia	Not reported	(Lakos et al., 2004)
alent chromium [Cr(VI)]	Male Sprague–Dawley rats (10 weeks)	11 days	Severe erythema, edema, necrosis and epithelial desquamation	Not reported	(Lee et al., 2012)
1-(1-methyl-propoxycarbonyl)-2-(2-hydroxyethyl)-piperidine (insect repellant)	4-week-old male and female CD-1 mice	18 months	No changes	No evidence of a compound-induced neoplasia	(Wahle et al., 1999)
2,4-dichlorophenoxyacetic acid sodium Dimethylamine	Male Fl-hybrid rats of the inbred strains WELS /FOHM (♀) and BD IX/Halle (♂)	120 hours	Not reported	Hepatic enzyme inducers	(Knopp and Schiller, 1992)
Ketoprofen	New Zealand white rabbits (5–6 weeks) Hartley albino guinea pigs (5–6 weeks)	24 - 72 hours, 7 days (rabbit) and 14 days (G. pigs)	Epidermal hyperplasia, keratinocyte hypertrophy and infiltration of inflammatory cells	Not reported	(Lee et al., 2007)
Myoga (R-(+)-limonene)	Female Hartley guinea pigs	24 hours	Allergic contact dermatitis	Not reported	(Wei et al., 2006)
Captopril	Male and female beagle dogs	90 days (orally)	Erythema, hyperkeratosis with parakeratosis and acanthosis	An increase in erythropoiesis due to RBCs hemolysis Hypertrophy and hyperplasia of juxtaglomerular cells	(Ohtaki et al.,1981)
30% Hydrochloric acid 11% Sodium hydroxide	Hairless, male SKH-1/h mice	Topical	The angiogenesis occurred faster after acid burns than after alkali burns	Not reported	(Goertz et al., 2013)
Red propolis extract	New Zealand rabbits Hartley guinea pigs	24 hours	Only mild erthyma	Not reported	(Ledón et al., 2002)
Polycyclic aromatic compound (benzo(a)pyrene), residual aromatic extracts and bitumens	female CF1 mice (11 Weeks)	6 hours	Not reported	skin penetration and systemic bioavailability depend on the viscosity of the compound	(Potter et al., 1999)

Table 2. Case studies and clinical investigations of dermal toxicity in pets and livestock

Xenobiotics	Animals	Exposure	**Toxic responses**		References
			Dermal	Others	
Phytolacca octandra (inkweed)	Friesian heifer calves (6-8)	Case study (ingestion)	Acute irritation and hepatogenons photosensitization	Liver and kidney changes	(Collett et al., 2011)
Gluten	3 Appaloosa mares	Case	Diffuse erythema, edema and dry gangrene, ulceration and severe photosensitivity dermatitis	Not reported	(Yeruham et al., 1999)
Froelichia humboldtiana	One 3-year-old Holstein crossbreed cow	14 days	Primary photosensitization, alopecia, cutaneous, edema and skin necrosis	No systemic changes	(Souza et al., 2003)
Diesel oil	2 cats	Case study	Alopecia , dry skin and severe orthokeratotic hyperkeratosis	Not reported	(Declercq and Bosschere, 2009)
Panicum coloratum	Lambs	Ingestion	Hepatogenous photosensitization	Hepatic necrosis, obstruction of bile ducts	(Bridges et al., 1987)

				and bile canaliculi by small aggregates of birefringent crystals	
Microcystis aeruginosa (freshwater cyanobacteria)	Sheep	Lake	Hepatogenous photosensitivity	Mortalities	(Carbis et al., 1995)
Ammi majus seeds (Claviceps purpurea sclerotia & furocoumarins)	Pigs	Ingestion	Cutaneous irritation in the unpigmented areas and primary photosensitization	Abortions	(López et al., 1997)
Enterolobium contortisiliquum	Cattle	Grazing	Hepatogenous photosensitization	Abortion	(Grecco et al., 2002)
Sporidesmin toxicosis	Sheep	Ingestion	Hepatogenous photosensitization, facial eczema	Severe icterus, hepatomegaly and liver necrosis	(Ozmen et al., 2008)

2.3 Photosensitization

Two types of photosensitization are mainly reported in the veterinary field, primary and secondary photosensitization (see Table 1 and 2). Primary type is a light-induced dermatitis and can be produced through exposure of the unprotected skin – e.g., lacking of hair, wool or pigmentation - to sunlight in presence of photodynamic agent (Klaassen, 2008; Scarth, 2006).

Mechanistically, the photodynamic molecules present in the skin are excited and energized by sunlight. When the molecules return to the ground state, energy is released and transferred to the receptor molecules. Then the chemical reaction between this molecule and various components of the skin takes place (Klaassen, 2008; Scarth, 2006). Dermal injury is described as a consequence of a reactive oxygen intermediate production and/or alterations in the permeability of the cell membrane. Many chemicals - including some that are of plant, fungal and bacterial origins - may contain photosensitizing agents.

However, in veterinary medicine the common cause of photosensitization is a plant-derived one (Souza et al., 2012; Scarth, 2006). Some medicinal compounds have a photoreactive effect such as hypericin (Carpenter and Kraus, 1991), hypocrellin A (Zang et al., 1992), indomethacin (Kimura and Doi, 1998), 5-Ethylamino-9-diethylaminobenzo[a]- phenothiazinium chloride and sulfonamides and tetracyclines (Moore, 2002; Scarth, 2006). The most susceptible animals are cattle, sheep, goat and horse; especially the dorsal and lateral surfaces of the body. Moreover, sunny climate acts as a predisposing factor. The secondary type of photosensitization is the most common and frequent observed type of photosensitivity in veterinary clinics. Phylloerythrin – a photosensitizing agent – is a phytoporphyrin and accumulates in the blood due to impairment of the hepatobiliary - Therefore, it refers as a hepatogenous photosensitization in some references - excretion. It is an efficient source of singlet Oxygen (Tønnesen et al., 2010) and is produced as a result of microbial breakdown of the chlorophyll in the gastrointestinal tract.

Physiologically, this porphyrin is absorbed into the circulation, conjugated in the liver cells and excreted in the bile. In case of hepatic diseases e.g., hepatic necrosis and cholangiopathies, the level of phylloerythrin increases in the blood. After sunlight exposure, phylloerythrin can absorb, release light energy and act as a photodynamic molecule. Thus a phototoxic reaction is initiated (Klaassen, 2008; Scarth, 2006). At the molecular level, the ATP-binding cassette transporter (ABCG2) is known as a phytoporphyrin transporter and responsible for its biliary excretion. Hepatogenous photosensitization is mainly caused by plants, fungi and algae (Collett, et al., 2011; Scarth, 2006). Such condition has been reported in animals due to hepatotoxicants e.g., pyrrolizidine alkaloid, cyanobacteria, Nolina sp, Agave lechuguilla, Holocalyx glaziovii, Kochia scoparia, Tetradymia sp, Brachiaria brizantha, Brassica napus, Trifolium pretense, Trifolium hybridum, Medicago sativa, Ranunculus spp, phosphorus and carbon tetrachloride. Both types of photosensitization exhibit similar clinical manifestations. Phototoxic reaction starts immediately when the photosensitive animals exposed to sunlight. The animals scratch the exposed areas of skin, severe phylloerythrinemia and the typical skin lesions are developed. Erythema and edema are rapidly appeared followed by pruritus, photophobia and hyperesthesia. In case of prolonged exposure, serum exudation, ulceration, scab formation and skin necrosis represent the main manifestations (Osweiler, 1996; Scarth, 2006).

In the secondary photosensitization, the classical signs of liver diseases are reported e.g., icterus. Evaluation of serum liver enzymes and liver biopsies are essential indicators of hepatic diseases. Genetic engineering is able to solve such case by breeding of disease resistant animals by marker assisted selection were performed (Phua et al., 2009). Together, these data indicate that the two types of photosensitization – primary and secondary – induce the same clinical manifestations. However, the mechanism of toxicological action is differing to some extent between both types.

2.4 Skin fibrosis

Skin fibrosis is a prominent and widespread fate of chronic exposure to the toxicants (see Table 1 and 2). Increase the dermis layer thickness, disturbances of hair follicles and sweat glands as well as alterations of the cutaneous blood vessels are the main characteristic features of the fibrotic skin. Accumulation of collagens type I, III and VII is occurred during fibrogenesis (Smith and Chan, 2010).

Expectedly, both number of alpha smooth muscle actin-positive myofibroblasts and collagen-modifying enzymes such as lysyl hydroxylase-2 are elevated in the fibrotic tissue (Rodero and Khosrotehrani, 2010). The early lesion is accompanied by perivascular inflammatory cell infiltrates, composed largely of T-lymphocytes and monocytes. Eventually the skin becomes atrophic. Vascular rarefaction leads to tissue hypoxia and induction of the hypoxia-inducible factor-1 (HIF-1) with increased local production of vascular endothelial growth factor (VEGF) and other angiogenic factors. Evidence of tissue hypoxia can even be found in clinically uninvolved apparently "normal" skin of patients. Hypoxia itself serves as a potent stimulus for fibroblast activation, epithelial–mesenchymal transition (EMT) and progression of fibrosis (Smith and Chan, 2010; Seok et al., 2013)

Taken together, these data conclude that skin fibrosis is the pathological sequences of a repetitive skin exposure to the chemical insults. Skin fibrogenesis, cellular organization and the relevant signaling pathways are the pivotal topics to be intensively studied.

2.5 Dermal hyperplasia and carcinogenesis

Skin cancer in veterinary field is not commonly induced by chemical exposure (Osweiler, 1996). Experimentally (see Table 1), ultraviolet (UV) radiation-induced cutaneous injury is a widely used model to investigate the key events during skin damage, wound healing and cancer pathogenesis. It was shown that the UV irradiation is able to induce CYP1A1 in the skin and liver of rats and mice. In mice, 7, 12-dimethylbenz[a]anthrancene (DMBA) is used as a skin model tumor initiator and the phorbolester 12-O-tetradecanoylphorbol-13-acetate (TPA) is frequently used as a skin model tumor promoter (Melnikova and Ananthaswamy, 2005).

Distribution of FasL-mediated apoptosis may play a crucial role in the development of skin cancer. Squamous cell carcinoma is produced in the sites of dermatosis in some dogs and the lesions are similar to solar keratosis in human (Hill et al., 1999).

3 Cellular and molecular mechanisms of dermal intoxication

The wound healing is a well-orchestrated and tightly-controlled process resulting in skin regeneration. The restored skin is not fully identical to the intact one. This process is composed of both molecular and cellular key events. Understanding the biological meanings behind these key events is fundamental in order to produce a new therapeutic candidate. Wnt/β-catenin, CNN1, TGF-β, Hedgehog and Notch signaling pathways are the most studied pathways during cutaneous injury and healing (Seok et al., 2013). As an example, deletion of nucleotide excision repair (NER) genes such as XPA and XPC increases the susceptibility of mice to ultraviolet (UV)-induced skin cancer. This process is a P53-mediated and associated with increased proliferation and decreased apoptosis of keratinocytes (Ananthaswamy et al., 1999; Melnikova and Ananthaswamy, 2005).

There are strikingly altered patterns of gene expression in skin from treated animals compared to controls. Prominent perturbations were seen in genes associated with transforming growth factor-ß and Wnt signaling, extracellular matrix, innate immunity and hypoxia (Seok et al., 2013). The number of genes differentially expressed in skin biopsy microarrays greatly exceeds the number in explanted dermal fibroblasts, suggesting partial extinction of the activated phenotype with ex vivo propagation. Microarray studies also highlight the tremendous patient-to-patient variability in the molecular fingerprint of scleroderma (Lakos et al., 2004).

4 In Vivo, In Vitro and Non-Animal Alternative Methods for Dermal Toxicity Testing

The main advantage of the experimental *in vivo* studies is reflecting the architecturally, functionally and metabolically intact animal and human systems. However, the experimental animals have a different skin thickness and permeability as well as different toxicokinetic abilities compared to animals and human. Other detrimental factors in case of topical administration of the chemicals are grooming behavior of animals and/or the contact with the cage. To test the skin penetration, responses, pharmaco-toxicokinetic, the mode of action, carcinogenicity and teratogenecity of the new therapeutic agents or already marketed ones, topical application on the clipped skin of different experimental animals can be performed (Kimura and Doi, 1998; Thougaard et al., 2010).

Therefore, dermal application is used for bioavailability testing e.g., measuring the compound or its metabolites in the blood or excreta, dermal adverse effects of some compounds e.g., skin biopsy and residual analysis e.g., measuring the compound or its metabolites in all tissues. Moreover, the application site should be prepared at least one day before dermal application. After the planned exposure time, the test preparation is removed. Furthermore, the treated skin should be carefully observed for visible symptoms of dermatitis and irritation. Rodents are usually used to investigate the percutaneous penetration efficiency of compounds to compare between the *in vitro* and *in vivo* dermal toxicities and to study the toxicity of different chemicals and medications (Reifenrath et al., 2011).

The transgenic mice were intensively used to address several aspects of skin disease e.g., using of the XPA gene-deficient mouse system to understand the acute inflammation, photobiological reactions, immunosupression and skin carcinogenesis (Ananthaswamy et al., 1999). Ibuki (Ibuki et al., 2007) used the Skh:hr-1 hairless mouse model to elucidate the role of cutaneous heme oxygenase during skin diseases. Another example is eosinophil depleted (ΔdblGATA0) mice to investigate the role of eosinophil during dermal inflammation and fibrosis. Xenograft models are widely used to investigate the metastasis and invasiveness of the tumor cells as well as the tumor microenvironment (Halaschek-Wiener et al., 2003). Recently, varieties of cell-based methods are established for risk assessment, safety measurements and categorization of chemicals that are topically applied to the skin. These models include monolayer cultures of rabbits, rodents and human skin – keratinocyte – cells, 3-dimensional keratinocyte cultures and co-cultivation of two or more than two cell types (Seok et al., 2013).

The ability of normal human keratinocyte assays to predict the irritation response in humans is fully described (Clothier and Khammo, 2006). As alternative method, it was shown that the dermal fixed dose procedure can be used to determine the dose of chemicals that causes clear signs of non-lethal toxicity (Stallard et al., 2004). The 3D models mimic the human or animals skin pieces structurally and to some extent functionally. A full-thickness skin explanation consists of pieces of skin from humans or animals can be used for in vitro testing applications (Reifenrath, 2007). The advancement in bioinformatics is partially able to predict the potential toxicities of the substance in humans using the in vitro and animal data (Seok et al., 2013). In conclusion, the quantification of dermal toxic responses can be determined through direct irritation of the skin of rodents, rabbit or porcine and in vitro by using keratinocyte cultivation models. To identify the median lethal dose (LD_{50}), the death of experimental animals or cells is usually used as an endpoint.

5 OMICS

Recently, what is called “omics” technologies was introduced in the fields of system biology and toxicology (Seok et al., 2013; Zhou et al., 2013). The main goal of “omics” technologies is to give the scientists a comprehensive overview on the entire genome, transcriptome, proteome, microbiome, metabolome and lipidome (Blumenberg, 2012). In the field of dermatology, these new technologies are known as “SKINOMICS” (Kimball et al., 2012). The first DNA microarrays applications in the dermatology were developed at the University of Stanford (Schena et al., 1995).

By using the skin model, the researchers are able to explore the stem cells, inflammation, cancers, signaling pathways, development, (de)differentiation and different pathological conditions (Blumenberg, 2012). The SKINOMICs technologies can be used in the investigation of the adverse drug reactions and the chemical safety as well as the risk assessment of chemicals (Zhou et al., 2013). The gene expression during epidermal injury is well characterized both *in vivo* and *in vitro* models (Hu et al., 2010). Conclusively, recent advances in OMICs technologies will aid the scientist to elucidate several toxic mechanisms and the regeneration process after the dermal exposure to chemicals.

6 Concluding Remarks

Dermatotoxicity represents a major health concern in pets and companion animals and to a lesser extent in farm animals. Several issues of dermatotoxicity, e.g., mechanisms of the intoxication are not fully understood in both experimental and clinical context. Furthermore, exposure time and concentration of the chemical are playing pivotal roles during toxicity and healing processes. Skin thickness and grooming behavior represent the main challenges in the *in vivo* studies. Still there is a discrepancy between the *in vivo* and in vitro systems in term of toxicity testing. This discrepancy stands as an obstacle in front of the developing an efficient bioinformatic prediction tool.

The lack of knowledge *in vivo* hampers the progress of an alternative method. Moreover, implementation of the advanced technologies is required to investigate the disruption and regeneration of the dermal system. Therefore, systematical attempts should be considered to follow up the dermal toxicity and regeneration; 1) Integration of the advanced technologies in the dermatology research is the first step; 2) A careful *in vivo* studies should be done in different scales e.g., acute, subacute and chronic levels; 3) Development of robust, well-characterized and highly reproducible alternative in vitro systems with a good comparability to the *in vivo* situation is a major bottleneck; 4) Improvement of the predictivity of the bioinformatics tools; 5) Prediction of the severity, level of markers and wound healing in a clinical condition; 6) Using of the prediction tool in the prognosis and diagnosis fields.

Conflict of interest

No conflict of interest is declared by the authors.

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