Studies and Works

Diphoterine®

An effective cleanser against chemical burns
# CONTENTS

## INTRODUCTION 3

## I. MECHANISM OF THE DECONTAMINATION SOLUTIONS 4

### I.1. Action mechanism of water and Diphoterine® 4

#### I.1.a. Efficiency of the chemical cleansing 4

#### I.1.b. Diphoterine® cleansing: the interest of amphoteric, hypertonic and chelating solutions 9

### I.2. Efficiency spectrum of Diphoterine® 11

## II. PHYSIOPATHOLOGY OF THE CHEMICAL BURN 13

### II.1. Physiopathology of acids burns 13

### II.2. Physiopathology of alkalis burns 14

## III. TOXICOLOGICAL AND EPIDEMIOLOGICAL STUDIES ABOUT DIPHOTERINE® 16

### III.1. Study of the oral and cutaneous LD₅₀ - Ocular and cutaneous irritation tests 16

### III.2. Safe used of Diphoterine® and residue tests of acid and alkali cleansing 16

### III.3. Epidemiological studies 17

## CONCLUSION 19

## BIBLIOGRAPHIC REFERENCES 20

## APPENDIX (attached documents)
Introduction

The constant progress of the chemical industry has created huge quantities of molecules, and more than 135,000 of those circulate daily in Europe. The efficiency of those products entailed an increase of the risk that becomes, in many cases, intolerable.

The chemical burn is created by a xenobiotic that has the property to react with the components of the skin or the eye surface. These xenobiotics are either corrosives or irritants. The chemical burn is due to the chemical exchange developed during the oxydo-reducing, acido-basic, chelating and solvatation reactions. The severity of the burn depends on the reaction energy and on the kind of exchange. The corrosive is often the sign of a serious and irreversible damage. The toxic or harmful properties of the chemical products are not involved here, they reveal themselves later.

It is admitted that during the accident, the efficacy of the first aid treatment often determines the evolution of the burn. Indeed, if the aggressive product is removed before the start of the burn process, there will not be any pathology. Then, several works and observations on major corrosives like caustic soda, sulphuric acid and phenol, show that the water cleansing is not efficient enough when the products are concentrated.

Diphoterine® is in accordance with the D.93/42/CEE European directive the medical devices, and not to the D 65/65 CEE directive because “Its principal action inside or on the human body is neither obtained by pharmacological nor immunological means nor by metabolism”. It does not allow a burn to heal, nor does it stop the evolution of the chemical product already inside the body. It is a first aid rinsing solution. The diversity and the important quantity of chemical products all over the world show the importance of establishing a sole protocol: the goal of Diphoterine® is to optimise the efficacy of the cleansing on most of the chemical products: acids, alkalis, oxidising agents, reducing agents, solvents, antimitotics...

The file has two main objectives: first, to present all the studies and experiments carried out on this subject until now, in order to show the efficacy of Diphoterine®, first molecule of the solutions produced by the PREVOR Laboratory - and second to give proofs that its use does not entail any secondary risks, neither concerning the reaction with the chemical products, nor concerning its own toxicity. Before introducing these studies, it is important to review the action mechanisms and the physiopathology of the burn.
I. MECHANISM OF THE DECONTAMINATION SOLUTION

I.1. Action mechanism of water and of Diphoterine®

I.1.a. Efficiency of the chemical cleansing

The efficacy of a cleansing solution is measured by keeping track of the evolution of the xenobiotic concentration on the surface of the body and also inside of the tissues, since the interest of a cleansing solution is to remove the xenobiotic from inside of the tissues. In fact, two competitive phenomena happen during the rinsing: on one hand the xenobiotic is washed away by the rinsing fluid, and on the other hand it penetrates into the tissues. Several experiments allow one to understand all these phenomena, and also to understand the action mechanism of water.

(i) First experiment: In vitro evaluation of an external rinsing efficacy

Through this experiment, we set up an in vitro efficacy of an external rinsing with different cleansing solutions (water, physiological serum, Diphoterine®). The sweeping effect, the dilution effect and the amphoteric effect are studied at the same time, independently of the other phenomena. The experiment consists of measuring the evolution of the concentration of a strong acid (HCl), or a strong alkali (NaOH), rinsed with different solutions. The sweeping and the dilution effects are measured by the variation in the pH value of the solution, taking into account that the pH is the logarithmic measure of the H⁺ ions concentration.

Materials and method

We take a 25 mL beaker, into which we pour 10 mL of 1N hydrochloric acid ((36,5g/l), FLUKA 84425) or 1N caustic soda ((40g/l) FLUKA 72072). A magnetic stirrer homogenises the solution, and the pH is measured with a Schott pH electrode (pH-meter CG 837 n° 259009, Prolabo electrode n° 06-934.006). The different cleansing solutions (demineralised water, NaCl 0,9%, Diphoterine® D580204) are "mixed" with a borosilicated 25 ml graduated burette (Prolabo). The table below shows the results of the rinsing of hydrochloric acid (HCl) and alkali (NaOH).

<table>
<thead>
<tr>
<th>Table n°1</th>
<th>HCl (final pH = 5.5)</th>
<th>NaOH (final pH = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demineralized water</td>
<td>155 mL</td>
<td>126 mL</td>
</tr>
<tr>
<td>NaCl 0,9%</td>
<td>144 mL</td>
<td>121 mL</td>
</tr>
<tr>
<td>Diphoterine®</td>
<td>35 mL</td>
<td>39 mL</td>
</tr>
</tbody>
</table>
Graphs 1 and 2 represent the variations curves in pH obtained with different rinsing solutions, respectively in the case of an acid and of an alkali.

**Graph 1 : External cleansing of 10mL of 1N hydrochloric acid (HCl)**

**Graph 2 : External cleansing of 10 mL of 1N caustic soda (NaOH)**

*Analysis*

We can note that *in vitro*, the three solutions cleanse the xenobiotic, acid or basic. But rinsing with Diphoterine® is four times faster than rinsing with the two other solutions. Indeed, the active site of Diphoterine® able to make a link with the H⁺ ion, has absorbed more H⁺ ions per unit of volume combined than water or physiological serum in the acid case. The same is true for the OH⁻ ion in the case of caustic soda. Diphoterine® did not neutralise either the corrosives or the irritant in a strictly speaking chemical way, otherwise the final pH would have been basic or acid. The amphoteric power of Diphoterine® makes it possible to approach.
This experiment about the *in vitro* simulation of an external rinsing on commonly used corrosives such as 1N caustic soda or 1N HCl showed the *in vitro* efficacy of a Diphoterine® cleanser compared to other rinsing solution such as water and the physiological solution. This active capacity of the Diphoterine® reduces considerably the contact time of the xenobiotic inside of the skin.

We can note the importance of the osmotic pressure in this experiment, because the volume of physiological serum necessary to reach the physiological pH zone is always lower than the volume of water. This model was confirmed by a recent studies of Pr. B. Kirchhof’s team (*N. Schrage: Chemische Elemente in der Hornhaut. Analytik und Experimente zur Lokaltherapie am Auge, 1997, ISBN 3-00-0011619-8 Dept. Opht. Univ. Aachen-Germany*)

**(ii) Second experiment : *In vitro* evaluation of the external rinsing’s efficacy**

The interest of an effective rinsing is not only to remove the toxic content on the skin or the eyes, but also to permit external and internal decontamination.

**Witness experiment : *in vitro* evaluation of a xenobiotic penetration**

The penetration phenomenon are complex, but concerning the surface of the skin and the eye, the classical diffusion laws are good models. There are two different kind of diffusion laws ; first the passive diffusion (or first Fick law) which is proportional to the xenobiotic’s concentration gradient, and second the diffusion due to the flow generated by the difference between the osmotic pressures. So in order to evaluate the penetration parameters of the xenobiotic, it is important to determine the time necessary for the toxic product to go through the semi permeable membrane separating the two physiological areas. They represent either the skin or the eye, and also the inside of the tissue. The time spent by the xenobiotic (NaOH, 1 mole per litre) to go through the membrane is evaluated.

**Materials and methods**

The following setting was necessary to carry out the experiment : we have two beakers (respectively 150 and 75 ml). The bottom of the 150 ml beaker (Ø = 5,8) is replaced by a semi permeable membrane (cellophane), and it is filled with 25 ml of physiological serum (NaCl 0,9%). The second beaker contains 50 ml of 1N caustic soda NaOH. The homogeneity is maintained by a magnetic stirrer, and the pH is measured with a Scott pH electrode (pH-meter CG 837 n° 259009, Prolabo electrode n° 06-934.006). The graph n°3 shows the penetration curve obtained.
We note that after 7 minutes, the pH is steady. We consider that the penetration is finished. In the second experiment, we will compare the efficacy of 4 rinsing solutions (water, physiological serum, NaCl isotonic solution and Diphoterine®)

**Experiment**

The aim of this experiment is to compare the rinsing capacity by studying the evolution of the xenobiotic’s concentration inside the beaker representing the eye anterior chamber. In order to reproduce reality in the best way, it is filled with physiological serum, which allows us to recreate the osmotic effects. The first beaker is cut and its bottom is replaced by a cellophane semi-permeable membrane. This beaker is filled with 25 mL of physiological serum. The second compartment or external chamber is contains a beaker filled with 50 mL of 1N caustic soda, which simulate the xenobiotic. The two chambers are placed so that they are in contact with each other and the levels of the two liquids are equal in order to avoid hydrostatic pressure. This model has been confirmed by the published studies of Pr. B. Kirchhof team (1997). The penetration of the xenobiotic in the anterior chamber is measured with a pH-meter. The liquid flow is 151 mL ± 3 mL. The sizes of the two beakers are studied in order to have equal liquid volumes taken into account.

Two series of cleansing will be done: a rinsing with delay (late rinsing) carried out 3 minutes after the contact with the toxic content. We estimate that this time is sufficient for 10 mL of 1N caustic soda (40g/l) to induce a serious burn. The second rinsing is an early one, 6 seconds after the first contact with the soda. The graphs n°4 and 5 show the results.
Results

Graph 4: Late rinsing of 1N caustic soda

Graph 5: Early rinsing of 1N caustic soda

Analysis

The analysis of the pH evolution curves in the internal chamber show that it is possible to remove the xenobiotic from this internal chamber because of the external rinsing. The late cleansing is obtained when the internal chamber is saturated. The efficacy of this external rinsing increases from water to physiological serum, to the isotonic solution and to Diphoterine®. The osmotic pressure is very important, since the hypertonic solution is better than the isotonic, the isotonic being even better than the hypotonic solution. But we note that the amphoteric properties give an active cleanse superior to the passive rinsing. The difference in the speed between the cleansing with Diphoterine® and the other rinsing solutions show that hypertonicity cannot explain alone this effectiveness. A Diphoterine® rinsing is much quicker, it is an active cleanser compared to others which carry out a passive rinsing. The time ratio between the rinsing with
Diphoterine® and the rinsing with the isotonic solution is 1/4. In the case of rinsing with water, the OH− ions diffuse from the internal chamber to the external chamber (Fick law). But water has a tendency to enter into the internal chamber, due to the osmotic pressure, and slows down the outburst of the OH− ions, and consequently the rinsing.

As regards to the hypotonicity, water flows from the hypotonic environment to the hypertonic environment, so from the internal chamber to the external one, entailing the outburst of the OH− ions. The more hypertonic the external chamber is, the more effective the rinsing will be. The rinsing with Diphoterine® is more effective than a rinsing with an isotonic solution. This shows that in addition to its hypertonicity, compared with the internal chamber, Diphoterine® own properties allow it to increase the efficacy of the rinsing: its chelating power makes for an active rinsing.

Thanks to these experiments, the importance of the activity of Diphoterine® compared with the passive rinsing carried out by other bathing means like water and physiological serum can be clearly displayed. The interest of Diphoterine® is to allow an immediate and effective rinsing, and to prevent the penetration of the product by a dynamic action, which is not the case with what the passive cleansing obtain with water or with physiological serum.

**I.1.b. Diphoterine® cleanser : the interest of amphoteric, hypertonic and chelating solution**

The diversity of the chemicals increases the accident risk and then, imposes the mastery of the early rinsing. In fact, when the accident happens, it is not always possible to be sure of the kind of toxic content involved. Therefore the rinsing solution must be effective at last as the common cleansing solutions (water, physiological serum). In order to guarantee this polyvalence, we use amphoteric agents: these molecules have several active sites (for example against acids), and also antagonistic sites. These antagonistic sites are then in self-balance. To prove this property, we just have to study the active power of Diphoterine® on an acid and on an alkali. In this case we study only the active properties and not the sweeping effects. We just have to perform an experiment that is made daily in our laboratory, called NEP¹ procedure. This procedure allows us to measure the active power in moles/litres of the solution, as well as the efficacy volume, that means the volume of rinsing product necessary to reduce the concentration of the toxic content to a value close to the toxic limit. With the acids and the alkalis, a product is not corrosive or irritant any more when its pH is between 5,5 and 9 (5,5 < pH < 9).

**Materials and methods**

We pour 1 ml of the toxic product to test in a beaker. Its concentration is 1N. The rinsing solution is poured with a graduated burette. The pH is measured according to the volume poured. The experiment is reduced to the comparison between water and Diphoterine®, since the first experiment showed us that the activity of physiological serum was similar to the activity of water. Here we test an acid, 1N hydrochloric acid (HCl) and an alkali, 1N caustic soda (NaOH). The results are displayed in graph n°6 below.

---

¹NEP : Norme d’Essai Prevor (PREVOR testing norm)
We notice that Diphoterine® has a large absorption capacity on acids and alkalis, and for similar volume, Diphoterine® absorbs a larger quantity of acid and alkali than water does. Therefore, Diphoterine® has the same efficacy on the acid and on the alkali, as for the active power and the efficacy volume. The table 2 give 2 examples of this absorption power.

### Table n°2

<table>
<thead>
<tr>
<th></th>
<th>pure acids</th>
<th>pure alkalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption power</td>
<td>0,1 mole/l</td>
<td>0,13 mole/l</td>
</tr>
<tr>
<td>Absorption volume</td>
<td>25 ml</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

The same experiment can be carried out on an oxidising agent, for example chromic acid (H₂CrO₄), replacing then the pH electrode by a electrode to measure the potential. We have the same kind of curve (graph n°7).
The potential variation, compared with the other tested solutions, clearly shows the effective action of Diphoterine® on an oxidising agent like chromic acid.

**I.2. Efficiency spectrum of Diphoterine®**

Diphoterine® was conceived in order to have an activity spectrum as wide as possible. Instead of beginning with different kinds of chemicals, we gathered all chemical products according to their kind of elementary reaction. In fact, in order for a chemical reaction to occur, the chemical has to undergo a transformation or metabolism that will reduce its energy. This transformation can be done by a chain of elementary reactions. Without this metabolism, the product can act directly by a single elementary reaction which will be our reference for the classification. Actually the chemical reactions come down to ten elementary reactions. These reactions were the subject of a book on toxicology published by the PREVOR Editions (F. Burgher et al. *Le risque Chimique et la Santé au Travail : Essai de toxicologie réflexive chapitre III pp. 267-295 (1996)*). The amphoteric characteristics of Diphoterine® were conceived against these elementary reactions. The aim is that the elementary reaction is done with Diphoterine® instead of the body elements, in order to tie up the toxic content.

The whole molecules existing in the world represent so wide a range that it is a priority impossible to test all the reaction possibilities. Our research allows us to establish for a positive list of the products tested, on which the cleanser with Diphoterine® and its derived solution - as Hexafluorine® - are effective (list available on demand). We test the physicochemical properties of the mixture in order to be sure of the ability to rinse the product, then we examine the capacity to neutralise the irritant or corrosive effects with Diphoterine® by studying either the characteristics of the residues when they can be identified with certainty, or in the case of complex products or mixtures, the activity of an equimolar mixture of the analysed product and Diphoterine® with corrosion and irritation tests for the skin and for the eyes. We were able to ratify the activity of Diphoterine® on all categories of corrosive and irritant chemical products and the efficacy of the Diphoterine® was tested on more than 300 chemicals among which some examples.

**Acids**
- Concentrated hydrochloric acid (HCl)
- 98% sulphuric acid (H_2SO_4)
- Nitric acid (HNO_3)
- Hydrobromic acid (HBr)
- Glacial acetic acid
- Suphonic acid (C_7H_8O_3S)
- Paratoluene sulphonic acid (C_7H_8O_3S)
- Adipic acid (C_6H_10O_4)
- Caproic acid (C_6H_12O_2)
- Fumaric acid (C_4H_4O_4)

**Alkalis**
- Caustic soda (NaOH)
- Potassium hydroxide (KOH)
- Ammonia solution (NH_4OH)
- Trimethylamine solution (C_3H_9N)
- Methylamine (CH_3N)
- Amines (RNH_2)
- Sodium silicates
- Anhydrous piperazine (C_6H_10N_2)
- Quicklime (Ca(OH)_2)
### Oxidising Agents

- Peroxides (benzyl peroxide, hydrogen...)
- Peracids (peracetic acid RCOOOH)
- Potassium permanganate (KMnO₄)
- Potassium dichromate (K₂Cr₂O₇)
- Methyl ethyl cetone peroxide

### Reducing agents

- Hydrazine (N₂H₄)
- Phenylhydrazine
- Sodium thioglycolate

### Complexing agents

- Mercury
- Nickel salts
- Iron sulphate
- Copper

### Solvents

- Acetone
- Toluene
- Dimethyl formamide (DMF)
- Dimethyl sulphoxide (DMSO)

The complete list of tested products is given in **appendix 5** and can be consulted on the web (http:\\www.prevor.com).
II. PHYSIOPATHOLOGY OF THE CHEMICAL BURN

The constituents of the skin and the cornea are equivalent although its thickness and its rule are different. The fundamental differences come from the thickness of its coats, the vascularisation, the eye essential function, the absence of the cornea coat and the presence of the cutaneous appendix (sweat gland and pilosebaceous follicle). The stroma must be transparent in order to assume its functions. During the acid or the basic splashes, we assist to releasing more or less mass of $\text{H}^+$ or $\text{OH}^-$ ions respectively.

II.1. Physiopathology of acid burns

Acids are entities which can be release $\text{H}^+$ ions or fix $\text{OH}^-$ ions. When $\text{H}^+$ ions come massively in contact with the skin, the penetration is done quickly through the inter and intra cells space. Brewitt and Honeger (1979) have shown that with a high concentration, we observe the direct effect on the cells' membranes, with the cell breaking membrane and the bursting of the cell. The interruption of the osmotic balance has an important rule, but the breaking and the crossing of the $\text{H}^+$ ions are not always systematicals. Inside the tissues, the $\text{H}^+$ ions will react essentially with the proteins and will produce structural and irreversible changes. These changes are translated by the coagulation phenomenon, come in the same time with the heat production (Morley and al. 1996). This phenomenon is well known with the white egg albumin, which coagulate in the presence of the acetic acid and/or the heat. This phenomenon provokes unavoidably the cell's death. The necrosis aspect describes in microscopy corresponds to the structures' coagulation which keep during a certain amount of time their structural appearance before disappearing. The acid creates injuries in the cells and the interstitial conjunctive tissues (Pereleux, 1986).

In the cutaneous and ocular level, acid burns will be represented by the following sequence:

1$^{\text{st}}$ phase: on the skin or on the cornea, the epithelium coat can be destroyed immediately according to the concentration and to the acid's nature of the acid. Nevertheless, this destruction is not always observed.

The $\text{H}^+$ ion continues its progress if the concentration is sufficient; it penetrates into the stroma and coagulates the collagen. This penetration will generally stop at this level. On the skin, when taking into account the vascularisation of the derma, the burn can move if the ion $\text{A}^-$ is toxic by successive penetration into the derma, the hypoderma and then into the organism : for example, this is the case of the anions fluorides $\text{F}^-$ of the hydrofluoric acid, which is cytotoxic (Itchak and all. 1997).

2$^{\text{nd}}$ phase: the protein coagulation draws on the one hand the reduction of the $\text{H}^+$ ions concentration and on the other hand the decrease of the tissue's permeability, slowing down the $\text{H}^+$ ion diffusion.

3$^{\text{rd}}$ phase: the necrosis cells on the surface are being destroyed and we observe the development of a vesicle due to the arrival of the serum under the injured cells. The epithelium is also destroyed and we observe an ulceration. The elimination of the derma is not important and the coagulated proteins make a kind of natural crust.

4$^{\text{th}}$ phase: the cells and coagulated fibres of the derma are progressively eliminated by the inflammatory reaction. When a safe conjunctive tissue is restored, the epithelium regrows from the peripheric to the centre. In spite of the fibroblastics and myofibroblastics repairing function, we rarely observe a perfect healing. The acid burns are not as serious as their spectacular aspect. In
the burn ladder, they are less serious than burns due to corrosive agents when the A⁻ anion did not have its proper toxicity.

**II.2. Physiopathology of alkali burns**

Alkalis are entities liable to release OH⁻ or to fix H⁺. In reaction to the water, we observe the freeing of the OH⁻ ions. During an ocular and a cutaneous burn, the OH⁻ ions will hydrolyse the proteins and will continue to penetrate into the stroma, pursuing the cellular proteins' dissolution. The alkali burn is characterised by an important cell destruction all the more so in the rapid penetration point of view, than in the depth of the destruction. The mass arrival of the OH⁻ ions on the cornea entails the following unfolding:

**1st phase**: rapid and total destruction of the epithelium by its cellular proteins' solubilisation. This destruction is proportional to the product's concentration.

**2nd phase**: since the hydrolyse reaction did not reduce the OH⁻ ions' activity, the penetration pursues and the cells are deeply solubilized. This reaction is slow to finish since the pH of the anterior chamber needs more than 9 hours to return to a normal value after a 6N soda burn (Laux and al. 1975).

**3rd phase**: the cells' restoration is difficult since the very corneal graft needs to save sides. The fibroblastics' proliferation with opacification is observed. The presence on the skin of a keratin coat, very resistant to the alkalis, will slacken the effects on the surface and hold up the basis penetration, but as soon as the product is penetrated, the tissular hydrolyse will occur very deeply. The restoration is possible contrary to the eye after the necrosis removal. The fibrous retractile reaction can bring a stenosis, a retractile or cheloid scar (Flyn and al. 1984).

The *in vivo* studies have been done, comparing the rinsing with water, to the physiological serum, and to the rinsing with Diphoterine® (Josset and al. 1986, *Appendix 6*). This study allows the evaluation of histological injuries, also the measurement of the intra and extra-ocular pH during the first seconds after the burn. We observe that Diphoterine® allowed the preservation of the endothelial cells and then a very favourable prognostic evolution.

From the chemical and histologic results obtained (Josset and al. 1986, *appendix 7*), we built a physicochemical model, applying the Fick diffusion laws and also the laws about osmotic pressure. From this model, we determined the material penetration coefficients and the penetration coefficients of soda in the cornea. This kinetic study shows that the diffusion of a product is intrinsic on one hand (Fick's law) and potentiated by the osmotic pressure on the other hand. The balancing by osmotic pressure (in the case of an isotonic solution) does not let according to the *in vitro* experiments - the evolution of the product stop. It is then necessary to make an **active rinsing** which will reverse the evolution of the toxic content. Moreover, the histological results give us precious elements in order to understand the burn's mechanism. When we cleanse with water, we note an important oedema in comparison with the one seen during a rinsing with an isotonic solution. The only difference between these two experiments is the regulation of the osmotic pressure. With just this only regulation, we note that the penetration of the toxic content is limited. The introduction of the **active rinsing** concept as a second element allows the penetration to stop. These results tally with the *in vitro* studies and allow us to consider that the above observed biological phenomenon noted during the burn and that the influence of the physicochemical phenomenon is primordial in the first instants. Indeed, the biological results, show that the efficacy curve of the isotonic solution has a similar shape. But the action of Diphoterine® is faster thanks to its regulating effect of the osmotic pressures. With Diphoterine®, we observe a quite immediate come back of the extra-ocular pH to a physiological value. This
study also confirms the in vitro studies about the complete rinsing as well as for an external or internal decontamination. More, the absorption capacity of the rinsing solution permits to forecast the possibilities of controlling the evolution of the toxic content in the tissues (N. Schrage and al., First International Congress on the Evolution of the Knowledge about Chemical Burns, La Baule, France, Oct. 1997. appendix 9).

**ps**: When some of these studies were published, the exact commercial name of the product had not yet been chosen, which is the reason why there are different designations (amphoteric solution, absorbing isotonic solution and then Diphoterine®), according to the date of publication.
III. TOXICOLOGICAL AND EPIDEMIOLOGICAL STUDIES ABOUT DIPHOTERINE®

Several studies have been done about Diphoterine® for the toxicological and the epidemiological validation. The toxicological study about Diphoterine® was done considering its usage instructions and the risks due to its use. Diphoterine® has to be used immediately after the accident in order to cleanse a corrosive. It's conceived to be used only when the accident happens. Its use is therefore different from these of antidotes like hydroxocobalamine or calcium gluconate, administered to move a deleterious balance or under certain circumstances to reduce the concentration of a toxic content when it is already in the tissues. The same is true, if we compare its action to the action of atropine, against organophosphorous compounds, it does not release a metabolic process to increase the organism’s defences.

III.1. Study of the oral and cutaneous LD₅₀ - Ocular and cutaneous irritation tests

The acute toxicity of Diphoterine® can be measured, in the case of our using procedure, by the study of the effects on the skin or on the eye on one hand, and on the other hand by the study of the possible systemic effects that xenobiotic could generate. All these works were carried out by independent and certified laboratories. All these results and procedures are in appendix 1 of this document.

<table>
<thead>
<tr>
<th>Kind of study</th>
<th>Study N°</th>
<th>Laboratory</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin irritation</td>
<td>133/3</td>
<td>SAFEPHARM</td>
<td>0.9 non irritant</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>133/4</td>
<td>SAFEPHARM</td>
<td>1.3 non irritant</td>
</tr>
<tr>
<td>Oral LD₅₀</td>
<td>6564 TAR</td>
<td>CIT</td>
<td>&gt;2.000 mg/kg non toxic</td>
</tr>
<tr>
<td>Cutaneous LD₅₀</td>
<td>133/9</td>
<td>SAFEPHARM</td>
<td>&gt;2000 mg/g</td>
</tr>
</tbody>
</table>

III.2. Safe used of Diphoterine® and residue tests of acid and alkali cleansing

Many studies had been published regarding the management of the chemical burn and particularly the protocol using the water rinsing. This protocol with water offer the advantage of safe use. The water did not bring any supplementary toxicity due to it used. This is not the case with certain buffer solution as phosphate buffer who, during the precipitation with some product as calcium, released the H⁺ ions, potentiating by this fact the burn. This introduce, in addition to the intervention's rapidity, the protocol unity and the polyvalence of the solution, the concept of safe used. It is primeval that no toxicity nor no deleterious effects are observed after the used of a rinsing solution. Diphoterine® hold this property by construction thanks to its amphoteric power (Josset and al. (1986)) and to it polyvalence, tested on more than 300 greater corrosives agents, representing the most commonly used products (Appendix 3,5,12). The Diphoterine® amphoteric's property always allow him to auto-equilibrate the reactions what ever the product accused and without practically a heat emission. Like this, the Diphoterine® and its derived solutions did not bring any risk of supplementary toxicity and the safe used is optimal. Those characteristics had been confirmed by in vitro experiment on more than 300 products (Appendix 3) To show the innocuousness of Diphoterine®, the residue tests of acid and alkali rinsing with Diphoterine® had been done on two antagonists products : pure chlorhydric acid and pure caustic soda. The results are in the following table:
<table>
<thead>
<tr>
<th>Kind of study</th>
<th>Study N°</th>
<th>Laboratory</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular irritation test of</td>
<td>6463 TA1 (10.07.1990)</td>
<td>CIT</td>
<td>no reaction: non irritant</td>
</tr>
<tr>
<td>acids residues cleanse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye irritation test of</td>
<td>6462 TA1 (10.07.1990)</td>
<td>CIT</td>
<td>no reaction: non irritant</td>
</tr>
<tr>
<td>alkali residues cleanse</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The very low gravity's dispersal observed during the epidemiological studies confirmed this safe used obtained in the use of Diphoterine®.

The toxicological studies show that Diphoterine® is at the same time not toxic in it intrinsic used and also that the residue of cleansing are neutral. This is very important because it is the proof that the capture of the acid and alkali is sufficiently strong that they are not able to react again on the eye. In the others world, we observed with this experiment that the absorption effect is irreversible and did not transformed the Diphoterine® in dangerous molecule. This confirmed the theoretic principles of amphotere.

### III.3. Epidemiological studies

In the countries where these toxicological studies were done, it was not possible for ethical reasons, to set up randomised studies. Indeed we can not burn volunteers with chemicals, knowing the risk incurred in case of a placebo rinsing, more, the pertinence of this kind of studies is valid only in the case where the study is done in duplicate blind. Now the conditions of application of the water rinsing and the cleanser with Diphoterine® prevent from the strict application of these principles. In fact, the application procedure of a water rinsing changes according to the country. For example for the eye, the recommendation goes from 15 to 45 minutes of rinsing with a flow around 12l/min, which can represent several hundreds litres of water, when 500 ml of Diphoterine® is sufficient.

Two problems come in addition. The first one is the impossibility to have real reproducible accidents to study. In fact, on one hand the accident is rare, and on the other hand, the diversity of the involved chemical products characteristics (nature, concentration, temperature...) makes the comparison difficult and forces us to obtain a great number of cases. That means including in the study many potential sites, and makes the comparative evaluations more difficult. The second difficulty comes from the dependence of the observable. In fact the (observable) evolution of a burn depends on two criterion: the causal agent and the efficacy of the rinsing method. When the causal agent is only fairly aggressive (solvent, weak acid), there is just a slight evolution of the burn.

The procedure for the use of Diphoterine® has an interest only if it is begun in the first seconds after the accident. The chemical accidents, except for the military risk, are more theoretical than real, in the industry or at home. Taking into account the organisation of first aid in case of a domestic accident, it is difficult to intervene in less than 15 minutes. It is then not possible to carry out a prospective study on domestic accidents. Furthermore, a study on healthy volunteers would not give other results other than the tolerance of Diphoterine®. This observation makes it difficult to compare the rinsing solutions because they are not the fundamental and unique causes of the difference.
Then we proved prospective studies, in collaboration with occupational doctors, by comparing the results of accidents that have happened in the industry. The first study is a multicentric study, carried out by the French National Institute for Research and Safety (INRS) which involved more than 29 doctors - 180 files - during 7 years (1988-1996). A procedure which (appendix n°2) had been done beforehand to quantify the results. The doctors had the choice of their first aid rinsing method.

The second study (appendix n°11) was carried out in a single Rhône-Poulenc establishment in La Rochelle, in collaboration with the ophthalmologists of the city and the hospital doctors. The advantage of this study was that the observations were done by the same people and that the accidents were induced by very varied products. The major drawback is that the study had to be split up into two different steps (water rinsing, Diphoterine® rinsing), in order not to disturb the application of a unique procedure.

The third study (appendix n°12) also took place in a single factory : Martinswerk in Bergheim (Germany). Due to the uniqueness of the people observing the patients, the interest of this study is that it is only about accidents involving strong alkalis (pH 14).

Comments on the studies

The results of the INRS study (Appendix 2) clearly show that Diphotérine®’s procedure (3 minutes of bathing) is at least as good as the water one (15 minutes). It also indicates that Diphotérine® seems to reduce the accidents’ consequences seriousness (decrease of the amount and the length of the sickness left), and decreases the amount of secondary care. These results are very precisely confirmed by the two other studies ((Doctor GIRARD, Rhône Poulenc 1993. Appendix 5); Doctor Konrad, Martinswerk 1995 Appendix 12).

In fact, the study from Doctor GIRARD (Rhône Poulenc, Appendix 5) clearly shows the reduction of the amount of chemical burns entailing sickness left from 7% to 0%, and the decrease of the percentage of these accidents needing secondary care from around 30% to less than 5%. As for the Martinswerk study, it confirms these results on strong alkalis since the average length of the sickness left, 8 days after a water rinsing is reduced to 0,18 days after a Diphoterine® rinsing. No secondary care was necessary in the cases cleansed with Diphoterine®, when 75% of the accidents rinsed with water needed medical care. In addition, the standard deviation was large with water (m=8 ± 8,12 days) when Diphotérine®: this standard deviation shows that the use of Diphotérine® is safer than the use of water.

It is important to notice that for the INRS study, the ratio between the amount of accidents and the diversity of the products did not allow to have a sufficient statistical power to show the significant difference between the rinsing methods. But this already proves the interest of an active cleanser compared to a passive bathing since we obtain at least the same results within only three minutes of rinsing. This is important because in the case of an extensive burn, it is very difficult to shower the injured during 15 minutes, due to the risk of hypothermia and this time is infrequently respected.
Conclusion

The gravity of the burn depends on the kind of agent (basic or acid for example), but also on the concentration, the temperature and the length of the exposure. Each of these parameters can potentiate in an exponential way the seriousness of the burn: it is in order to stop these burns that the PREVOR laboratory conceived Diphoterine®, a rinsing solution containing a molecule with amphoteric hypertonic and chelating properties, able to make a link with the active site of the aggressive agent, and to inhibit corrosive or irritant potential, and allowing elimination of the histotoxic agent by a sweeping effect:

- **absorbing multisite molecule**: Diphoterine® owns at least one antagonistic site for each of the 5 corrosive and irritating reactions. Its link energy is calculated so that the corrosive agent is more attracted by Diphoterine® than by that of the skin or the eye. For the rinsing of solvents, the mechanism of action is indirect in order to stop the solvation effect. The hypertonicity of Diphoterine® blocks the flow of the solvent. Graphs 4 and 5 show us the role of hypertonicity and osmotic pressure in the cleanser; the more hypertonic the solution is, the quicker the rinsing will be. It is thanks to its amphoteric, hypertonic and chelating properties that Diphoterine® is quicker and more effective than water and other rinsing solutions. These properties permit Diphoterine® to assume the active rinsing, which is neither the case of water nor isotonic saline solution.

- **stops the action of corrosive and irritant products**: the aggressive site of the corrosive or the irritant is attracted from the beginning of the rinsing by the antagonistic site of Diphoterine®, and ends up making a link with it. This link allows the destruction of the aggressive agent effects.

- **eliminates quickly the aggressive agent**: the link between Diphoterine® and the aggressive agent allows the increase of the sweeping effect of the rinsing. The hypertonicity makes easier the elimination of the toxic agent easier, allowing the osmotic pressure to regulate and to go back as quick as possible to a physiological state.

- **stops the evolution of the burn**: the neutralisation of the aggressive agent affects and its quick elimination the indispensable elements to be suppressed for the deleterious chemical reaction. Lacking "fuel", the evolution of the burn is immediately stopped.

- **decreases the secondary consequences of chemical accidents**: the quick elimination of the chemical product from the surface of the skin or the eye permits the burn to stop quickly and then decreases such consequences as secondary care and after-effects.

Diphoterine® then stops the action of the chemical aggressive product and eliminates it quickly. Thanks to the combination of amphoteric and chelating properties and to its hypertonicity, it provides for an optimal decontamination to occur.
BIBLIOGRAPHIC REFERENCES

F. Burgher, J. Blomet, L. Mathieu.
Le Risque Chimique et Santé au Travail

Falcy, J. Blomet
Evaluation de l’efficacité des premiers soins lors de projections de produits chimiques
INRS : Document pour le médecin de travail, n°70, 2è trimestre 1997

H. Brewitt and Honegger
Early Morphological Changes of the Corneal Epithelium after Burning with Hydrochloric Acid : A scanning electron microscope study
Ophthalmological, Basel 1979, 178: 327-336

M. Falcy, J. Blomet
Premiers soins en cas de projection oculaire
DMT n°53 1er trimestre
INRS : Document pour le médecin de travail, n°53, 1er trimestre 1993

J.P. Faure et al.
Table ronde sur les brûlures de la cornée

W.J. Flynn, T.F. Mauger and R.M. Hill
Corneal Burns: A Quantitave Comparison of Acid and Base

Itzchak Beiran, Benjamin Miller and Yedidia Bentur.
The efficacy of calcium gluconate in ocular hydrofluoric acid burns.
Human and Experimental Toxicology (1997) 16, 223-228

P. Josset, B. Pelosse, H. Saraux
Intérêt d’une solution isotonique amphotère dans le traitement précoce des brûlures chimiques basiques cornéo-conjonctivales.
Bull. Soc. Opht France 1986 ; 6-7 : 765

J. J. R. Kirpatrick and D. A. R. Burd
An algorithmic approach to the treatment of hydrofluoric acid burns.
Burns (1995) vol. 21, 7: 495-499

Hydrofluoric acid burns : a review.
Laux U., Roth H. W., Krey H. Steinhardt B.
Die Wasserstoffionenkonzentration des Kammerwassers nach Alkaliverräetzungen der Hornhaut
und deren therapeutische Beeinflussbarkeit. Eine tierexperimentelle Studies.
S.E. Morley, D. Humzah, J.C. McGregor and P.M. Gilbert
Cement-related burns
Burns, 1996, Vol. 22, 8: 646-647

A. Pereleux
Les Brûlures Chimiques Oculaires

N. Schrage
Chemische Elemente in der Hornhaut. Analytik und Experimente zur Lokaltherapie am

N Schrage et al.: Retour aux équilibres physiologiques des électrolytes de la cornée conditions
indispensables de la « restitution ad integrum »
La Baule, 1er Congrès International sur l’Évolution des Connaissances sur la Brûlure Chimique
(CIECBC), La Baule (1997)

N Schrage et al.: Rinsing therapy in severe alkali burns of rabbit eye.
Congrès du Joint European Research Meetings in Ophtalmology and Vision (JERMOV) 1996

N. Schrage
Chemische Elemente in der Hornhaut. Analytik und Experimente zur Lokaltherapie am Auge,