ADVANCES IN THE SUBSTITUTION OF PHTHALATES

STUDIES TOWARDS THE SUBSTITUTION OF ORTHO-PHTHALATES IN PLASTISOLS

INDEX

1 PURPOSE OF THE STUDY

The purpose of this study is to devise alternatives to the use of *ortho*-dialkylphtalates (*o*-DAPs) as plasticizers for plastisol printing pastes. Substitutes should be printing pastes free of *o*-DAPs.

2 STRATEGY

The strategy employed in this substitution study is based in the following type of experiments:

- Technical experiments, in order to prepare plastisol prints containing the alternative plasticizers proposed as substitutes concerned phthalates.
- Quality measurements, to assess the properties of representative plastisol printed samples.
- Toxicity measurements, to establish the level of toxicity of the proposed substitute plasticizers with respect to *ortho*-dialkylphtalates (*o*-DAPs).

3 TEAMS INVOLVED IN THE STUDY

Alternative proposal study for phthalates in PVC plastisol has been carried out by a team from the University of Santiago de Compostela, Spain (USC), led by Prof. F. Javier Sardina. This team was also in charge of gathering and analyzing the available information on the hazards (toxicity) posed by phthalates and the substitutes under evaluation.

Preparation of the required printed textile samples and the quality studies on the finished samples were carried out in cooperation with a team from Technical Advice, S. L. (Barcelona, Spain) led by Mr. Joan Roca.

Experimental toxicity measurements on the chemicals under evaluation were carried out by two teams from the University of Santiago de Compostela, Spain (USC), led by Profs. Laura Sánchez Piñón and M. Isabel Loza.

4 INTRODUCTION

4.1 Scientific and technological background

Plasticisers are non-volatile solvents incorporated into the formulation of plastics to confer flexibility on them by reducing the viscosity of the melt as well as by lowering the glass transition temperature. They separate the polymer chains and enable their deformation to be more easily accomplished.

ortho-Dialkylphtalates (o-DAPs, figure 1) are a very common class of plasticizers used in plastics. They can be employed in the preparation of a broad range of articles including flooring, clothing, packaging, and children's soft plastic toys. However, some of these substances have toxicity hazards associated with them and, accordingly, have been regulated, e.g. in the European Union several phthalates are regulated in plasticized materials belonging to toys and childcare articles under the Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

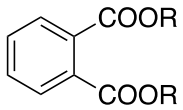

Figure 4.1. ortho-Dialkylphtalate structure

The textile positional printing is an important finishing technique in which a color pattern is applied to the fabric by employing pigments or dyes embedded in a polymer paste. Plastisol pastes employed for textile printing usually consists of solid plastic particles dispersed in a plasticizer. When heated, the plastic dissolves in the plasticiser, forming a liquid which is applied over the fabric or textile. After cooling, a flexible solid plastisol layer is produced. Phthalates are the most widely plasticizers employed in plastisol printing.

4.2 Mechanism of plasticizer action

A plasticizer can interact with the polymer chains of the plastic in several different ways, all of which will contribute to an increase in the flexibility of the plasticized material. There are several different theories about the mechanism of plasticizer

action.[1] Two of these theories are the gel theory and the lubricity theory. The first one considers that plasticizers break the polymer chain-chain attachments of a gel structure, and prevent their reformation by masking these attachment centres. In the lubricity theory, the function of plasticizer would be to reduce intermolecular frinction between polymer chains.

In summary, plasticizers are adhered to or adsorbed into the plastic particles and there they interact with the polar groups of the polymer reducing the friction between chains and increasing the flexibility points.

5 METHODOLOGY

This study was developed acording to the following key steps:

- Literature revision to search the most suitable alternatives to ortho-phthalates as plasticizers to be used in plastisol printing.
- Revision of the available toxicolgocial data for the alternative plasticizers and carrying out experimental toxicity determinations when the available toxicological data is deemed insufficient.
- Finally, the performance of the alternative candidates will be tested.

5.1 Selection of plasticizer alternatives to phthalates in plastisols

There are several families of plasticizer candidates that could be employed as alternatives to phthalates, such as: adipates, citrates, trimellitates, phosphates, benzoates or vegetable oils.[2] After reviewing the bibliography and the existing plasticizers databases, some suitable candidates were found for their use in plastisol prints instead of phthalates.[3]

Considering the objectives of this study, the principal criteria for choosing candidates were:

1. Their use in plastisols
2. Their availability in the market as commercial products

[1] *Encyclopedia of Polymer Science and Technology*, Wiley & Sons,
[2] *Handbook of Plasticizers*, George Wypych, 3rd Ed. **2017**; *PVC formulary*, George Wypych, 3rd Ed. **2017**
[3] *Databook of Plasticizers*, Anna Wypych, 2nd Ed., **2017**

The most suitable alternatives found in the acientific and technical literature are shown in the following table 1:

Table 1. Initial list of plasticizer candidates for the substitution study

	Branded name	Common name of active plasticizer	CAS Number	Available Hazard Information[4]
1	**ATBC**	**Tributyl *o*-acetylcitrate**	77-90-7	Not classified
2	**Diplast D/ DEHA/DOA**	**(2-ethylhexyl) adipate**	103-23-1	Not classified
3	**Hexamoll DINCH**	**Cyclohexane-1,2-dicarboxylic acid diisononyl ester**	474919-59-0	Not classified
4	**TOTM**	**Trioctyl trimellitate**	3319-31-1	Not classified
5	**Plasthall DOTP/DEHT**	**Dioctyl terephthalate**	6422-86-2	Not classified
6	**Benzoflex 181**	**2-ethylhexyl benzoate**	5444-75-7	Not classified
7	**Kalama K-Flex 850P**	**Diethylene glycol dibenzoate**	120-55-8	Not classified
8	**Kalama K-Flex 850P**	**Dipropylene glycol dibenzoate**	27138-31-4	Aquatic Chronic 3
9	**Plastoflex 2307**	**Epoxidized soybean oil**	8013-07-8	Not classified
10	**Eastman TXIB**	**2,2,4-Trimethyl-1,3-pentanediol diisobutyrate**	6846-50-0	Aquatic Chronic 3
11	**Sancticizer Platinium P1400/ Flexidone 300**	**1-Dodecyl-2-pyrrolidinone**	2687-96-9	Harmonised Skin Corr.1B Skin Sen. 1 Aquatic Acute 1

[4] Data ased on Harmonised Classification & Labelling (Table 3.1 of Annex VI) to European Regulation EC No. 1272/2008 and Registration Dossier from ECHA webpage

				Aquatic Chronic 1
12	**Monoplex DOS/ DEHS**	**di-(2-ethylhexyl) sebacate**	122-62-3	Not classified
13	**Mesamoll ASE**	**Alkylsulphonic phenyl ester**	91082-17-6	Not classified
14	**Flexricin 15**	**Ethylene glycol mono-ricinoleate**	106-17-2	Not classified
15	**Flexricin 13**	**Glycerol mono-ricinoleate**	1323-38-2	Not classified
16	**Flexricin P-3**	**Butyl ricinoleate**	151-13-3	Not classified

5.2 Known hazards of the candidates

The information available about the alternative plasticizers shows different positions between notifiers and the ECHA, so there is no agreement about their classification. Most of these substances are not classified by CLP, and the notifications about dangers are not supported by enough data.

For this reason, an experimental study was designed and launched to determine their toxicity levels.

6 TOXICITY MEASUREMENTS

Toxicity studies of the employed finishing agents were performed employing zebrafish embryos as model organism. Details on the toxicity study performed are shown in Annex I.

6.1 Test conditions

- *Type of study*: acute toxicity on zebre fish embryos
- *Model organism*: *Danio rerio*, wild type
- *Source*: the embryos used in the toxicity studies came from broodstock crossing.
- *Assay conditions*: The water temperature of the breeding fish is maintained by heaters at 26 °C and is permanently monitored by thermometers inside the fish tanks. In addition, parameters that affect the well-being of aquarium

fish should be measured weekly, mainly: ammonium, nitrite and nitrate concentrations, chlorine concentration, pH and water hardness. If any parameter is altered, it must be restored to its optimum value.

The rate of fertilization of eggs has been higher than 70%, a proportion recommended by the OECD.

6.2 Methodology

Embryos have been exposed to five increasing concentrations of the substance to be analyzed in 24-well plates. Four of these wells do not present the chemical in solution and constitute the internal negative control. Additionally, the experiment was accompanied by an external negative control plate (without a chemical) and a positive control with a substance whose toxicity in the model organism used was demonstrated, in this case 3,4-dichroloaniline 4 mg / L (CAS No. 95-76-1).

Compound toxicity was evaluated using the OECD Test Guideline No. 236: Fish Embryo Acute Toxicity (FET) Test. The mortality rate in the negative control was less than 10%, greater than 30% in the positive control and no more than one in the internal negative control. Also, the mortality at each concentration has been corrected in relation to mortality in the negative controls by Abbott correction.

After the start of the test, observations are made every 24 hours until the end of the test (96 hours), monitoring the lethal characteristics that cause the death of the embryo.

6.3 Toxicity studies of the commercial formulations used for textile finishes

After performing the *Test No. 236: Fish Embryo Acute Toxicity (FET) Test* (OECD, 2013a) on 11 phthalate alternatives, only in 5 of them a LC_{50} estimate was obtained (see following table). This was due to lack of mortality at the highest soluble concentration in the 6 remaining compounds. Toxicity in those compounds where LC_{50} could not be determined has been indicated as Maximum Toxicity Expected, taking into account the highest tested concentration. Toxicities varied from very

toxic 1-dodecyl-2-pirrolidinone (LC_{50} = 0.962 mg/l) to non-toxic 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (LC_{50} = 302.765 mg/l).

Table 2. Information summary for the Phthalate alternatives analyzed.

Phthalate Alternatives	CAS No.	Bibl. LC_{50} (fishes)	Exp. LC_{50}	NOEC	LOEC	[]Max. Tested	Toxicity	Solubility problems	Embryo Malformations
Tributyl *o*-acetylcitrate	77-90-7	38-60	5.12	3	4.4	40	Toxic	YES	YES
(2-ethylhexyl) adipate	103-23-1	> 0.78	ND (> 300)	ND	ND	300	Non Toxic*	YES	NO
Trioctyl trimellitate	3319-31-1	> 100	ND (>1000)	≥ 1000	> 1000	1000	Non Toxic*	YES	NO
Dioctyl terephthalate	6422-86-2	> 984	ND (>40)	ND	ND	40	Moderately toxic*	YES	NO
Diethylene glycol dibenzoate	120-55-8	3.9	1.96	0.5	1	8	Toxic	NO	YES
Dipropylene glycol dibenzoate	27138-31-4	3.7	5.26	1.5	3	24	Toxic	NO	YES
Epoxidized soybean oil	8013-07-8	900	ND (>20)	ND	ND	20	Moderately toxic*	YES	NO
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	> 1.55	302.77	50	100	400	Non Toxic	NO	YES
1-Dodecyl-2-pyrrolidinone	2687-96-9	No data	0.96	< 0.5	≤ 0.5	8	Very toxic	NO	YES
di-(2-ethylhexyl) sebacate	122-62-3	No data	ND (>300)	> 300	≥ 300	300	Non Toxic*	YES	NO
Alkylsulphonic phenyl ester (Mesamoll)	91082-17-6	> 100	ND (>100)	ND	ND	100	Non Toxic*	YES	NO

All concentrations are expressed in mg/l. "Bibl. LC50" and "Exp. LC50" stands for Bibliographic and Experimental LC50 estimates respectively. "[]Max. Tested" refers to Maximum Concentration Tested. * in "Toxicity" column indicates maximum toxicity expected since LC50 was not achieved at maximum concentration tested. ND = not determined.

Conclusions

In some compounds there has been important difficulties in achieving the required concentrations. Oily drops were observed for several substances and concentrations. The absence of higher solubility of some of these compounds in

water made very difficult to derive a LC_{50} value. In addition, it is difficult to assess to what extent the dissolved compound is available to the embryos.

Nevertheless, for these compounds, it has been demonstrated that at the maximum soluble concentration they do not have lethal effects under the test conditions. For those soluble, 4 out of 5, were cataloged as toxic or very toxic. The exception has been 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate.

Developmental delay only appeared in 4 compounds, for the ones that showed good solubility. This could indicate that developmental delay on fishes is dependent of the availability (i.e. solubility) of the compound.

Finally, comparing the toxicity results on phthalates and their alternatives is complex. The feasibility of the substitutions will depend on the particular phthalate to be droped and its possible alternatives. Notwithstanding, the alternatives seem to be less toxic.

7 QUALITY MEASUREMENTS

7.1 Preparation of the textile samples

In progress. To be completed in September 2018.

7.2 Evaluation of plastisols obtained

In progress. To be completed in September 2018.

7.3 Conclusions of the technical studies

In progress. To be completed in September 2018.

8 CONCLUSIONS OF THE TECHNICAL AND TOXICITY STUDIES

In progress. To be completed in September-October 2018.

9 ANNEX I: DETAILS ON TOXICITY MEASUREMENTS

9.1 Test conditions

- *Type of study*: acute toxicity on zebre fish embryos.
- *Model organism*: *Danio rerio*, wild type.
- *Source*: the embryos used in the toxicity studies came from broodstock crossing.

9.2 Methodology

- *Assay conditions*: The water temperature of the breeding fish is maintained by heaters at 26 °C and is permanently monitored by thermometers inside the fish tanks. In addition, parameters that affect the well-being of aquarium fish should be measured weekly, mainly: ammonium, nitrite and nitrate concentrations, chlorine concentration, pH and water hardness. If any parameter is altered, it must be restored to its optimum value.

The rate of fertilization of eggs has been higher than 70%, a proportion recommended by the OECD.

The following table shows the general conditions under which the toxicity analyses are carried out:

Conditions of Experiments	
Photoperiod	Darkness
Method design	Static
Temperature	26 ± 1ºC
pH	6,5 - 7,5
Hardness of water	6ºd

It has been described that keeping the entire process in the dark does not affect the toxicological result. Also, the physical-chemical conditions of the water must be kept stable within the viability ranges of the species. The ordinary procedure followed for the analyzes is the preparation of 1 L of test substance at the highest concentration to be analyzed, from which the different test concentrations are

obtained. Freshly prepared compound is always used, unless other methodology is advised.

Embryos have been exposed to five increasing concentrations of the substance to be analyzed in 24-well plates. Four of these wells do not present the chemical in solution and constitute the internal negative control. Additionally, the experiment was accompanied by an external negative control plate (without a chemical) and a positive control with a substance whose toxicity in the model organism used was demonstrated, in this case 3,4-dichroloaniline 4 mg / L (CAS No. 95-76-1).

If the chemicals under study are not soluble in water, a solvent should be used, the toxicity of which must be analyzed on a last outer plate at the same concentration as that used to dissolve the chemical.

The mortality rate in the negative control was less than 10%, greater than 30% in the positive control and no more than one in the internal negative control. Also, the mortality at each concentration has been corrected in relation to mortality in the negative controls by Abbott correction.

The light/dark cycle has been established as permanent darkness, with a static method design. The temperature has been maintained at all times at 26 ± 1 ° C, with a pH of 7.3 and a water hardness of 6 °d.

After the start of the test, observations are made every 24 hours until the end of the test (96 hours), monitoring the lethal characteristics that cause the death of the embryo.

Analysis of data: Compound toxicity was evaluated using the OECD Test Guideline No. 236: Fish Embryo Acute Toxicity (FET) Test. Briefly, five concentrations of each compound are tested in 20 embryos of D. rerio, in triplicate. Positive, negative and internal controls are disposed to validate the results. The test starts at 0 hpf (hours post fecundation) and ends at 96 hpf. The ecotoxicological estimates calculated from the mortality toxicity assays were LC50, LOEC and NOEC:

- LC_{50}, or *Lethal Concentration 50* or *median lethal dose*, represents the concentration of a substance, which kills half the members of a tested population. It is calculated extrapolating the concentration from the concentration-response curve got by probit regression of the observed results.
- NOEC stands for *No Observed Effective Concentration*. It is the highest concentration tested (only from the tested concentrations) not statistically different from the negative control.
- LOEC stands for *Lowest Observed Effective Concentration*. It is the lowest concentration tested (only from the tested concentrations) at which the effect of the compound is statistically different from the negative control. LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) are calculated by two samples Fisher's exact test comparing the mortality each concentration against negative control. In ascending order, the first tested concentration significantly different from the negative control will be the LOEC, and the immediately lower will be the NOEC.

It has been shown that ignoring mortality in the negative control, even when it is less than 10% accepted by the test, can cause an underestimation of the LC_{50} value. Therefore, data were corrected for mortality in the negative control using the Abbott's formula, where P is the percentage of mortality in the embryos subjected to the toxic and P_0 is the percentage of mortality of the embryos in the negative control.

$$\% \text{ Corrected} = \frac{P - P_0}{100 - P_0} \times 100$$

LC_{50} is estimated by interpolation of the function calculated by the probit regression from mortality data. LOEC (lowest observed effect concentration) and NOEC (no observed effect concentration) are calculated by two samples Fisher's exact test comparing the mortality each concentration against negative control.Toxicity studies of the commercial formulations used for textile finishes.

9.3 Toxicity studies of the commercial formulations used for textile finishes

Two different groups of compounds have been analyzed for the determination of toxicity under the Fish Embryo Toxicity (FET) Test (OECD, 2013). Sixteen candidates as phthalates substitutes were initially selected:

Alternatives to Phthalates:

- Tributyl o-acetylcitrate
- (2-ethylhexyl) adipate
- Cyclohexane-1,2-dicarboxylic acid diisononyl ester
- Trioctyl trimellitate
- Dioctyl terephthalate
- 2-ethylhexyl benzoate
- Diethylene glycol dibenzoate
- Dipropylene glycol dibenzoate
- Epoxidized soybean oil
- 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TXIB)
- 1-Dodecyl-2-pyrrolidinone
- di-(2-ethylhexyl) sebacate
- Alkylsulphonic phenyl ester (Mesamoll Trademark)
- Ethylene glycol mono-ricinoleate
- Glycerol mono-ricinoleate
- Butyl ricinoleate

Results

After performing the *Test No. 236: Fish Embryo Acute Toxicity (FET) Test* (OECD, 2013a) on 11 phthalate alternatives, only in 5 of them a LC_{50} estimate was obtained (see following table). This was due to lack of mortality at the highest soluble concentration in the 6 remaining compounds. Toxicity in those compounds where LC_{50} could not be determined has been indicated as Maximum Toxicity Expected, taking into account the highest tested concentration. Toxicities varied from very toxic 1-dodecyl-2-pirrolidinone (LC_{50} = 0.962 mg/l) to non-toxic 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (LC_{50} = 302.765 mg/l) (see figure).

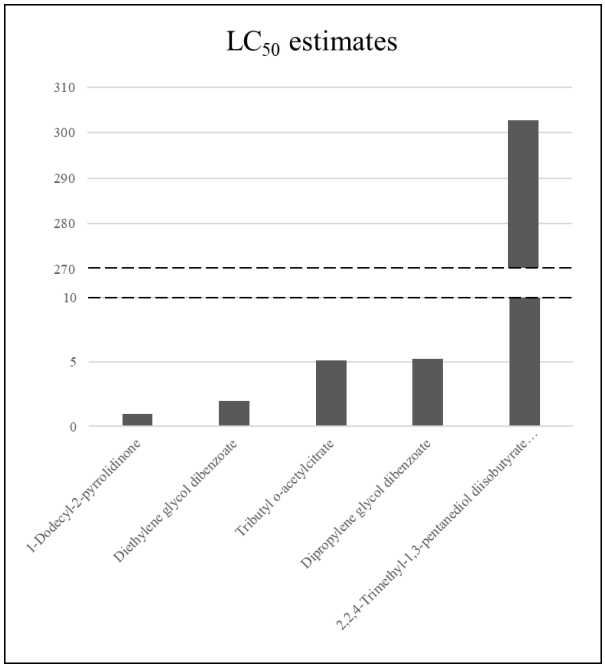

Figure 9.1 Graphical representation of LC50 estimates (mg/l). Lower value means higher toxicity

Only four compounds were relatively soluble at the tested concentrations, that match with those with toxicological estimates: Diethylene glycol dibenzoate, Dipropylene glycol dibenzoate, 1-Dodecyl-2-pyrrolidinone and 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate. It should be noted that LC50 estimate is available also for Tributyl o-acetylcitrate because of mortality related to tested concentration, although it was not completely dissolved. Related to this information, embryo malformation was only detected in this 5 compounds with concentration-mortality response. More striking is the appearance of developmental delay in only the 4 compounds easy to solubilize.

The difference between a toxicological estimate of ND (not determined because mathematical reasons) and "higher than X value" is that the results were not concentration-related. For example, if there is lower mortality in the highest concentration than in any of the lower, whatever the reason.

In general, results are comparable to those found in the scientific literature for toxicity in fishes, especially because several data are expressed as "toxicity higher than…" highlighting the difficulty in dissolving the compounds in water. The only exception, and it is not an relevant one, has been the Tributyl o-acetylcitrate, with

an experimental estimate of 5.12 mg/l, whereas the bibliographic estimate on Lepomis macrochirus ranged from 38 to 60 mg/l.

Table 3 Information summary for the Phthalate alternatives analyzed.

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