PETITIONER SUMMARY DATA SHEET

("P-SDS")

SUBSTANCE: Perfluoro{acetic acid, 2-[(5-methoxy-1,3-dioxolan-4yl)oxy]}, ammonium salt

USE OF SUBSTANCE : additive

REF N. not yet assigned

CAS. N.: 1190931-27-1

SOCIETY: SOLVAY SPECIALTY POLYMERS

PERSON RESPONSIBLE FOR THE TECHNICAL DOSSIER:

ADDRESS OF THE RESPONSIBLE:

PHONE: +39 [redacted] FAX: +39

e-mail :

Where the technical dossier does not contain all the requested data (see below) give reasons:

In April 2013, Solvay Specialty Polymers submitted a technical dossier and a P-SDS for the application for the safety evaluation of Perfluoro{acetic acid, 2-[(5-methoxy-1,3-dioxolan-4yl)oxy]}, ammonium salt.
This technical dossier contains additional information requested by the EFSA Working Group on Food Contact Materials of the Scientific Panel on food contact materials, enzymes, flavouring and processing aids (CEF), i.e. EFSA Question number EFSA-Q-2013-00363.
Additional non-toxicity data are provided with respect to the specific migration of *confidential information has been removed* using an analytical method with a target detection limit of 1 ppb. In addition, an in-vitro micronucleus assay for *confidential information has been removed* is provided.

The initial P-SDS submitted in 2013 has been revised and the new information related to the additional information can be recognised as text on a grey background, marked REV.

Hard copies of Annex 13-15, related to additional information, are provided in the present dossier, while on CD ROM copies of Annex 1-15 are provided.

REMARKS:

CONFIDENTIALITY

Certain sections of this filing contain confidential business information that should not be released to third parties. The information marked "Confidential" should be reviewed only by authorized members of EFSA and the European Commission in connection with its evaluation of the suitability of the petitioned substance for use in food contact materials.
More specifically the following information is considered confidential information:

- spectroscopy data *(see 1.2.9)*
- the proprietary manufacturing process *(see 1.2.10)*
- purity% *(see 1.2.11);*
- impurity *(see 1.2.12);*
- thermal decomposition products *(see 2.2.5)*
- maximum percentage in formulation *(see 3.5)*
- migration report – i.e. the nature of the decomposition products; the analytical method for the determination of the residual content of F-Diox Acid Ammonium Salt is not confidential *(see 5 and 6)*
- gene mutation assay on decomposition product *(see 8.1.4)*

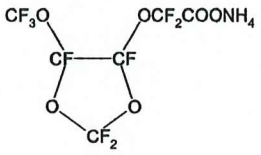
Clearly, public disclosure of this information would harm Solvay Specialty Polymers' competitive position, as competitors could potentially improve their own process by learning the details of Solvay Specialty Polymers' process.
The confidential information has been removed from the "non-confidential" version of the filing, provided on CD-ROM.

The information considered to be treated as confidential can be recognized as text on a yellow background.

INFORMATION REQUESTED		INFORMATION PROVIDED
1.	**IDENTITY OF SUBSTANCE**	
1.1	**individual substance:**	No
1.2	**defined mixture:**	yes
1.2.1	**chemical name:**	Acetic acid, 2,2-difluoro-2-[[2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl]oxy]-,ammonium salt (1:1)
1.2.2	**synonym(s):**	F-Diox Acid Ammonium Salt, C6O4 Cyclic,
1.2.3	**trade name(s):**	Cyclic C6O4 Ammonium Salt, cC6O4 Ammonium Salt
1.2.4	**CAS Nr:**	1190931-27-1
1.2.5	**constituents:**	The substance is a process mixture of four diastereoisomers and each pair contains a 50:50 mixture of two enantiomers.
1.2.6	**proportions in the mixture:**	The substance is a process mixture of four diastereoisomers with the following proportions: • ammonium difluoro {[(4S,5R)-2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl] oxy} acetate (~25%w/w) • ammonium difluoro {[(4R,5S)-2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl] oxy} acetate (~25%w/w)

- ammonium difluoro {[(4S,5S)-2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl] oxy} acetate (~25%w/w)
- ammonium difluoro {[(4R,5R)-2,2,4,5-tetrafluoro-5-(trifluoromethoxy)-1,3-dioxolan-4-yl] oxy} acetate (~25%w/w)

Ref.Annex 4 (File N°8)

1.2.7	**molecular and structural formula:**	Molecular formula: C6H4O6NF9 Structural formula: 
1.2.8	**molecular weight (Mw) and range:**	357 g/mol
1.2.9	**spectroscopic data:**	*confidential information has been removed*
1.2.10	**manufacturing details**	*confidential information has been removed*
1.2.11	**purity (%):**	*confidential information has been removed* F-Diox Acid Ammonium Salt is manufactured and used only as aqueous solution: typically concentration in water is 25% -55%
1.2.12	**impurities (%):**	*confidential information has been removed*
1.2.13	**specifications:**	
1.2.14	**other information:**	No other information is given
1.3	**Non-defined mixture:**	no
1.4	**polymer used as additive1:**	no

2. PHYSICAL AND CHEMICAL.PROPERTIES OF SUBSTANCE

2.1	**physical properties**	
2.1.1	**melting point (°C):**	64.5°C Melting point has been established according to OECD 102 method
2.1.2	**boiling point (°C):**	The boiling point of the notified substance could not be determined since at 138°C-140°C it starts decomposing.

2.1.3	**decomposition temperature (°C):**	Decomposition starts at 140°C → Ref. Annex 5
2.1.4	**solubility (g/l):**	>667 g/L water Water solubility has been established according to OECD 105 method
2.1.5	**octanol/water partition(log Po/w)**	log Pow = -0.38 at 22 °C Partition coefficient has been established according to OECD 107 method
2.1.6	**other information related to lipophilicity:**	No other information is submitted
2.2	**chemical properties**	
2.2.1	**nature:**	The pH of the notified substance in aqueous solution (20g/l) is 10
2.2.2	**reactivity:**	The notified substance is not reactive under the conditions described in section 3. Nevertheless due to its poor thermal stability it starts decomposing at temperature exceeding 140°C.
2.2.3	**stability:**	The notified substance, due to its poor thermal stability, starts decomposing during the thermal treatments described in section 3. → Ref. Annex 5
2.2.4	**hydrolysis:**	Not relevant, hydrolysis will not occur.
2.2.5	**intentional decomposition/ transformation:**	*confidential information has been removed*
2.2.6	**unintentional decomposition/ transformation product(s):**	-
2.2.7	**interaction with food substances:**	There are no known interactions with food substances.
2.2.8	**other information:**	

3. INTENDED APPLICATION OF SUBSTANCE

3.1	**food contact material:**	The notified substance is used in the manufacture of fluorinated polymers like: – Tetrafluoroethylene homopolymer – Copolymers of tetrafluoroethylene with perfluoroalkylperfluorovinyl ethers – Copolymers of tetrafluoroethylene with hexafluoropropene These fluoropolymers are processed to produce a range of articles: – Parts for food processing equipment (fitting, valves) – Tubes, sheets, pipes

		– Tapes, cast films – Antistick coatings on cooking utensils All of the articles manufactured from these polymers and copolymers are for repeat use
3.2	**technological function:**	The substance is used as an emulsifier/dispersing agent during the polymerization process of fluoropolymers. Its presence ensures that the polymer to be synthesized forms a stable dispersion of particles.
3.3	**maximum process temperature (°C):**	Typical temperatures encountered in the manufacturing of finished articles are; – Tubes, sheets and pipes up to 420°C for 5 min max. – Tapes, cast films and parts for food processing equipment up to 380°C for 3 min max. – Anti-stick coatings are dried and then baked at temperatures up to 420 °C for 15 min max.
3.4	**maximum percentage in formulation:**	*confidential information has been removed*
3.5	**conditions of contact in practice**	
3.5.1	**contact food:**	All types of foodstuffs
3.5.2	**time and temperature:**	The maximum temperature of contact is experienced by coated cookware, such as coatings on frying pans and articles for oven baking. Coatings for cookware are used at temperatures up to 230 °C for, usually up to one or two hours. There may be excursions up to 250 °C for 15 minutes.
3.5.3	**surface to volume ratio:**	This will vary from article to article, but the conventional surface to volume ratio of 6 mg/dm² is applicable.
3.5.4	**other information:**	All the articles manufactured from these fluoropolymers are for repeated use.
3.6	**treatment of food contact material prior to use:**	Cookware and bakeware are usually washed in hot soapy water before use; other articles, such as components of food processing machinery, are rinsed with hot water.
3.7	**other uses:**	Lubricant powder grades, manufactured with these fluoropolymers may be used as additives in a variety of polymers which may be extruded and moulded into articles for contact with all types of foodstuffs, at all temperatures for repeat use. Lubricant powders are used in these polymers to reduce friction and wear.
3.8	**other information:**	No other information on intended application of the substance is submitted

4. AUTHORISATION OF SUBSTANCE

4.1	**EU countries**	
4.1.1	**in Member States:**	No
4.1.2	**notified as "new substance" in the context of 6th Amendment of Directive 67/548/EEC:**	Yes REACH Registration Number: 01-2119879655-19-0000
4.1.3	**other information:**	-
4.2	**non-EU countries**	
4.2.1	**in USA:**	no
4.2.2	**in Japan:**	no
4.2.3	**in other countries:**	no
4.2.4	**other information:**	-
4.3	**other information:**	-

5. DATA ON MIGRATION OF SUBSTANCE

5.1	**specific migration (SM):**	SM not determined. Instead, the residual content of F-Diox Acid Ammonium Salt in a PTFE film has been determined and the worst case migration was calculated assuming 100% migration, see section 6. → Ref Annex 7
5.2	**overall migration (OM):**	OM not determined
5.3	**Quantification and identification of:** **a) migrating oligomers and** **b) reaction products derived from monomers and starting substances and additives**	F-Diox Acid Ammonium Salt is thermally unstable, see 2.2.5. The residual content of two thermal decomposition products of F-Diox Acid Ammonium Salt in a PTFE film was determined. The worst case migration was calculated, assuming 100% migration of the decomposition products, *confidential information has been removed* which were found to be formed during thermal treatment of the F-Diox Acid Ammonium Salt (see 2.2.5). *confidential information has been removed* The analytical method to determine the residual content of F-Diox Acid Ammonium Salt is not confidential. → Ref Annex 7
		REV The specific migration of the main decomposition product, *confidential information has been removed* has been determined → Ref Annex 13

5.3.1 test sample:	Cast film, obtained from an aqueous dispersion of PTFE (polytetrafluoroethylene), containing 50 ppm of F-Diox Acid Ammonium Salt referred to dry polymer. A substrate (polyimide) is impregnated with the aqueous dispersion, the film is subsequently dried and then sintered at 370°C for 2.4 min. The impregnation process is repeated three times in order to reach the requested thickness (60 µm) The resulting cast film obtained on both sides of the substrate is detached from the support and cut in foils, having a nominal thickness of 60 µm. The sample is considered to represent the worst case as aqueous dispersions contain the highest content of the notified substance (50 ppm) and are treated at the lowest temperature and for the shortest period (see 3.4). As a result, the sample also represents the worst case with respect to residual levels of the thermal decomposition products, *confidential information has been removed* if present at all, taking into account their boiling points (67°C and 170°C respectively).
	REV The test sample was the same cast film tested in 2013. The FCM WG requested to test a realistic test sample, i.e. produced in a way as occurring in practice (and not by a layer-by-layer procedure) and of a typical film thickness. This film is considered to represent the worst case and represents a realistic sample. Film casting is a well-known industrial process commonly adopted for processing PTFE aqueous dispersion. The cast film tested has been obtained using this dip-coating process and it is a realistic sample, not manufactured according to laboratory procedure, with a typical film thickness. The cast film is considered to represent the worst case because it is being processed at lower processing temperatures with shortest processing times, which would result in a worst case residual level of *confidential information has been removed* → Ref Annex 15
5.3.1.1 chemical composition:	The film sample tested is a PTFE film, manufactured with 1% of F-Diox Acid Ammonium Salt in the first step of the polymerisation process (see 3.4). The content of F-Diox Acid Ammonium Salt in the aqueous PTFE dispersion is 50 ppm referred to dry polymer, which is considered to represent the worst case with respect to the highest content of F-Diox Acid Ammonium Salt.
5.3.1.2 physical composition:	homogeneous film
5.3.1.3 density, melt flow index of polymer:	Polymer density = 2.2 g/cm3
5.3.1.4 dimensions of test sample:	18 cm x 30 cm sheets, thickness 60 µm.
5.3.1.5 dimensions of test specimen	- 0.5 gram for the determination of *confidential information has been removed* - 1 sheet (18 cm x 30 cm) for the determination of

		confidential information has been removed
5.3.2	**treatment of test sample prior to testing:**	The film was cut into small pieces, prior to analysis
5.3.3	**test food(s)/food simulant(s)/extraction solvent(s):**	*confidential information has been removed*
		REV The specific migration of *confidential information has been removed* was determined in 3% acetic acid and olive oil. → Ref Annex 13
5.3.4	**contact mode:**	total immersion
		REV The specific migration of *confidential information has been removed* was determined in 3% acetic acid and olive oil by total immersion. → Ref Annex 13
5.3.5	**contact time and temperature:**	*confidential information has been removed* 1 hour at 100°C in an conventional oven, followed by 1 hour at 70°C in the headspace oven. *confidential information has been removed* 4 hours at reflux temperature.
		REV As a worst case, tests were performed 4.5 hours at 100°C in 3% acetic acid and 3 hours at 175°C in olive oil, by total immersion, in a gas-tight system (head-space vial). After the migration period, the headspace vials containing the sample and the simulant were subjected to HS-GC-MS analysis. → Ref Annex 13
5.3.6	**surface to volume ratio in extraction tests:**	*confidential information has been removed* 0.5 gram polymer film was extracted with 5 ml extraction solvent (HFE 7500). As on average 0.8 dm² of film sample had a weight of 1 gram, the surface to volume ratio in the extraction tests was 0.4 dm²/5 ml *confidential information has been removed* 18 cm x 30 cm of film sample was extracted in 100 ml extraction solvent; this equals to a surface/volume ratio of 5.4 dm²/100 ml
		REV 0.3 dm² polymer film was immersed in 5 ml food simulant → Ref Annex 13
5.3.7	**analytical method:**	*confidential information has been removed* HS-GC-MS (headspace gas chromatography with mass detection), using the standard addition method. *confidential information has been removed* GC-MS analysis, using an internal standard

REV
The specific migration of *confidential information has been removed* was determined using HS-GC-MS (headspace gas chromatography with mass detection).
→ Ref Annex 13

5.3.8 detection/ determination limit:

confidential information has been removed 3 µg absolute (equal to the intercept of the calibration curve. Taking into account the surface/volume ratio applied in the extraction test, the detection limit of *confidential information has been removed* is 0.045 mg/6 dm².

confidential information has been removed 228ng/ml, corresponding to the level in the lowest calibration solution. Taking into account the concentration factor prior to GC-MS analysis and the surface/volume ratio applied in the extraction test, the detection limit of *confidential information has been removed* is 0.001 mg/6 dm²

REV
The detection limit of the HS-GC-MS method was 20 ng/vial or 20 ng/vial/0.3 dm². This corresponds to 0.4 µg/6 dm².
→ Ref Annex 13

5.3.9 recovery:

confidential information has been removed:
As the standard addition method was applied, recovery is not applicable.
confidential information has been removed
The recovery of *confidential information has been removed* was determined after reflux for 4 hours in HFE7100/acetone (8:2). The following results were obtained:
→ Ref Annex 7

Component	level added (µg/ml)	level found (µg/ml)	Recovery (%)
confidential information has been removed	2.38	2.22	93
		2.53	106
		2.32	97
		Average ± Sd (n-1)	99 ± 7

REV
The recovery was determined by analysing spiked food simulants, after storage for 4.5 hours at 100°C (3% acetic acid) and 3 hours at 175°C (olive oil). Recovery of *confidential information has been removed* was determined in food simulants, both in the absence and the presence of film.
After storage for 4.5 hours at 100°C (3% acetic acid) and 3 hours at 175°C (olive oil) the recovery of *confidential information has been removed* was as follows:
→ Ref Annex 13

Food simulant	Level added (ng/vial)	Level found (ng/vial)	Recovery (%)[2)]	Average recovery (%)
3% acetic acid,	103.5	64.1	62.0	

in the absence of polymer		69.6	67.3	66.1 ± 3.8
		71.6	69.2	
3% acetic acid, in the presence of polymer	103.5	68.4	66.0	64.1 ± 7.1
		58.3	56.3	
		72.5	70.1	
olive oil, in the absence of polymer	103.5	75.5	73.0	72.2 ± 6.0
		80.4	77.7	
		68.1	65.7	
olive oil, in the presence of polymer	103.5	93.2	90.0	83.6 ± 6.5
		86.7	83.8	
		79.8	77.1	

5.3.10 other information: -

5.3.11 results:

confidential information has been removed:
The residual content of *confidential information has been removed* was found to be not-detectable. As a result, worst case migration of *confidential information has been removed* is < 0.045 mg/6 dm².

confidential information has been removed
The residual content of *confidential information has been removed* was found to be not-detectable. As a result, the worst case migration of *confidential information has been removed* is < 0.001 mg/6 dm².

In the GC-analysis for residual *confidential information has been removed*, minor additional peaks were detected in the chromatogram, where absent in the chromatogram of the blank solvent.
Based on the calibration curve of *confidential information has been removed*, the total area of these additional peaks would result in a worst case migration of 0.0024 mg/6 dm².

→ Ref Annex 7

confidential information has been removed

REV
The migration of *confidential information has been removed* was not detectable or < 0.4 µg/6 dm².

→ Ref Annex 13

6. DATA ON RESIDUAL CONTENT OF SUBSTANCE IN THE FOOD CONTACT MATERIAL

6.1 actual content: Actual content determined

→ Ref Annex 7

6.2 substance: F-Diox Acid Ammonium Salt

6.3 test sample: The same film was tested as described in section 5.3. i.e. a cast film, obtained from an aqueous dispersion of PTFE (polytetrafluoroethylene), containing 50 ppm of F-Diox Acid Ammonium Salt referred to dry polymer.

6.3.1 chemical composition see 5.3.1.1

6.3.2 physical composition homogeneous PTFE film

6.3.3	**density, melt flow index of polymer**	see 5.3.1.3
6.3.4	**dimensions of test sample**	18 cm x 30 cm sheets, thickness 60 µm
6.3.5	**dimensions of test specimen:**	one sheet (18 cm x 30 cm) per test
6.4	**treatment of sample:**	the sample was refluxed for 4 hours in HFE7100/acetone (8:2)
6.5	**test method:**	After the reflux period, the extract was cooled down and a residual content of F-Diox Acid Ammonium Salt was determined by LC-MS analysis. For quantification, standard solutions of F-Diox Acid Ammonium Salt were prepared and analysed.
6.5.1	**detection/ determination limit:**	The detection limit was 4 ng/ml, and is based on the signal obtained for the lowest standard solution.
6.5.2	**precision of test method:**	The precision of the test method can be obtained from the standard deviation of the triplicate recovery experiments. Sd(n-1) = 5% repeatability r = 2.83 * Sd(n-1) = 14%
6.5.3	**recovery:**	The recovery of F-Diox Acid Ammonium salt was determined after reflux for 4 hours in HFE7100/acetone (8:2). The following results were obtained:

Component	level added (µg/ml)	level found (µg/ml)	recovery (%)
F-Diox Acid Ammonium Salt	0.21	0.27	129
		0.25	119
		0.26	124
		Average ± Sd(n-1)	124 ± 5

6.5.4	**other information:**	-
6.6	**results:**	The residual content of F-Diox Ammonium Salt was found to be not-detectable or < 4ng/ml. After applying the surface/volume ratio used in the extraction test, the non-detectable residual content corresponds to < 0.0004 mg/6 dm².
6.7	**calculated migration (worst case):**	The calculated worst case migration of F-Diox Ammonium Salt is 0.0004 mg/6 dm².
6.8	**residual content versus specific migration:**	-

7 MICROBIOLOGICAL PROPERTIES OF SUBSTANCE

7.1 Is the substance used as an anti-microbial agent?	Not applicable

8. TOXICOLOGICAL DATA

8.1 Genotoxicity

8.1.1 Gene mutation in bacteria:

The test item C6O4 CYCLIC was examined for the ability to induce gene mutations in tester strains of Salmonella typhimurium and Escherichia coli, as measured by reversion of auxotrophic strains to prototrophy.
The five tester strains TA1535, TA1537, TA98, TA100 and WP2 uvrA were used.
Experiments were performed both in the absence and presence of metabolic activation, using liver S9 fraction from rats pre-treated with phenobarbitone and betanaphthoflavone.
The test item was used as a solution in sterile distilled water.

The test was performed according to the EC Method B.13/14 and the OECD Guideline 471.

The test item C6O4 CYCLIC was assayed in the toxicity test at a maximum dose level of 5000 µg/plate and at four lower concentrations spaced at approximately half-log intervals: 1580, 500, 158 and 50.0 µg/plate.
Concentrations are expressed in terms of active ingredient. No precipitation of the test item was observed at the end of the incubation period at any concentration. No toxicity was observed at any dose level with any tester strain.
In Main Assay I, using the plate incorporation method, the test item was assayed at the following dose levels expressed in terms of active ingredient: 5000, 2500, 1250, 625 and 313 µg/plate. No toxicity was observed at any dose level with any tester strain both in the absence and presence of S9 metabolic activation.
As no relevant increases in revertant numbers were observed at any concentration tested, a pre-incubation step was included for all treatments of Main Assay II. The test item was assayed at the same concentrations employed in Main Assay I.
Toxicity, as indicated by thinning of the background lawn and/or reduction in revertant numbers, was observed at higher dose levels with all tester strains, both in the absence and presence of S9 metabolic activation.
No precipitation of the test item was noted at the end of the incubation period at any concentration tested, in any experiment.
The test item did not induce two-fold increases in the number of revertant colonies in the plate incorporation or pre-incubation assay, at any dose level, in any tester strain, in the absence or

presence of S9 metabolism.
It is concluded that the test item C6O4 CYCLIC does not induce reverse mutation in Salmonella typhimurium or Escherichia coli in the absence or presence of S9 metabolism, under the reported experimental conditions.

→ Ref. Annex 8

8.1.2 In vitro mammalian cell gene mutation test:

The study was performed to investigate the potential of cC6O4 Ammonium Salt to induce mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y according to the EC Method B.17 and the OECD Guideline 476.
The assay was performed in two independed experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment period of 24 hours in the absence of metabolic activation and 4 hours in the presence of metabolic activation.
The highest concentration tested, 3640 µg/ml, applied in the pre-experiment was chosen with regard to the molecular weight of the test item corresponding to a molar concentration of about 10 mM. The concentration range of the main experiments was limited by cytotoxic effects and precipitation of the test item. Tested concentrations are reported below:
Experiment I:
Without S9 mix: 56.9; 113.8; 227.5 and 455 µg/ml
With S9 mix: 56.9; 113.8; 227.5; 455 and 910 µg/ml
Experiment II:
Without S9 mix: 28.1; 56.3; 112.5; 225 and 337.5 µg/ml
With S9 mix: 56.3; 112.5; 225; 450 and 675 µg/ml
No substantial and reproducible dose dependent increase in mutant colony numbers was observed in both main experiments. No relevant shift of the ratio of small versus large colonies was observed up to the maximal concentration of the test item.
Appropriate reference mutagens (MMS and CPA) were used as positive controls and showed a distinct increase in induced mutant colonies, indicating the sensitivity and validity of the tests.
In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported the test item did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y with and without S9.
Therefore the tested substance is considered to

be non-mutagenic in this mouse lymphoma assay.

→ Ref. Annex 9

8.1.3 In vitro mammalian chromosome aberration test :

According to the EC Method B.10 and the OECD Guideline 473 the test item C6O4 CYCLIC was assayed for the ability to cause chromosomal damage in Chinese hamster ovary cells, following *in vitro* treatment in the absence and presence of S9 metabolic activation.
Two main experiments for chromosomal damage were performed.
In the first experiment, the cells were treated for 3 hours with C6O4 CYCLIC in the presence and absence of S9 metabolism. The cells were then harvested after 20 hours, corresponding to approximately 1.5 cell cycle. Dose levels of 5000, 2500, 1250, 625, 313, 156, 78.1, and 39.1 µg/ml were used in the absence and presence of S9 metabolism. Solutions of the test item were prepared in sterile distilled water of injectable grade.
No induction of chromosomal aberrations were obtained in the first main assay but, following treatment with the test item in the presence of S9 metabolism, marked increases in the number of endoreduplicated cells over the concurrent negative control were observed.
A second main experiment was performed where cells were treated with C6O4 CYCLIC in the absence of S9 metabolism and harvested after 20 hours at the dose levels of 2000, 1330, 889, 593, 395, 263, 176 and 117 µg/ml. A continuous treatment until harvest was used.
A modified dose range was adopted for this treatment in order to investigate more closely those doses most likely to exhibit endoreduplication. Both experiments included appropriate negative and positive controls. Two cell cultures were prepared at each test point. Dose levels were selected for the scoring of chromosomal aberrations on the basis of the cytotoxicity as determined by the reduction of population doubling.
One hundred metaphase spreads were scored for chromosomal aberrations from each culture, with the exception of one replicate culture treated with Cyclophosphamide (first main experiment), where 75 metaphases were examined.
For the first experiment, following treatment with the test item C6O4 CYCLIC, no statistically significant increase in the incidence of cells bearing aberrations including or excluding gaps was observed in the absence or presence of S9 metabolism. In the presence of S9 metabolism, marked increases in the number of endoreduplicated cells over the control were observed. An increase in the number of endoreduplicated cells was also observed in the

absence of S9 metabolism at the highest dose selected for scoring.
For the second experiment, following treatment with the test item in the absence of S9 metabolism, slight increases but not statistically

significant in the incidence of cells bearing aberrations, excluding gaps, over the control values were
observed at all dose levels selected for scoring. More remarkable increases of aberrant cells were observed when including gaps.

significant in the incidence of cells bearing aberrations, excluding gaps, over the control values were observed at all dose levels selected for scoring. More remarkable increases of aberrant cells were observed when including gaps.
In the presence of S9 metabolism, statistically significant increases in the incidence of aberrant cells including and excluding gaps were observed at the highest dose selected for scoring (2000 µg/ml).
The incidences exceeded the range of the historical values for negative controls when excluding gaps. Moreover marked increases in the number of endoreduplicated cells over the controls were seen at the intermediate and high dose levels selected for scoring.
Statistically significant increases in the number of cells bearing aberrations (including and excluding gaps) were observed following treatments with the positive controls Cyclophosphamide and Mitomycin-C, indicating the correct functioning of the test system.
On the basis of these results it is concluded that C6O4 CYCLIC induces chromosomal aberrations in Chinese hamster ovary cells after in vitro treatment under the reported experimental conditions.
It is also concluded that the test item inhibits cell cycle progression and chromosome segregation under the reported experimental conditions.

→ Ref.Annex 10

8.1.4 Other information:
In vivo mammalian chromosomal aberration test

According to OECD Guideline 475 and under GLP conditions, cC6O4 ammonium salt was tested to investigate its potential to induce chromosome aberrations in bone marrow cells of rats (Wistar, Harlan) *in vivo*.

Pre-experiment toxicity tests were carried out in order to determine the maximum doses to be tested for cytogenetic assay.
Animals orally treated with 1250 mg/kg b.w. showed clinical signs like reduction in spontaneous activity, ruffled fur and hunchback posture indicating bioavailability of the test item. Since no substantial differences among sexes were observed, only male animals were used for the cytogenetic assay.

Doses selected for the cytogenetic assay were: 312.5, 625, 1250 mg/kg b.w. (referred to the dry salt).
The test item was administered at a volume of 10 ml/kg b.w. and 24 hours after treatment bone marrow cells were collected for chromosomal aberration analysis. An additional group at the highest dose was sampled after 48 hours post treatment. Seven males per test item groups (included positive and negative controls) were evaluated for the occurence of cytogenetic damage. 100 well spread metaphases per animal were scored for gaps, breaks, fragments, deletions, multiple aberrations, exchanges and chromosomal disintegrations.

The study was considered acceptable since the positive control showed a statistically significant response (7.3% aberration rate excluding gaps) and the aberration frequency excluding gaps of the vehicle control was below 2%.

The mitotic indices were not relevantly reduced after treatment, indicating that the test item did not have cytotoxic effects in the bone marrow.
No statistically significant increment of the aberration frequencies occured as compared to the control value. No relevant dose dependent increases in the aberration rates of the treated animals were observed.
In conclusion it can be stated that cC6O4 Ammonium Salt did not induce chromosome mutations in the bone marrow cells of the rat *in vivo*.

→ Ref.Annex 11

8.1.4 Other information:
Gene mutation in bacteria on *confidential information has been removed*

confidential information has been removed

8.1.4 Other information:
***In-vitro* micronucleus test on** *confidential*

confidential information has been removed

information has been removed

8.2 General toxicity

8.2.1	**Subchronic (90d) oral toxicity:**	-
8.2.2	**Chronic toxicity/ carcinogenicity:**	-
8.2.3	**Reproduction/teratogenicity:**	-
8.2.4	**Other information:**	-

8.3 Metabolism

8.3.1	**Absorption, distribution, biotransformation and excretion:**	-
8.3.2	**Accumulation in man:**	-
8.3.3	**Other information:**	-

8.4 Miscellaneous

8.4.1	**Effects on immune system:**	-
8.4.2	**Neurotoxicity:**	-
8.4.3	**Introduction on peroxisome proliferation:**	-
8.4.4	**Other information:**	-

LIST OF ANNEXES

Annex 1: *confidential information has been removed, P-SDS item 1.2.9;*

Annex 2: *confidential information has been removed, P-SDS item 1.2.9;*

Annex 3: *confidential information has been removed, P-SDS item 1.2.10;*

Annex 4: *confidential information has been removed P-SDS item 1.2.11, 1.2.12;*

Annex 5: TGA Curve F-Diox Acid Ammonium Salt, *P-SDS item 2.1.3, 2.2.3;*

Annex 6: *confidential information has been removed P-SDS item 2.2.5;*

Annex 7: *confidential information has been removed P-SDS item 5 and 6;*

Annex 8 RTC report no. 76250: C6O4 Cyclic – Bacterial Mutation Assay 20 October 2009, *P-SDS item 8.1.1;*

Annex 9 HARLAN report no. 1305602 cC6O4 Ammonium Salt – Mutations in L5178Y $TK^{+/-}$ mouse lymphoma cells (fluctuations method), 14 July 2010, *P-SDS item 8.1.2;*

Annex 10 RTC report no. 76240: C6O4 Cyclic – Chromosome aberrations in Chinese Hamster Ovary cells in vitro, 9 October 2009, *P-SDS item 8.1.3.*

Annex 11 [redacted] report no. 1305601: cC6O4 Ammonium Salt – Chromosome aberrations in Bone Marrow Cells of the Rats in vivo, 13 July 2010, *P-SDS item 8.1.4.*

Annex 12: *confidential information has been removed P-SDS item 8.1.4*

Annex 13: *confidential information has been removed P-SDS item 5.3;*

Annex 14: *confidential information has been removed P-SDS item 8.1.4*

Annex 15 *confidential information has been removed P-SDS item 5.3.1*