

Effects of Produced Water on the Marine Phytoplankton *Phaeodactylum tricornutum*

A study of the effects Produced Water, from the Petroleum Industry, can have on Marine Phytoplankton, and the possibilities of a simple yet highly modifiable experimental design

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Abstract

1 Abstract

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3 Produced Water (PW) is a complex mixture of many chemical compounds, ranging from 4 heavy metals and dispersed oil to Polycyclic Aromatic Hydrocarbons (PAHs), Alkylphenols 5 (APs), and even production chemicals. In the following study the effects of PW discharged 6 from the Norwegian Petroleum Industry was studied on the marine phytoplankton 7 Phaeodactylum tricornutum, a species of marine diatoms. The experimental design of this 8 study was focused on simplicity, and the ability to modify the methods for future studies was 9 discussed. The results from this study shows effects of PW as a mixture of all the compounds 10 mentioned above. The toxic effects, seen in the results as growth inhibition or effects on 11 different parameters of algal growth, was compared to effects seen in early life-stages of fish, 12 zooplankton and other microalgae species. The many different parameters measured in this experiment could all be an indication of growth, but as they all indicated growth by different 13 reactions or factors in algal growth, the results were hard to discuss and any direct 14 15 conclusions or correlations was difficult to justify. The most interesting results was found in a 16 delay in pH change, visible in the pH results from the exposure group with the highest PW 17 concentration, 10% PW. When this lack of pH increase, which was unexpected with algal 18 growth, occurred, while other tests showed growth in all cultures, a possibility of growth and 19 even photosynthesis without the use of CO2 was suggested. This result was in part correlated 20 with a study on volatile hydrocarbons and their effect on Lipid : Chlorophyll a ratio in algal 21 cells, although any definite conclusions was not justifiable based on this study alone. The 22 differences between the results from all tests show that the ability to test and consider many 23 factors and parameters are important when studying microalgae. Many earlier studies assume 24 that algal growth rates can be directly extracted from one parameter measuring growth, but 25 this study suggest heavy considerations of the actual chemical reactions behind results from a 26 growth experiment are required to properly understand what any result actually show. 27

Keywords: Produced Water, *Phaeodactylum tricornutum*, BTEX, PAHs, Alkylphenols,
Chlorophyll-a, Fluorescence, North Sea Petroleum Industry.

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32 Sammendrag – Norwegian/Norsk

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Produsert Vann (PW) er en kompleks blanding av mange kjemiske stoffer, blant annet 34 tungmetaller, løste oljepartikler, Polysykliske Aromatiske Hydrokarboner (PAHs), 35 Alkylfenoler (APs) i tillegg til kjemikalier brukt under produksjon. I dette studiet ble 36 37 effektene av PW fra den Norske Petroleumsindustrien studert på den marine fytoplanktonet 38 Phaeodactylum tricornutum (P. tricornutum), en marin diatomeer. Dette studiets 39 eksperimentelle design var fokusert på enkelhet og evnen til å modifisere metodene for 40 fremtidige studier ble diskutert. Resultatene fra dette studiet viser effektene av PW som en 41 blanding av alle stoffene nevnt over. De toksiske effektene, som vist i resultatene som 42 veksthemning eller effekter på forskjellige parametere av algevekst, ble sammenlignet med effekter vist på tidlige livsstadier hos fisk, zooplankton, og andre arter microalger. De mange 43 forskjellige parameterne målt i dette forsøket kunne alle indikere vekst, men siden alle 44 45 indikerer vekst basert på forskjellige reaksjoner eller faktorer i algevekst, var resultatene 46 vanskelige å diskutere og direkte konklusjoner eller korrelasjoner var vanskelige å 47 rettferdiggjøre. De mest interessante resultatene ble funnet i form av en forsinket pH 48 forandring, som kunne sees i pH resultatene fra kulturene eksponert for den høyeste 49 konsentrasjonen PW, 10% PW. Når den manglende pH økningen, som normalt er forventet 50 ved algevekst, viste seg, mens andre tester viste vekst i alle kulturene, kunne en mulighet for 51 vekst og kanskje også fotosyntese uten bruk av CO2 forslås. En delvis korrelasjon mellom 52 dette resultatet og en studie på flyktige hydrokarboner, og deres effekt på Lipid : Klorofyll a 53 forhold i algeceller ble diskutert, men noen definitiv konklusjon var ikke rettferdiggjort av 54 resultatene fra dette studiet alene. Forskjellene mellom alle testene i dette studiet viser at 55 evnen til å teste mange faktorer og parametere er viktig når man studerer mikroalger. Mange 56 tidligere studier antar at vekstraten til alger kan direkte måles fra en parameter som måler vekst, men dette studiet forslår at en må ha god forståelse rundt kjemien og betrakte alle de 57 58 kjemiske reaksjonene som står bak resultatene fra et vekstforsøk.

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63 Abbreviations

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65	AP	Alkyl phenol
66	AH	Aromatic Hydrocarbon
67	BTEX	Benzene, Toulene, Ethylbenzene, and Xylenes
68	⁰ C	Celsius
69	$\rm CO_2$	Carbon Dioxide
70	Chl-a	Chlorophyll-a
71	Chl. Fluor.	Chlorophyll Fluorescence
72	cm	Centimeter
73	DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
74	DEG	Diethylene Glycols
75	dH ₂ O	Distilled Water
76	FRI	Fluorescence Response Index
77	Ft	Fluorescence Yield (Actual Fluorescence)
78	F-QY	Fluorescence Quantum Yield
79	Fv/Fm	Potential Photosystem II efficiency
80	H_2O	Water
81	HCI	Hydrogen Cloride
82	HD-PE	High-Density Polyethylene
83	LD-PE	Low-Density Polyetnyelene
84 05	MEG	Niono etnylene giycols
03 06	NaOн	Nanometer
00 87		The Norwagian Oil Industry Association
0/	OCP	International Association of Oil and Cas Producers
80	ОЛ	Polycyclic Aromatic Hydrocarbon
90	PS Can	Photosynthetic Canacity
91	PW	Produced Water
92	mL	Milliliter
93	mm	Millimeter
94	TOC	Total Organic Carbon
95	ug	Microgram
96	um	Micrometer
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176 Chapter 1. Introduction

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178 1.1 Produced Water

179 Produced Water (PW) is a term used for any water used in production of a product. It is used 180 in the petroleum industry where it is a term for the water created within an oil well and the 181 water that follows the oil from drilling to the refinery (Neff, 2002). Legislation surrounding 182 PW is mainly focused on oil-content, but it is known that PW is a complex mixture of oil 183 droplets, other hydrocarbons, alkylphenols (APs), organic acids, and heavy metals. In addition 184 to all these chemical components, chemicals used during production, transport, and treatment 185 of the oil or the PW itself can be found in PW at varying levels (Neff, 2002). Using data from 186 the public report from Statoil's Åsgard oilfield it is shown that between 2002 and 2012 a 187 900 000 000 m³ of Produced Water was released from the combined production at the Åsgard 188 oilfield (Sekkesæter and Myrhaug, 2013). Noting that this report consist of data from 10 years 189 of production, other studies measure a total amount of PW discharged into the North Sea to be 190 more than 500 000 000 m³ per year (OLF 2011, OGP 2011). Although these numbers sound 191 high, it is discussed in both reports (OLF 2011 and OGP 2011) that even though the amount 192 of discharged PW increases, more and more PW is re-injected before shutdown of oil wells, 193 and the total oil content in PW is reduced in the later years. It is also worth noting that the 194 levels and content of PW discharged to the North Sea is not the worst compared to for 195 example North America, Africa or the Middle East (See OGP 2011 Figure 4.1c). Produced 196 Water from the North Sea Petroleum Industry is interesting when studying the total 197 environmental impact from the petroleum industry, because the PW is dispersed and spread to 198 fisheries in Norway, the UK, and Skagerrak. The effects of PW on fish have been studied on 199 fish from larvae stages to adult fish and effects have been seen in larvae at even low 200 concentrations, while adult fish experience a toxic effect in the highest concentrations closest 201 to the offshore facilities (Meier et al., 2010). In many reports it is also stated that the major 202 studies performed on toxic effects from PW discharge show lesser to no effects on fish on a 203 population level, and that the rapid dispersion caused by waves and water-flow around the 204 offshore facilities lead to non-toxic concentrations even at just >100m away from the source 205 of discharge. It is therefore also found in most studies that effects on community levels are 206 low or non-existent for fish and zooplankton throughout the North Sea. However, even though 207 the effects of PW have been studied in fish, only a few studies have tried looking for toxic 208 effects in the lowest stage of the food chain, the phytoplankton.

209 As mentioned above, PW is a complex mixture with many chemical components and

210 production chemicals, and many of these single components have shown both toxic and

211 helpful effects on the growth of algae. It is commonly known that release of wastewater, from

212 urban localities or other industries, can lead to algal blooms and have harmful effects on both

213 freshwater and marine environments.

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215 1.2 Toxicity testing: Growth Experiment

216 The toxicity tests performed on Produced Water samples in earlier studies have been 217 performed by micro assays, captured or placed individuals of fish, and most often by 218 preparing test solutions based on predicted or calculated levels of single components from 219 Produced Water. This study uses a basic growth experiment with different concentrations of 220 PW to measure different parameters of growth and assess what effects can be seen in a marine 221 phytoplankton. The goal of the experimental setup was to create a basis for an experiment, 222 which could be modified heavily, and be performed easy and cheap. The effects of PW, 223 visualized as growth inhibition could be studied, and the multiple parameters tested all 224 measured growth. The key however, was that since each parameter studied was based on 225 different reactions within the chemistry of microalgae, many different findings could be 226 suggestive of many different conclusions. The experimental design has its problems, and 227 lacks the precision of a well-funded multidisciplinary experiment, but its simplicity leads to 228 an ability to modify all parameters and test for the almost unlimited amount of effects that one 229 could expect a complex mixture like PW to have on a relatively sensitive algal culture. The 230 growth experiment itself can therefore be seen as a pilot study on growth of microalgae in 231 natural concentrations of PW. The concentrations used in this experiment was adopted from a 232 study on early life stages of fish, where the highest concentration was 10% and resembled a 233 radius very close to the offshore facility, while the lowest concentration of 0,01% resembled a 234 more general ecosystem-wide chronic effect of PW (Meier et al., 2010). The focus on 235 relevance to the natural environment was hindered a bit by the lack of funding and time, but 236 the improvements and further possible studies are discussed fully in Section 4.5.

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240 1.3 Aims

241 The aims of the study was focused around simplicity and based on the theory of a pilot

- 242 project. The first goal was to examine if the natural concentrations of PW, mentioned in
- section 1.2, could have any effect on microalgae. Earlier studies have shown effects on fish in
- 244 multiple stages, but the official reports from OLF 2011 and OGP 2011 suggest low to non-
- existent toxic effects, especially in the lowest parts of the food chain. This means that the first
- 246 goal of the study was to disprove this assumption that Produced Water does not affect the
- 247 lowest parts of the food chain. The second goal of the study was to review literature and
- earlier studies on Produced Water to examine if any correlations between the results found in
- the growth experiment on *P. tricornutum* and effects on larvae-stadium fish, zooplankton, or
- 250 other microalgae, could be found. Finally, it was important for me to look at the ability to
- create a cheap project, which was highly modifiable, and which could be used to study further
- effects of PW on microalgae, and possibly study how the findings of such studies could be
- 253 used in methods surrounding PW treatment or Bio-fuel production.
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285 Chapter 2. Materials & Methods

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287 2.1 Experimental design

288 The tanks was set up according to Table 1. Each exposure group consisted for 3 LD-PE 289 tanks, which was treated the exact same way throughout the experiment. The growth 290 experiment lasted for 7 days after inoculation. Each day from Day 1-7 pH, Turbidity, and all 291 the fluorescence tests were performed on a sample from all 15 tanks. The tests were not 292 performed randomly or blindly, but the test-order was chosen following the simple rule of 293 starting with the presumed lowest concentration. This is a technique used to reduce the chance 294 of higher concentration samples affecting lower concentration samples, if the washing steps 295 between each tests are not performed correctly. For the Chlorophyll a analyses, 1 tank was 296 chosen from each exposure group, and a sample from these tanks were used for filtration 297 throughout the experiment.

298 Table 1: Experimental Setup for growth of P. tricornutum in Produced Water

Tank number:	1-2-3	4-5-6	7-8-9	10-11-12	13-14-15
Produced Water (%):	10	1	0.1	0.01	0
Produced Water (ml):	222	20,2	2,002	0,2	0
F/2 Medium (ml):	2000	2000	2000	2000	2000
Volume PW+Medium:	2222	2020,2	2002,002	2000,2	2000
<i>P. tricornutum</i> culture					
(ml):	5	5	5	5	5
Volume Total:	2227	2025,2	2007,002	2005,2	2005
Light intensity (nm):	710	710	710	710	710
Temperature (°C)	5	5	5	5	5

299

300 **Optimization** of growth was assured by the use of a good medium and high light intensity.

301 The experimental setup was designed to ensure that the major limiting factors on the growth

302 of *P. tricornutum* would be the result of possible contaminants from the Produced Water. The

303 only limiting factor on growth-rate is self-shading with increased cell-counts, and together

304 with turbidity this is taken into account in the Discussion. Other than self-shading, the

305 medium and high light intensity is assumed to provide enough nutrients and energy for

306 maximum growth throughout the growth period.

307 **Overall**, the goals of this experimental design was to be able to establish a fast growth of *P*.

- 308 *tricornutum*, and examine the effects of Produced Water on this growth early. It was also a
- 309 goal for this experiment to be easy and cheap, so one person could perform it over a short
- time, and on a small budget.

311 2.2 Sampling and preparation

The Produced Water sample was collected at the oil refinery at Mongstad, Norway. On-site technicians, who were following a sampling-procedure provided by Me, performed the sampling. The sample was collected at an "entry-point" where the sample would most closely resemble the produced water released on an offshore facility. This means that the water was not stored for days/weeks/months, or treated before sampling. The sampling procedure was as follows:

318 A 5L High-density Polyethylene (HD-PE) tank was filled almost up to the rim of the tank, to

319 reduce oxygen-sample interaction. It was then shipped overnight in a sealed container with

320 cooling elements. This ensured that neither light nor heat would affect the sample during the

321 overnight shipping. This procedure was designed after consulting papers surrounding

322 multiple similar experiments (Thomas et al., 2003, Brendehaug et al., 1992, Dórea et al.,

323 2007).

In the laboratory, the sample was kept at 5 °C before utilization. The sample was carefully
mixed before use, by turning the tank upside down multiple times, but without excessive use
of force.

The medium was prepared using a standard medium recipe for the laboratory at the Trondhjem Biological Station, which was derived from a standard F/2 medium recipe (*See Appendix 1*). Seawater was collected from the Trondheim Fjord by underwater cable. The seawater was filtered, and sterilized by autoclaving overnight. It was cooled down to room temperature before the addition of the media stocks. The finished medium was stored at 5 °C before utilization, and kept sterile by covering the top of the tank (*See Appendix 1 for full recipe*).

Inoculation took place the same day as the Produced Water sample arrived at the laboratory.

The medium was distributed into the 15 LD-PE tanks, and the correct levels of Produced

336 Water was added to create a medium with the correct PW concentration (*See Table 1*).

338 After addition of PW, 5 ml of concentrated *P. tricornutum* culture was added to each tank.

- The tanks were set up so that equal light intensity hit each tank. This was made possible by
- 340 the use of sheets of paper to cover the lamps, and distribute the light more even throughout

341 the room where the growth experiment was performed (*See Appendix 3*).

342 The choice of HD-PE over Glass-, Amber-, or Teflon-tanks for transport and LF-PE for the

343 growth-experiment, was made after consulting literature, surrounding sampling of oil-waste

- 344 (Thomas et al., 2003, Brendehaug et.al. 1992). In relation to Diatom growth, Glass and
- 345 Amber-glass was deemed not fit due to its ability to react with the sample, and releasing

346 metals and silicates into the medium during the experiment. The release of silicates into the

347 sample could positively interact with the diatom growth rates. Teflon, although preferred by

348 some scientists and laboratories, was deemed not fit for this experiment due mainly to its

349 price, and to the fact that LD-PE and HD-PE has been proven not to have a huge impact,

350 especially regarding overnight transport, cooled sample during transport, and the short growth

351 time of the experiment (*See Appendix 2*).

352 Day 0 results were collected by measuring pH, Turbidity, and In Vivo fluorescence in small
353 samples from each exposure group before addition of *P. tricornutum*.

354

355 2.3 Produced Water chemical composition

356 Statoil ASA perform bi-weekly tests on the chemical composition of the Produced Water

357 where the PW sample was taken from. This data was supplied to me, and protected under a

358 non-disclosure agreement. However, the average of some components from 1 year of testing

359 was allowed to be published with this study, and is presented in *Figure 1*.

360 There is however, no information about how the chemical composition was determined, so no

361 further procedures or details regarding these tests are presented or discussed. However, the

362 results are taken into account when discussing possible toxicity in the Discussion. It is

363 assumed that the results from the chemical composition tests are reliable, and that the

364 described content of the Produced Water sample is sound.

365

367 2.4 Chlorophyll a-analysis

- 368 The Chlorophyll a-analysis was performed using a standardized method (Mackinney, 1941).
- 369 Each day, 1 tank from each exposure group (chosen at day 1, based on proximity to average
- 370 within the exposure group) was tested for Chlorophyll a-levels. At day 1, 100 mL from each
- tank was filtered using water pressure, and the filters were dry-frozen until the analyses could
- be performed. After day 1, as the concentration of cells increased, less water could be filtered
- before the filter filled up. The reduced amount of filtered water was taken into account in thecalculations.
- 375 Chlorophyll a was extracted from the filters by adding 5 mL 85% Acetone, leaving them over
- night, before re-filtrating the extract through a 0.45 µm syringe-filter. The filtrated extract was
- then measured for absorbance in a spectrophotometer. The spectrophotometer was used
- instead of a fluorimeter (which is normally used), as the samples had levels beyond the
- 379 fluorimeters range of detection. (Mackinney, 1941).
- 380 The amount of Chlorophyll a in each extracted sample was calculated using the following381 formula:
- 382 Formula 1 $\mu g chl a / L = ((Abs665 Abs750) E*1000)/(74.5*L*F)$
- 383 Where Abs665 and 750 is absorbance measured at 665 nm and 750nm in a
- 384 spectrophotometer. E equals the extraction volume in milliliter, while F was the filtered
- amount in Liter. L is the length-way of light in the cuvette, normally 1 cm. The data was
- finally calibrated against a 0-test, and presented in *Figure 2*.

387 2.6 pH-analysis

- 388 The pH-analysis was performed using a new, but standardized pH-meter. The pH-meter was
- 389 calibrated each day before testing, using a built-in multi-point calibration procedure with 3
- buffer solutions (pH 4, 7, 10). Each day, pH-levels were measured for all tanks in all exposure
- 391 groups, and the results are presented in *Figure 4*.

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395 2.5 Turbidity

- 396 Turbidity (Absorbance) was tested using a standard spectrophotometer at 750 nm light
- 397 intensity. The absorbance measured at 750 nm was calibrated against a 0-test consisting of
- 398 distilled H2O. Turbidity was tested for all tanks in all exposure groups every day. It was
- measured using a standard 50 mm quartz cuvette, which was washed well with water and
- 400 distilled water between each use. The results from the turbidity tests are presented in *Figure 3*.
- 401 2.7 Fluorescence analyses
- The fluorescence tests was performed using the AquaPen-C designed by Photon System
 Instruments. It is a handheld fluorimeter, where you can perform many tests using a 10 mm
 cuvette. It is equipped with a Blue LED emitter, which is optimal for algal cultures (Photon
 Systems Instruments, 2015). All the tests performed using the AquaPen was done by adding
 5ml of the sample to a quartz cuvette after dark adaptation, in a dimly lit room. Some of the
 tests are light sensitive, to a less or more extent, so depending on the tests and sample, dark
 adaptation times varied.
- 409 Instantaneous Chl. Fluorescence was measured by adding 5ml of sample (before dark
 410 adaptation) to a cuvette and running the Ft program on the AquaPen. This program runs for a
 411 few seconds, before the number stabilizes and the result is presented as fluorescence yield (Ft)
 412 or minimum (actual) fluorescence (Šlapakauskas and Ruzgas, 2005). The results from these
 413 tests are presented in *Figure 5*.
- Fluorescence Quantum Yield (F-QY) was measured by adding 5ml of sample (after dark
 adaptation) to a cuvette and running the QY program on the AquaPen. The program runs for a
 few seconds, before the number stabilizes and the result is presented as Fv/Fm, which is an
 estimation of potential Photosystem II efficiency (Kitajima and Butler, 1975). The results
 from these tests are presented in *Figure 6*..
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Photosynthetic Capacity is a measure derived from a study suggesting a relationship
between low Fluorescence Response Index (FRI) and diminished Photosynthetic Capacity
(Cullen and Renger, 1979). It is of special interest to this study because other studies have
suggested a good correlation between DCMU-induced fluorescence and photosynthesis levels
(Samuelsson and Öquist, 1977). These relationships are more fully discussed regarding the

425 pH results in *Chapter 3.2: Algal growth*, and in the Discussion).

The Photosynthetic Capacity is derived from the FRI, which is calculated as a ratio between

- 427 minimum fluorescence and a DCMU-induced increase in fluorescence. It is performed using
- the same method as for Instant. Chl. Fluorescence above, but with the addition of another
- 429 similar test where you add a few drops of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) to
- 430 the sample before testing. DCMU is a known photosynthesis inhibitor, and interrupts the
- 431 photosynthetic electron transport chain. The addition of DCMU ultimately provides a visible
- 432 increase in fluorescence, which is a picture of the absorbed light energy normally used for
- 433 photosynthesis.
- 434 The ratio between Minimum or Actual fluorescence, which is presented above, and the
- 435 DCMU-induced fluorescence can be presented as a Fluorescence Response Index, which can
- 436 be related to Photosynthetic Capacity, and hence also Photosynthesis. The FRI is presented in
- 437 *Figure 7*, and represents the DCMU-induction ratio.
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440 2.8 Presentation of results

441 The results from all tests are presented as graphs, where the trend lines represent the average442 between the triplicates of each exposure group. The graphs from all tests except Chlorophyll a

443 also include error bars, which are determined from the standard deviation between the

triplicates. All the results have been corrected against Day 0 results and versus 0-controls likedH₂O.

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454 Chapter 3. Results

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456 3.1 Chemical composition of Produced Water sample

The Produced Water sample used for this project is tested for chemical composition 2 or 3 times a month by an independent laboratory on order from Statoil ASA. The data for 1 year of these tests were supplied, and have been presented in *Figure 1*. I did not perform the tests, and the details of the results or analyses cannot be disclosed in this report (*See Materials & Methods*). However, the averages from 1 year of test results show two predominant chemical components, which are of interest to this project, Methanol (CH₃OH) and Total Organic Carbon (TOC).

464 Methanol content is important for growth of diatoms, due to its inhibitory and stimulatory 465 effects on biomass production. Dewes et al. 2003 suggests that the effects of methanol on 466 growth is dependent on concentration and exposure time (Dewez et al., 2003). Methanol can 467 act as a very effect solvent for organic molecules including Chl-a, which means that a high 468 methanol concentration can affect buildup of Chl-a and other vital organic molecules in the 469 cells (Dewez et al., 2003). From the results of the chemical analyses, we note that the average 470 concentration of Methanol in this Produced Water source is 0.42%, with a 0.89% possible 471 deviation. This means that this source of PW can have a maximum concentration of ca 1.3%.

Total Organic Carbon (TOC) content is also important for growth of diatoms, as it can
affect uptake of CO2 and Dissolved Inorganic Carbon, and directly affect Photosynthesis, and
hence also growth (Goldman et al., 1971). The TOC concentration in the Produced Water
source is on average 0.68%, with a standard deviation of 0.32% giving a possible max of 1%.

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483 Figure 1: Chemical Composition of Produced Water Sample from Mongstad, Norway.

499 3.2 Algal Growth (Chlorophyll a, Turbidity and pH)

- 500 Many of the tests performed in this project can indicate Algal Growth. I have chosen the
- 501 results from the Chlorophyll a (Chl-a), Turbidity and pH tests to describe the bigger picture of
- 502 the results which is further discussed in the Discussion.
- 503 **Chlorophyll a** (**Chl-a**) content in µg/ml from each exposure group is presented in *Figure 2*.
- 504 Chl-a can be used as an indication of algal growth, and is often used to determine the "health"
- 505 of a body of water, and predict or monitor deadly algal blooms. However, studies also suggest
- 506 that Chl-a is not a good indicator for biomass (Ramaraj et al., 2013). I therefore use the Chl-a
- 507 concentrations presented in *Figure 2*, to assess if the growth presented in *Figure 3* and *Figure*
- 508 *4*, is an actual representation of *P. tricornutum* growth, and not contamination of organic or
- 509 inorganic matter, or an indication of other chemical reactions than photosynthesis.
- 510

511 Figure 2: Chlorophyll-a results from growth experiment on *P. tricornutum* in Produced Water



Chapter 3. Results

515 In Figure 2, we see that the Chl-a content follows a growth-trend, which indicates a slow

516 exponential growth with a carrying capacity indicated around Day 5 at concentrations

517 between 900-1200 µg/ml.

518 The highest Chl-a concentrations are present at Day 5, in exposure groups 0.01%-PW and

519 Control. This maximum is similar to a traditional "overshoot" of carrying capacity (Whittaker

and Likens, 1973). It is worth noting that all exposure groups hits the same carrying capacity

521 at around 1050-1100 μ g/ml at the end of the experiment, and that the level at days 3 and 4

522 indicates a slightly lower Chl-a content in exposure group 10%-PW. Chl-a content of each

523 exposure group is also an indication of the algal health, which is presented in *Section 3.3*, and

524 more deeply discussed in the *Discussion*.

525 **Turbidity** in each exposure group is presented in *Figure 3*, as Turbidity (absorption) at a

526 wavelength of 750 nm. Turbidity indicates algal growth by giving a representation of light-

527 absorbing matter, or particle concentration in a sample. The results in Figure 3 was presented

528 after correcting each result against a standard (distilled water), and Day 0 levels of turbidity

529 (0.05 in 10%-PW, and 0.002 in 0.01% PW) to account for the turbidity in the added Produced

530 Water.



531 Figure 3: Turbidity results from growth experiment on *P. tricornutum* in Produced Water

Chapter 3. Results

533 The results from the turbidity tests show a similar trend to that of Chl-a, and the visible

- 534 correlation between these results suggest that growth of *P. tricornutum* in each exposure
- 535 group can be indicated by either of the results.

536 However, the 10%-PW exposure group (indicated in figure 3 by black-dots/solid-line),

- 537 continues to increase after the final day of growth. The other 4 exposure groups show a
- 538 carrying capacity, and stabilization of turbidity/growth at day 5 around a Turbidity of 1.2.
- 539 This can be an indication that particles, which are not representing cells with Chl-a, is present
- 540 and growing in the 10%-PW after Day 6.
- 541 **pH-levels** in each exposure group throughout the experiment is presented in *Figure 4*. The
- 542 results were corrected with pH-levels from 10%-PW/media and 0%-PW/Media, to account for

543 potential pH-increases by higher PW concentrations. The pH-levels presented in figure 4 is

among the most exciting results from this experiment. The pH results together with

545 Photosystem II (PSII) efficiency suggest that there are multiple underlying reactions within

the culture, both performed by the algae and the media+PW combination, which are affectingeach other.

548 pH is widely used as an indicator for Algal growth in Aquatic and Marine environments

549 because, pH in a culture changes with phytoplankton uptake of carbon for photosynthesis

550 (Axelsson 1988, Hofslagare et al. 1985). This means that the pH results in this experiment can

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be discussed in relation to both diatom growth presented in this section, and photosynthetic

552 activity/health presented in *Section 3.3*.

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563 Figure 4: pH results from growth experiment on *P. tricornutum* in Produced Water



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566 From Figure 4, we can see that Exposure groups 1%,0.1%,0.01%, and 0%-PW show a similar 567 trend of exponential growth, and stabilizes at Day 4 around pH 10.2-10.3. This is not in 568 correlation with the trends presented in figure 2 and 3, which indicated a stabilization 569 (carrying capacity) around Day 5. Even more interesting is the fact that exposure group 10%-570 PW shows a slow almost stabilizing trend until Day 5, before growing linearly upwards until 571 the end of the experiment, where it ends around pH 9.5-9.6. at Day 7. An explanation of what 572 is shown in Figure 4 is the addition of H-ions from the PW. Together with the removal of H-573 ions caused by the uptake of CO2 reach steady state around Day 2, and this steady state is 574 probably changed around Day 5 as the amount of photosynthesis remove more H-ions than 575 what was added with the PW at Day 0.

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Chapter 3. Results

580	A short summary of this section is that the results from the Chl-a and Turbidity tests indicate
581	a slow semi-exponential growth from Day 1-5 with a stabilization at Day 5. The pH results
582	indicate that 4 of the exposure groups (from 0-1%-PW) growth exponentially to Day 4 before
583	stabilizing (if pH changes are assumed to be an indicator of growth), while the highest
584	exposure group (10%-PW) does not show any high increase in pH before Day 5 where it
585	linearly grows until the end of the experiment. This growth could be indicated in the turbidity
586	tests where the 10%-PW exposure group continues to increase after day 6. Finally, it is worth
587	noting that the 0.01%-PW exposure group along with the control-group seem to "overshoot"
588	the carrying capacity based on the Chl-a test at Day 5 but ultimately stabilizes along with the
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Chapter 3. Results

606 3.3 Fluorescence (Instant. Chl-Fluorescence, Fluorescence-Quantum yield,

607 Photosynthetic Capacity)

Since the pH results show a different trend compared to the Chl-a and Turbidity tests, the fluorescence tests presented in this section are meant to give further indication of what happens, in relation to photochemistry, in the exposure groups. Based on Photosynthetic activity, during the growth period. The term "Photosynthetic Activity" is based, mostly on assumptions and iteration from references, more than actual calculations and definitions. This concept coincides with my choice to focus more on the big picture in the results, and less on calculations and statistics.

615 Instantaneous Chlorophyll Fluorescence was measured using the PSI-AquaPen-C (See 616 Materials and Methods), and indicates steady-state yield of fluorescence in light (Maxwell 617 and Johnson, 2000). The results are presented in Figure 5, and was corrected against a 618 standard (Distilled Water). Figure 5 shows a slow trend from near-zero to 110000-130000 at 619 the end of the experiment, for the four lowest exposure groups (1%-0%-PW). Figure 5 also shows a slower increase from near-zero to ca. 55000 for the 10%-PW exposure group, which 620 621 may indicate a higher rate of photochemistry, or even a lower cell count. The Instant. Chl. 622 Fluorescence was used to for calculations in the Photosynthetic Capacity. The Instant Chl. 623 Fluorescence also shows that there is photosynthetic activity in the 10%-PW exposure group, 624 which means that the pH results showing a H-ion steady state must be the reason for the lack 625 of pH increase, and not growth of non-photosynthetic cells. This finding supports the theory 626 that there are growing cells which are producing Chlorophyll a, and performing 627 photosynthesis, but that the H-ion steady state hinders the pH increase normally seen with 628 photosynthetic activity. 629

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- 636 Figure 5: Instantaneous Chlorophyll Fluorescence (measured as Intensity) from growth experiment on *P*.
- 637 *tricornutum* in Produced Water



639 Fluorescence-Quantum Yield (F-QY) was also measured using the QY-program of the PSI-640 AquaPen-C. F-QY is an indication of Photosystem II (PSII) efficiency, and can indicate; 641 levels of damage on PSII or levels of Quenching by light-damage or chemical inhibition 642 (Maxwell and Johnson, 2000). The results are presented in Figure 6, and was corrected 643 against a standard (Distilled Water). Figure 6 shows that F-QY increases strongly from day 1 644 to day 2, and then increase slower towards day 3 and 4, with a maximum of around 6.5-7. At 645 day 5, F-OY has decreased to around 0.47-0.55 for the lowest exposure groups (1%-0%-PW), 646 and only decreased to about 6.2 for the 10%-PW exposure group. All groups decrease further 647 until the end of the experiment, but while the four lowest exposure groups decrease all the 648 way to 3.1-3.7, the 10%-PW exposure group only decrease to 0.6. All exposure groups seem 649 to be stabilizing at these final levels, although further testing would have given a clearer 650 picture of this. The four lowest exposure groups decline from day 4 to day 7, to a level lower 651 than Day 1. This may indicate that stress (possibly related to high cell-counts/competition), or 652 damage to PSII can have affected these exposure groups. However, F-QY can also, during 653 laboratory experiments, give a measure of linear electron transport, and indicate overall

box photosynthesis (Maxwell and Johnson, 2000).

- 655 F-QY can be related to carbon fixation during photosynthesis in PSII. From this we can
- 656 suggest that the slower decline in F-QY seen in the 10%-PW exposure group can be
- 657 correlated with the pH-increase happening from Day 5 (*See Section 3.2*), although this is an
- 658 interesting suggestion, it is only a speculation which will be discussed fully in the Discussion
- 659 Figure 6: Photosystem II Efficiency presented as Fluorescence Quantum Yield (F-QY) from growth experiment
- 660 on *P. tricornutum* in Produced Water



670 Photosynthetic Capacity is another parameter, which can indicate the health of a

- 671 phytoplankton cell. In this experiment, Photosynthetic Capacity is iterated from a
- 672 Fluorescence Response Index, which is based on DCMU-Induced increase in fluorescence.
- 673 The results were based on the Instant. Chl. Fluorescence, before and after addition of DCMU
- to the sample. The results from these analyses are presented in *Figure 7*.
- 675 DCMU-Induced increase in fluorescence can indicate the levels of cells, which are currently
- 676 actively performing photosynthesis (Cullen and Renger, 1979). Cullen and Renger suggests
- 677 that a DCMU-induced increase in fluorescence can be linked to the health of a phytoplankton
- 678 culture, and indicate the level of non-photosynthetic material or "dead cells" in a culture.
- 679 From *Figure 7*, we found that all exposure groups follow almost the same trend. They
- 680 increase rapidly from day 1 to 2 up to a level of 43-48, and then stabilizes (more or less in the
- same fashion) until day 4/5, before the all decrease until day 6. The results show many
- 682 scattering results, high deviations and errors, and no real dose-dependency.
- 683 However, Maxwell and Johnson, as well as Cullen and Renger mention that these kind of tests
- have many variables, and require a near perfect experiment-setup and performance (See
- 685 *Chapter 2*) (Maxwell and Johnson 2000, Cullen and Renger 1979). This is a reasonable
- 686 explanation for the high standard deviations. However, it is still possible to see a good trend
- 687 where all exposure groups follow a similar trend, except the 10%-PW which plateau at a
- higher level around day 3 and 4, and ends the experiment at a significant higher level than the
- rest of the exposure group. This result, together with the F-QY, suggest that the
- 690 Photosynthetic activity in the 10%-PW exposure group become better towards the end of the
- experiment, compared to the rest of exposure groups, which seem to fall off in both capacityand quantum yield.
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- 700 Figure 7: Photosynthetic Capacity presented as DCMU Induced Fluorescence increases from growth experiment
- 701 on *P. tricornutum* in Produced Water



Chapter 3. Results

713 3.4 Visual and Physical observations

- 714 I want to point out, for further reference, multiple visible and sensible changes throughout the
- 715 experiment. The main visible change was a change of color in all 15 tanks. The change of
- 716 color was distinct from clear to brownish water (*See Appendix 3*). This change was parallel to
- an increased amount of downfall, presumably from organic matter. In addition to the visible
- 718 changes, there was a distinct "gasoline"-smell in the 10%-PW tanks.
- This smell, although project to bias, seemed to reduce in power through the growth period.
- Although this observation is strictly based on my previous visual experiences, it is assumed
- that the change of color throughout the experiment could be a good indication of growth in all
- 15 tanks. The slight lighter color visible in tanks 1-2-3 (Appendix 3) could also be an
- 723 indication of reduced growth in these tanks, although this is also speculation.
- The reduction of "gasoline"-smell present in the highest 10%-PW exposure group could be an
- indication of volatile BTEX components escaping through the seal, as the plastic seal on a
- tank is not airtight. The change in smell could also be an effect of *P. tricornutum* absorbing,
- chemically altering, or removing BTEX compounds and possibly oil-droplets, from the
- media, hence reducing the amount of gases given off during sampling. It is again worth noting
- that these results are based on observations and not quantifiable. However, they are discussed
- further in the Discussion.
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759 Chapter 4. Discussion

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761 4.1 Toxicity of Produced Water

Toxicity of Produced Water was assessed, by comparing the dose-response in the results,
with the chemical composition of the Produced Water sample. From the results, presented in *Chapter 3. Results*, no visible dose-response between all 5 concentrations was found in any of
the tests. However, the 10%-PW exposure group deviates from the rest of the exposure groups
in all tests (to some degree).

767 From earlier studies (presented in the Introduction) it is suggested that many of the chemicals 768 used in the petroleum industry, and other components found in PW, can have toxic effects in 769 high concentrations on higher marine organisms, especially mussels and fish (Meier et al., 770 2010, Brooks et al., 2011). However, both Meier et al. 2011 and Brooks et al. 2011 suggest 771 that the highest effect of PW discharge is detected in organisms located close to the point of 772 discharge, which in relation to this study represent the 10%-PW exposure group. Previous 773 studies focus mainly on effects of Alkylphenols (APs), Heavy Metals, and Aromatic 774 Hydrocarbons (AHs) including BTEX compounds (Benzene, Toluene, Ethylbenzene and 775 Xylenes). Levels of Heavy Metals, BTEX cmpounds, and APs can be extracted from Statoil 776 Reports including composition of Produced Water effluents from North Sea petroleum 777 activities (See Introduction).

778 Alkylphenols have shown effects on the endocrine and reproductive systems in fish and other 779 vertebrates (Meier et al., 2010, Brooks et al., 2011). These effects are based on the chemistry 780 of APs, and for example their ability to mimic effects of sex hormones and inhibit or induce 781 endocrine- or reproductive processes. In phytoplankton a study on 4-Nonylphenol, a 782 compound in the AP family, showed different effects on phytoplankton cultures, based mainly 783 on species and concentration (Hense et al., 2003). A similar but longer study on the 784 ecotoxicology of 4-nonylphenol mixtures included a diatom (Melosira Sp.). However, in the 785 study, a species of fathead minnows was also studied, and the only publications from this 786 study are based on the effects on the fish. The effects on the diatom remains unpublished, but 787 taken into account in this study. This support the hypotheses that organisms in higher trophic 788 levels remain the focus of studies and publications.

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The study does mention one result from the diatom part of the experiment. It states that the

results suggest that 4-nonylphenol alters the Lipid : Chlorophyll a ratio in the phytoplanktoncells (Schoenfuss et al., 2004-2006).

793 The Lipid : Chlorophyll a ratio is an indicator of stress, where both the production of 794 Chlorophyll a and storage/depletion of lipids can indicate different types of stress-coping 795 mechanisms. In relation to the current study, the Lipid : Chlorophyll a ratio is interesting to 796 discuss, because the pH results (Figure 4) indicate low carbon uptake (which can be an 797 indication of lipid storage, growth rate etc.), while the chlorophyll a (Figure 2) and Instant 798 Chl. Fluor. (*Figure 5*) results indicate a lower growth rate in the 10%-PW exposure group. It 799 is also interesting to relate the knowledge of APs effects on Lipid : Chlorophyll a ratios to the 800 turbidity results (Figure 3), because turbidity can be an indication of increased particulate 801 lipids, increased cell size (increased lipid-storage), or light permeability of cell membranes, 802 all of which can be related to the lipid : chlorophyll a ratio. Sadly, the publications from the 803 mentioned study only focus on the results from the freshwater fish species. The correlations 804 mentioned in this section regarding the diatom part of the study, was derived from speculation 805 and should therefore be considered as assumptions, more than conclusions or suggestions.

806 Heavy Metals have shown effects on growth, duration of log phase, and motility in 807 phytoplankton. A study focusing on a mixture of heavy metals, also found results suggesting 808 how different concentration of multiple heavy metals in mixtures can lead to both synergistic 809 and antagonistic growth inhibitory effects on phytoplankton, including P. tricornutum 810 (Thomas et al., 1980). The effects of heavy metals on the more delicate processes inside of 811 phytoplankton cells are unknown or poorly studied, which makes relating heavy metal content 812 to results from the specialized Fluorescence tests (F-QY and PS Capacity) difficult to justify. 813 However, as heavy metals, both singular and in mixtures, have shown toxic effects on 814 phytoplankton it is interesting to relate the study from Thomas et al. 1980 to the lower growth 815 rates shown in the Chlorophyll a and Instant. Chl. Fluor. tests. Another study focusing on 816 precipitation of heavy metals, suggest that heavy metal concentrations alone does not 817 correlate with toxicity (growth inhibition), but this study does mention heavy metals 818 aggregate to larger particles (Azetsu-Scott et al., 2006). Although this study by Azetsu Scott 819 et al. 2006 suggest that heavy metals alone exempt no toxicity towards phytoplankton, and 820 hence works against the hypotheses suggested by Thomas et al. 1980 it does state a need for 821 more precise measurements.

The results from Azetsu-Scott et al. 2006 do suggest another hypotheses; that the turbidity results, which suggest a similar growth rate in all exposure groups, may be affected by increased particulate matter. Since the 10%-PW exposure group could have more particulate matter in the form of oil droplets or other agglomerates, the results suggest that the heavy metal content in the 10%-PW exposure group could increase turbidity by heavy metals aggregating to these larger particles, and affecting the turbidity results.

828 BTEX compounds found in effluents related to the petroleum industry have shown both 829 inhibitory and inducing effects on phytoplankton growth. A study focusing on the effects of 830 BTEX mixtures, and especially mixtures containing volatile hydrocarbons, showed the 831 different effects on 4 species of microalgae (Dunstan et al., 1975). The study performed by 832 Dunstan et al. 1975 show that a diatom (Skeletonema costatum) showed no growth 833 enhancement in any mixture or concentration, but showed an inhibitory effect in low 834 concentrations of the mixture containing volatiles. As the current study was performed using a screw-capped plastic bottle, and not plastic-capped glass bottle as the Dunstan et al. 1975 835 836 study, a suggestion might be made towards the pH results, showing a delayed growth in the 837 10%-PW exposure group, being a result of volatile hydrocarbons leaving the culture through 838 gaps in the plastic screw-cap cover. As the volatile hydrocarbons leave the culture, the 839 inhibitory effect of low-concentrations shown by Dunstan et al. 1975 might be reduced. 840 However, the BTEX compound, which showed the biggest influence on the diatom from 841 Dunstan et al. 1975, was Xylene, which we from the Åsgard 2012 Statoil report know to be 842 one of the lesser BTEX compounds released. The results from this study on the BTEX 843 compounds effects on diatoms support the hypotheses that growth is inhibited in 10%-PW, 844 but to a lesser extent that APs and Heavy Metals. The BTEX levels in produced water can 845 also be related to particle matter, Total Organic Carbon from the Chemical Composition tests, 846 and turbidity, but any direct toxicity from BTEX is not assumed present in this study. As 847 mentioned before, the results part of this study show that the pH results deviate the most from 848 the other tests. The 10%-PW exposure group show a delayed change until day 5 (Figure 4), 849 which should suggest a delayed growth. The Instant Chlorophyll Fluorescence results suggest 850 a slower growth rate (*Figure 5*), and the Chlorophyll a results show a similar, but less distinct, 851 effect around day 4 (Figure 2). This comparison support the theory that P. tricornutum 852 growth is slower in the 10%-PW exposure group, which would be an indication of the toxicity 853 of produced water suggested by the compounds above.

854 The results which indicate similar growth rate, and in some instances higher growth rate, in 855 the 10%-PW exposure group consist of; F-QY (Figure 6), Photosynthetic Capacity (Figure 856 7), and Turbidity (Figure 3). However, the F-QY and Photosynthetic Capacity results are 857 based on ratios between two data points, and not direct quantifiable numbers. This means that 858 these results only give an indirect indication of actual growth, and instead indicate 859 photosynthesis efficiency (or algal health). The turbidity results, although commonly used to 860 assess algal growth, can also be highly influenced by cell-structure, cell-size, contaminants 861 (for example oil-droplets dispersed in water), and dead organic matter, a theory that is 862 supported by the toxicity assessments above. This means that although the turbidity results 863 support a similar growth rate in all exposure groups, it is reasonable to assume that the results are affected by many confounding factors. 864

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866 **In summary**, the results, although dependent on the assumptions made above, suggest a 867 slower growth rate in the highest concentration 10%, but as the 1%-PW exposure group show 868 no visible effects no dose response between 0% to 1% was found. It is therefore assumed that 869 the produced water tested in this experiment have toxic effects on the growth of P. 870 tricornutum in the highest environmentally relevant concentration (10%-PW). This suggestion 871 is mainly based on the Instantaneous Chlorophyll Fluorescence, and pH results. It is however 872 interesting to note that although the pH of the 10%-PW exposure group is negative and almost 873 stagnant between day 2 and 4, the rest of the results suggest slow but visible growth. This 874 suggestion that growth occur in all exposure groups, while pH stays stagnant in the 10%-PW 875 exposure group, is supported by physical/visual observations made during the experiment 876 (See Section 3.4). This suggest that either; Growth occur in the 10%-PW exposure group 877 without the use of CO2 between day 2 and 4, or that compounds from the Produced Water act 878 as a pH buffer negating the expected pH changes normally observed with algal growth at a 879 concentration level of 10%.

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4.2 Produced Water Impacts on Marine Phytoplankton

- 886 Produced Water impacts on Marine Phytoplankton was brought up in the early hypotheses of
- this study. The question was if and how, the results from this experiment, and from earlier and
- future studies, could be related to the actual impacts produced water have on marine
- 889 phytoplankton in the natural environment. In Section 4.1, the toxicity of produced water
- 890 suggested by the results from this experiment is discussed. In this section however, the
- discussion centers on how produced water is released, and react, in the environment.
- Finally, the discussion focus on determining how the marine phytoplankton *P. tricornutum*can be related to communities of phytoplankton in the actual environment.

894 **Release of Produced Water** occur, as discussed in the introduction before, during, and after 895 the production part of an industry. The Produced Water, which this study focus on, is from the 896 Petroleum Industry in the North Sea. For the results in this study to be relatable to the natural 897 environment, it is necessary to discuss how the exact sample of PW used in this experiment 898 relate to the PW actually released into the ocean. The PW, which was sampled at Mongstad, 899 was collected at a point of entry, where (according to sources in Statoil ASA) the produced 900 water would be the best representation of the water discharged on an offshore facility. 901 Knowing this, it is still a few important points to consider when relating the results to the 902 actual environment. First, is the time the produced water sample used in the oil-pipelines, 903 where it endured different temperatures and interaction with metals and sedimentation from 904 the pipes themselves. The time spent in the pipeline is unknown, but it is assumed that both 905 interaction with oil, oxygen, and perhaps seawater, is natural in those conditions. The PW that 906 is released into the ocean from and offshore facility will, in most cases, undergo a treatment 907 process before discharge. This process focus on removing the biggest oil-particles, as this is 908 where the legislation is focused (Durell et al., 2006). The PW used in this experiment could 909 therefore be assumed to contain more oil-droplets, which has diffused into the water from 910 interaction with the oil in the pipelines, and has not been removed through a treatment facility. 911 The PW used in this experiment will also have had a longer interaction with oxygen (if 912 present in pipes), and some reactions could have changed the composition and chemistry of 913 the Produced Water. However, it is still assumed (based on the statements from Statoil 914 employees), that the produced water used in this experiment is relatable to the water 915 discharged offshore, as long as the differences mentioned above is taken into consideration.

916 **Phaeodactylum tricornutum** was chosen for this experiment, because of its reliability, its 917 ability to growth fast and in many different media/mixtures. The fact that P. tricornutum also 918 have been studied thoroughly was important for this study. As mentioned in Section 4.1, there 919 was a study by Dunstan et al. 1975, which studied effects of BTEX components of petroleum 920 origin on 4 different microalgae. The results from this experiment shows that there is a 921 species difference in toxicity of PW (Dunstan et al., 1975). This species difference was 922 suggested based on toxicity of Alkylphenols (Hense et al., 2003). It is therefore important to 923 examine how *P. tricornutum* relate to a community of phytoplankton, and discuss how the 924 results from this experiment can be an indication of possible effects PW has on the marine 925 environment. This study suggest that PW as a mixture has a small effect on algal growth, if 926 the growth is assessed by Chlorophyll a levels and Instant Chlorophyll Fluorescence. This 927 study also suggest that CO2 levels are not changing in the highest concentration exposure 928 group until later in the experiment based on the pH results. As both Instant Chlorophyll 929 Fluorescence and pH are results derived from chemical reactions related to photosynthesis 930 within the cells of the phytoplankton it is possible to suggest that PW discharged from an 931 offshore oil-facility will have an effect on the P. tricornutum community located close to the 932 point of discharge. However, since the results from this experiment is based on PW as a total 933 mixture, while earlier studies focus on different components of PW it is hard to relate the 934 results seen in this experiment to the results of the other studies. It is also hard to determine 935 how other species would react to the PW used in this study. The question related to Produced 936 Water impacts on a natural phytoplankton community is therefore based on the theory behind 937 a mixture of chemicals and pollutants, and the shown variance in composition and chemistry 938 of Produced Water. The suggestion from this study as an experiment, and a literature review, 939 is therefore that Produced Water as a mixture of many components can provide both 940 important nutrients and be a source of toxic chemicals to marine phytoplankton. The results 941 from many of the tests in this study can therefore be of importance when discussing the 942 legislation on Produced Water effluents, not only from the petroleum industry, but when 943 discussing any discharge of water used in production.

944

946 4.3 Presentation and Statistics

947 Earlier in the study, it has been mentioned that the lack of statistics and the simplicity of the 948 results was important to me. The results part of the study starts by showing the chemical 949 composition of Produced Water throughout a year of testing. The discussion sections above 950 also focus heavily on the lacks of this study, and how it is hard to relate results from this study 951 and previous study to the natural environment, and actual effects of PW on phytoplankton 952 communities. The reason statistics are used in scientific experiments is to be able to visualize 953 data, and to be able to find hidden correlations between different results and different tests. In 954 this study, each single test is its own result. Each graph presented in Chapter 3 can be a 955 picture of growth, and in many studies only one of these tests are used to determine growth. It 956 is therefore not important to this study that each graph and each data point be statistically 957 analyzed against each other. It is more important that the big pictures each graph show is 958 discussed in related to what each test actually mean. As an example from this study, it is not 959 the growth rates between days 1 and 2 that is important. It is not how the pH on day 4 relate 960 to the Chlorophyll a levels on day 6. The study is not looking for correlations between each 961 test, but is trying to show a simple graph, and then relating what this graph actually show to 962 results from previous studies.

963 Another important part of statistics in scientific studies is the ability to reproduce the results, 964 and for other scientists to be able to relate their data to the results from this experiment. 965 However, as mentioned before the Produced Water used in this experiment was the PW 966 arriving at Mongstad at the day of sampling. If the sampling was delayed 2 weeks, it is 967 possible that the results from some of the tests in this experiment would be different. 968 Questions that may rise when discussing the variability of Produced Water discharge can be; 969 how any experiment using produced water can be relatable to the actual natural environment? 970 Alternatively, how can anyone working with produced water or marine phytoplankton can use 971 the findings of this study in their discussion? The simple answer is that I would not 972 recommend any use of any data points or results from this study directly in discussion of other 973 studies. However, if one use other sources from similar studies, and have a wide knowledge 974 of the differences between those studies, the results from this study, especially regarding 975 photosynthetic activity and pH, can be of great use to future studies surrounding PW or any 976 industrial effluent, in regards to algae.

977 The results from this study also show the importance of focusing on the chemical reactions

- leading up to a result in a test, and not only looking at the data point from the test itself and
- 979 especially not after the data points have been subjected to many different statistical programs.
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981 4.4 Further Studies

982 Many improvements can be made to a study like this. With more funding and more time, it 983 would be possible to look at many parameters of phytoplankton growth, like CO2 uptake, cell 984 count, cell size, and perhaps the uptake of oil- or heavy metal-particles in the phytoplankton 985 cell. This study can in sort, be seen as a pilot study on phytoplankton and produced water. The 986 results of this study give an indication of possible effects, but many factors must be taken into 987 account, and more parameters could be studied. The results also suggest the possibility of 988 Produced Water influencing reactions within the cells of the diatoms, especially related to 989 photosynthesis. Possible future studies based on the results of this experiment could be:

Checking for bacterial growth within the culture. Even though actions were taken to try to
keep the experiment from being contaminated from bacteria, it is possible that there would be
bacterial growth throughout the experiment, which could affect the results.

993 Test of PW effects on a phytoplankton community. As mentioned earlier in the discussion, the
994 environmental relevancy of this experiment is lacking due to the choice of only 1
995 phytoplankton species. If one were to examine the effects on a community scale, it could be
996 possible to understand more deeply how PW affect the natural environment of marine
997 phytoplankton.

998 Perform a similar experiment in a larger scale to examine the results, which suggest that the

10%-PW exposure group seem to be doing better than the others towards the end of the

1000 experiment. With the addition of PW, a lot of possible nutrients and toxicants were added, and

1001 as shown by the experiment in this study, a longer study on a bigger scale would give more

1002 results that are more detailed and possibly show more trends that are interesting after Day 7.

1003 The discussions brought up in this study can also be important for further discussions

1004 surrounding the increasing use of statistics, and the importance of discussing all factors within

1005 a study as a whole, from planning phase to conclusions.

1006

1007 Chapter 5. Conclusion

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1009 The findings of this study on Produced Water effects on marine phytoplankton was presented 1010 in *Chapter 3*, and discussed in *Chapter 4*. The goals of the study, have been examined 1011 throughout both the experiment and the literature review. The natural concentrations adopted 1012 from Meier et al. 2011 did not show any dose-response, other than a few tests giving very 1013 interesting results for the highest concentration 10%-PW. The goal was to examine if these 1014 natural concentrations would have any effect on *P. tricornutum*. It is possible to say, based on 1015 the literature review and comparison to the results from this study, that in high concentrations 1016 of PW the natural community of marine phytoplankton could be affected. The highest 1017 concentrations do however represent a low environmentally relevant radius around an 1018 offshore facility. The pH results differ greatly from the rest of the tests, and together with the 1019 Photosystem II Efficiency (PSII) and Chl-a data, a suggestion can be made that P. 1020 tricornutum is growing from Day 1, but the addition of 10%-PW creates a H-ion steady state 1021 which is causing the pH to stay low, while growth continues. At Day 4, the pH rises, and the 1022 PSII efficiency in the 10%-PW exposure group does not fall with the other groups to a level 1023 lower than Day 1. This could support the theory that the H-ion steady state is holding off the 1024 expected pH increase. The results of the Instant Chl. Fluorescence and F-OY together with 1025 this pH trend, could suggest that the 10%-PW exposure group is actually doing better than the 1026 other groups, towards the end of the experiment. This is a theory, which should be studied 1027 further, and could have importance related to PW treatment and Bio-Fuel production. The 1028 final goal is intertwined in the methodology of the study and the final discussions surrounding 1029 presentation and statistics. The small conclusion presented in Section 4.4 shows that a simple 1030 experiment with a cheap and highly modifiable methodology can have many possibilities for 1031 future studies.

Overall, the study takes the form of a pilot study, and the results are interesting and provides multiple points to think about and discuss. This study shows interesting results regarding pH changes in relation to industrial effluents, and the photosynthetic rate and efficiency of marine phytoplankton in polluted waters. This shows the importance of looking more deeply at the lowest parts of the food chain.

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1140	Appendix	1 –	F/2	Medium	Recipe
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1142 **f/2 Medium**

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Stocks per liter

- 1144 **S** 1145
- 1146 (1) NaNO₃ 75g
- 1147 (2) NaH₂PO₄.2H₂O 5.65g
- 1148 (3) Trace elements (chelated)
- 1149 NA₂ EDTA 4.16 g
- 1150 FeCl₃.6H₂O 3.15 g
- 1151 CuSO₄.5H₂O 0.01 g
- 1152 ZnSO₄.7H₂O 0.022 g
- 1153 CoCl₂.6H₂O 0.01 g 1154 MnCl₂.4H₂O 0.18 g
- 1154 MiliCi2.4H2O 0.18 g 1155 Na2MoO4.2H2O 0.006 g
- 1155 (4) Vitamin mix
- 1157 Cyanocobalamin (Vitamin B₁₂) 0.0005 g
- 1158 Thiamine HCl (Vitamin B₁) 0.1 g
- 1159 Biotin 0.0005 g
- 1160

1161 Medium per liter

- 1162 NaNO₃ 1.0 ml
- 1163 NaH2PO4.2H2O 1.0 ml
- 1164 Trace elements stock solution (1) 1.0 ml
- 1165 Vitamin mix stock solution (2) 1.0 ml 1166
- 1167 Make up to 1 liter with filtered natural seawater. Adjust pH to 8.0 with 1M NaOH or HCl. For
- agar add 15g per liter Bacteriological Agar. Sterilize by autoclaving for 15 minutes at 15 psi
- and use when cooled to room temperature.
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1179 Appendix 2 – HD-PE Resistance Charts

HDPE Chemical Resistance Chart

Acetaddehyde R R R Acetaddehyde R R R Add turnes R R R Berzaid defyde R ND Ferror Collondo R R R Berzaid defyde R ND Ferror Sold (SOS) R R R Berzaid defyde R R R Burzaid R R R Burzaid defyde R R R Burzaid R R R Burzaid R R R Burzaid R R R Burza	Chemical Name	Resistano 20oC(68F)	ce Level 60oC(140F)	Chemical Name	20oC(68F)	Resistance Leve 60oC(140F)
Lostic and (10x) R R Cational calo R R Acetic and (paraller) R R Causic coda & potash R R Acetic and (paraller) R R Causic coda & potash R R Acetiona R R Causic coda & potash R R Other fetones R R Collorine, dy LR NR Acatylene R R Chlorine, dy LR NR Acatylene R R Chlorine, dy LR NR Aduminum chloride R R Chlorides of Na, K, Ba R R Aluminum chloride R R Chlorides of Na, K, Ba R R Aluminum chloride R R Chlorides of Na, K, Ba R R Aluminum chloride R R Chlorides of Na, K, Ba R R Aluminum chloride R R Chlorides of Na, K, Ba R R Auminum chloride R	Acetaldehvde	В	В	Carbon disubbide	R	NB
Acetic analyticity Acetic analyticity Receipt	Acetaidenyde	D	D	Carbon disciplide	D	
Acete analysis of the state of	Acetic acid (10%)	n	n	Carbonic acid	ND	ND
Acette antryothole H H Causale Social Social Social Social Social Social A potentin H H Other ketones R R Collations of Na, K, Ba R R Acchoritie R R Collations of Na, K, Ba R R Acchoritie R R Chiorose of Na, K, Ba R R Acchories R R Chioroseotic acid R R Aliphatic cesters R R Chiorosome NR NR Aluminum subptate R R Chiorosoliphonic acid NR NR Aluminum subptate R R Coper saits (most) R R Ammonia, aqueous R R Coper saits (most) R R Aromatic solvents R R R Coper saits (most) R R Ammonia, aqueous R R R Coper saits (most) R R Ammonia, aqueous R R R Coper saits (most) R	Acetic acid (glac./ann.)	н	н	Carbon tetrachioride	NR	NR
Acotone H H Collusios pant H H Other ketones R R Collorates of Na, K, Ba R R Accontinitie R R Chiorates of Na, K, Ba R R Acid hames R R Chiorates of Na, K, Ba R R Actinames R R Chiorates of Na, K, Ba R R Alphatic cesters R R Chiorobencem NR NR Aluminum chionide R R Chiorobencem NR NR Aluminum chionide R R Chiorobencem NR NR Ammonia, anyotous R R Chiorobencem R R Ammonia, anyotous R R Chiorobencem R R Ammonia, anyotous R R Chiorobencem R R Ammonia, anyotous R R R R R Ammonia, anyotous R R R R	Acetic anhydride	н	н	Caustic soda & potash	н	н
Other steames R R Chlorates of Na, K, Ba R R Acctorytine R R Chlorine, vert LR NR Acctorytine R R Chlorine, vert LR NR Acctorytine R R Chloroacetic acid R R Alchabite setters R R Chlorobenzene NR NR Aluminum chloride R R Chlorobenzene NR NR Aluminum chloride R R Chlorobenzene NR NR Aluminum chloride R R Chlorobenzene NR NR Ammonia, anhydrous R R Chlorobenzene NR NR Ammonia, anhydrous R R Chlorobenzene NR NR Ammonia, anyaeoua R R Chlorobenzene NR NR Ammonia, anyaeoua R R Chlorobenzene NR NR Ammonia, anyaeoua R R R </td <td>Acetone</td> <td>R</td> <td>R</td> <td>Cellulose paint</td> <td>R</td> <td>R</td>	Acetone	R	R	Cellulose paint	R	R
Acetylene R R Chlorine, vdy LR NR Acid funes R R Chlorides of Na, K, Ba R R Aliphatic esters R R R Chlorobenzene NR NR Aliphatic esters R R R Chlorobenzene NR NR Aliphatic esters R R Chlorobenzene NR NR Aluminum subplate R R Chlorobenzene NR NR Aluminum subplate R R Chlorobenzene NR NR Ammonum choride R <t< td=""><td>Other ketones</td><td>R</td><td>R</td><td>Chlorates of Na, K, Ba</td><td>R</td><td>R</td></t<>	Other ketones	R	R	Chlorates of Na, K, Ba	R	R
Accifures R R Chlorine, wet LR NR Acid funes R R Chloroacetio acid R R Aliphatic esters R R Chloroacetio acid R R Aliphatic esters R R Chlorobertzene NR NR Aluminum robinide R R Chlorobertzene NR NR Aluminum robinide R R Chlorobertzene NR NR Aluminum robinide R R Chlorobertzene NR NR Ammonia, anhydrous R R Copper salts (moth) R R Ammonia, anucous R R Copper salts (moth) R R Ammonia, anductos R R Copper salts (moth) R R Anine R R Copper salts (moth) R R Andine R R Pertroi chloride R R Anonatic solvents R R R Pertroi chloride R R Berozic acid R R <td>Acetonitrile</td> <td>R</td> <td>R</td> <td>Chlorine, dry</td> <td>LR</td> <td>NR</td>	Acetonitrile	R	R	Chlorine, dry	LR	NR
Add Tumes R R R Chlorides of NR, K, Ba R R Alcohols R R R Chloroberzene NR NR Alky dhoindes R R R Chloroberzene NR NR Alky dhoindes R R R Chloroberzene NR NR Aluminum chloride R R R Chloroberzene NR NR Aluminum sulphate R R Chloroberzene NR NR Aluminum sulphate R R Chloroberzene NR NR Ammonium chloride R R Copper salts (most) R R Armmonium chloride R R Copper salts (most) R R Annonium chloride R R Cresplic acids (50%) R R Anine R R R R R R Aromatic solvents R NR Eaters ND ND Bernoic acid R R R R R Bernoic acid R R R R R Boroic acid R R R R Boroic acid <t< td=""><td>Acetylene</td><td>B</td><td>B</td><td>Chlorine wet</td><td>LB</td><td>NB</td></t<>	Acetylene	B	B	Chlorine wet	LB	NB
Activities R R Controlation (A, N, but A R R Alightatic esters R R Chlorobenzane NR NR Alightatic esters R R Chlorobenzane NR NR Aluminium chloride R R Chlorobenzane NR NR Aluminium sulphate R R Chlorobenzane NR NR Auminium sulphate R R Copper salts (most) R R Ammonia, anhydrous R R Copper salts (most) R R Ammonia, anhydrous R R Copper salts (most) R R Anine R R Detrogents, synthetic R R Anine R R Detrogents, synthetic R R Berzidelyde R NR Esters ND ND Boric acid R R R R R Boric acid R R R R	Acid fumes	D		Chlorides of Na K Ba	D	D
Autonicous In In In In In In Allynatic centre R R R Chloroform NR NR Allynatic centre R R Chloroform NR NR Alluminium sulphate R R Chloroform NR NR Aluminium sulphate R R Chlorobenzene NR NR Aluminium sulphate R R Chlorobenzene NR NR Aluminium sulphate R R Chlorobenzene NR NR Ammonium chloride R R Chlorobenzene NR NR Ammoniu, aqueous R R Chlorobenzene NR NR Ammonium chloride R R Chlorobenzene NR NR Amalescella R R Chlorobenzene NR NR Am	Acid iumes	B	R	Chloroacetic acid	R	R
Aliphatic esters R R Chlorobenzene NR NR Alum R R Chloroburg NR NR Aluminum chloride R R Chlorobad NR NR Aluminum sulphate R R Chrorobad R R Ammonia, anhydrous R R Corper salts (most) R R Ammonia, anyaueous R R Corper salts (most) R R Ammonia, anyaueous R R Corper salts (most) R R Ammonia, anyaueous R R Detergents, synthetic R R Animate R R Detergents, synthetic R R Animate R R Detergents, synthetic R R Acomatic solvents R R R Eaters ND ND Berazilelinyde R ND Ferricus aubstrate R R Berazilelinyde R ND Ferricus aubstrate R R Berazilelinyde R R Fluorine, wet NR NR Diracid R R R Fluorine, wet NR NR Dirac				enerodoodo dold		
Alky dioindes R R R Aluminium chloride R R Chiorosubphonic acid (80%) R NR Aluminium uphate R R Chromic acid (80%) R NR Ammonium diphate R R Chromic acid (80%) R R Ammonium diphate R R Corper salts (most) R R Ammonium diphate R R Corper salts (most) R R Ammonium diphate R R Corper salts (most) R R Ammonium diphate R R Corper salts (most) R R Ammonium diphate R R Corper salts (most) R R Ammonium diphate R R Corper salts (most) R R Amine R R R Detergents, synthetic R R Anontic solvents R R R R R R Ber R ND Esters ND ND R Boric acid R R R R R Boric acid R R R R R Boric acid R R	Aliphatic esters	R	R	Chlorobenzene	NR	NR
Alum R R R Chlorosuphonic acid NR NR Aluminium sulphate R R Chromic acid (80%) R NR Aluminium sulphate R R Citric acid R R Anmonia, anhydrous R R Copper salts (most) R R Ammonia, anhydrous R R Copper salts (most) R R Ammonia, anhydrous R R Cyclohexane NR NR Annonia, anay lacetae R R Cyclohexane NR NR Aninie R R Esters ND ND ND Acomatic solvents R R R R R Beera R R Ferrous sulphate R R Berzolc acid R R R R R Brines, saturated R R R R R Butyl acetae R R R R R Calcium chloride R R R R R Calcium chloride R R R R R Calcium chloride R R R R	Alkyl chlorides	R	R	Chloroform	NR	NR
Aluminium chloride R R Chromic acid (80%) R NR Aluminium sulphate R R Citic acid R R Ammoniue, anhydrous R R Coper salls (most) R R Ammoniue, anhydrous R R Coper salls (most) R R Ammoniue, foliofe R R Coper salls (most) R R Ammoniue, foliofe R R Coper salls (most) R R Ammoniue, foliofe R R Coper salls (most) R R Ammoniue, foliofe R R Esters ND ND Beer R R R R R Beroid acid R R Ferrio sulphate R R Bromide (K) Soltion R R Fluorine, drip remass ulphate R R Bromide (K) Soltion R R Formaldehyde (40%) R R Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formit pices R R R R	Alum	R	R	Chlorosulphonic acid	NR	NR
Aluminium sulphate R R Citric acid R R Ammonia, anhydrous R R Copper salls (most) R R Ammonia, anhydrous R R Copper salls (most) R R Ammonia, aupuous R R R Cyclohexane NR NR Ammonia, aupuous R R R R R R Annonia, aupuous R R R R R R Acontia caid R R R R R R Berzaidehyde (K) solution R R R R R Brines, saturated R R R R R Calcium chloride R R R R R Calcium chloride	Aluminium chloride	R	R	Chromic acid (80%)	R	NR
Ammonia, aqueous R R Copper salts (most) R R Ammoniu, folonde R R Creeytic acids (50%) R R R Ammoniu, aqueous R R R Creeytic acids (50%) R R R Ammonium chlonde R R R Detergents, synthetic R R R Ammonium chlonde R R R R R R R Annaine R R R R R R R Acornatic solvents R R R R R R R Beer R R R R R R R R Berzoic acid R R R Fluorinated refrigerants R NR NR Burdi acetate R LR R Fluorine, dry NR NR R Burdi acetate R R R R R R R Chemical Name 20oC(68P) 60oC(140P) Chemical Name 20oC(68P) 60oC(140P) Chemical Name 20oC(68P) 60oC(140P) R R R Galcium choride	Aluminium sulphate	R	R	Citric acid	R	R
Ammonia, annyonous H H Copper sate (most) H H Ammonia, annyonica, annyonou R R Cyclohexane NR NR Ammonia, quecous R R Cyclohexane NR NR Annyol acetate R R Cyclohexane NR NR Aniline R R Esters ND ND Ascorbic acid R R Esters ND ND Berzaidehyde R R Fatty acids (>C6) R R Berzaic acid R R Ferroic shoride R R Brines, saturated R R Flourine, dry NR NR Brines, saturated R R Flourine, dry NR NR Brines, saturated R R Flourine, dry NR NR Brines, saturated R R R Formical acid R R Calcium choride R R R Formical Name 200C(68F) 600C(140F) Formical Ame 200C(68F) 600C(140F) Chemical Name 200C(68F) 600C(140F) Formical Calcium choride R R R R <t< td=""><td></td><td></td><td></td><td>o</td><td></td><td></td></t<>				o		
Ammonia, aqueous R R Cresylic acids (50%) R R Ammonium chloride R R R Detergents, synthetic R R Amine R R R Ether R R Anomatic solvents R R R Ether R R Acomatic solvents R R R Ether R R Beer R R R Ether R R Benzoic acid R R Ferrous sulphate R R Boric acid R R Flourine, wet NR NR Brines, saturated R R Flourine, wet NR NR Brines, saturated R R R Flourine, wet NR NR Brines, saturated R R R Flourine, wet NR NR Calcium chioride R R R Mercury R R Calcium chioride R R R Mercury R R Glycolic acid R R R Mercury R R Glycolic acid R R Moiest air R <td>Ammonia, annydrous</td> <td>н</td> <td>н</td> <td>Copper salts (most)</td> <td>н</td> <td>н</td>	Ammonia, annydrous	н	н	Copper salts (most)	н	н
Ammonium chloride R R Cyclohexane NR NR Anvil acetale R R Betragents, synthetic R R Anine R R Emulatifiers, concentrated R R Anormatic solvents R NR Esters ND ND Accorbia coid R R R R R Benzolchyde R ND Ferric chloride R R Benzolca coid R R Ferrous sulphate R R Brines, saturated R R Fluorine, type NR NR Brines, saturated R R Fluorine, type NR NR Buryl acetate R LR Fluorine, type NR NR Chemical Name 20c0C(68P) 60cC(140P) Chemical Name 20cC(68P) 60cC(140P) Formit juces R R Mercury R R Glycole acid R R R Methanol R NR Glycole acid R R R Methanol R R Glycole acid R R R Motistair R R Glycole a	Ammonia, aqueous	R	R	Cresylic acids (50%)	R	R
Amyl acetate R R Anilne R R Anilne R R Anomatic solvents R NR Ascorbic acid R R Berr R R Berraidelyde R ND Berraidelyde R ND Berraidelyde R ND Boric acid R R Brines, saturated R R Brines, saturated R R Broic acid R R Brines, saturated R R Broic acid R R Broine acid R R R R Fluorine, wet NR Butyl acetate R R Chemical Name 200C(68F) 600C(140F) Formaidehyde (40%) R R R R R Mercury R Glycolic acid R R R Glycolic acid R R R Glycolic acid R R Noist air R R R Napthalene R R Hydrochoric acid (50%) R R Nitric acid (50%)	Ammonium chloride	R	R	Cyclohexane	NR	NR
Anime R R Emulaitiers, concentrated R R Aromatic solvents R NR Esters ND ND Ascorbic acid R R Esters ND ND Beer R R R Esters ND ND Benzoic acid R R Ferrous sulphate R R Boric acid R R R Ferrous sulphate R R Boric acid R R R Fluorine, dry NR NR Boric acid R R R Flourine, dry NR NR Boric acid R R R Flourine, dry NR NR Bury lacetate R LR Flourine, dry NR NR Chemical Name ZooC(68F) 60oC(140F) Chemical Name ZooC(68F) 60oC(140F) Formit juces R R Mercury R R Glycolic acid R R Mercury R R Glycolic acid R R Moltases R R Glycolic acid R R Moltases R R Glycolic acid	Amyl acetate	R	R	Detergents, synthetic	R	R
Aromatic solvents R R NR Ascorbic acid R R R Beer R R ND Berzaidelyde R ND Berzaidelyde R ND Berzaidelyde R ND Berzaidelyde R ND Berzaidelyde R ND Berzaidelyde R R R Boric acid R R R Boric acid R R R Brines, saturated R R R Broinelde (K) solution R R R Broinelde (K) solution R R R Calcium chloride R R R Calcium chloride R R R Calcium chloride R R R Calcium chloride R R R Fruit juces R R R Glycole R R R Glycole R R R Glycole acid R R R Glycole acid R R R Hexamipe R R R Glycole acid R R R Hexamipe R R R Hexamiphene diamine R R Hydrochlorica cid (0%) R R H Hydrochlorica cid (0%) R R H Hydrochlorica cid (0%) R R H Hydrochlorica cid (0%) R H H H H H H H H H H H H H H H H H H H	Aniline	R	R	Emulsifiers, concentrated	R	R
Aromatic solvents R NR Esters ND ND Ascorbic acid R R Ether R R Berzalchyde R ND Farty acids (>C6) R R Benzalchyde R ND Ferric ofloride R R Boric acid R R R Ferrico floride R R Boric acid R R R Fluorinated refrigerants R NR Brines, saturated R R R Fluorine, dry NR NR Borid (Solution R R R Fluorine, dry NR NR Bury tacetate R LR Fluorine, dry NR NR Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formit juices R R Mercuric choride R R Glycoli R R Methanol R NR Glycoli acid R R Methylene choride R R Glycoli acid R R Molasses R R Glycoli acid R R Molasses R R <td< td=""><td></td><td></td><td>-</td><td>,</td><td></td><td></td></td<>			-	,		
Ascoribic acid R R R R Berrer R R R Benzoic acid R R ND Benzoic acid R R R Boric acid R R R Boric acid R R R Brines, saturated R R R Brines, saturated R R R Brines, saturated R R R Borine & R Boric acid R R R Brines, saturated R R R Calcium chloride R R R Calcium Calci (10%) R R R Calcium Calci (10%) R R R Calcium Calci (10%) R R R Calcium Calcium R R R Calcium R R R Calcium Calcium R R R Calcium R R R Calcium Calcium R R R Calcium R R R Calciu	Aromatic solvents	R	NR	Esters	ND	ND
Beer R R Fartic coloride R R Benzaldehyde R ND Ferric coloride R R Boric acid R R R Ferrous sulphate R R Boric acid R R R Fluorinated refrigerants R NR Brines, saturated R R R Fluorine, dry NR NR Buryl acetate R LR Fluorine, dry NR NR Buryl acetate R LR Fluorosilic acid R R Calcium chloride R R Mercuric chloride R R Formica ixid R R Mercuric chloride R R Glycolis R R Mercuric chloride R R Glycolis acid R R Methanol R R Glycolic acid R R Moist air R R Glycolic acid R R Moistair R R Hydrocholic acid (10%) R R NItrates of Na, K and NH3 R Hydrocholic acid (10%) R R Nitric acid (20%) R R Hydrocholic acid (Ascorbic acid	R	R	Ether	R	R
Benzaldehyde R ND Ferric chiolde R R Berzoic acid R R R Ferric chiolde R R Boric acid R R R Ferric chiolde R R Brines, saturated R R Fluorine, dy NR NR Broinde (K) solution R R Fluorine, dy NR NR Calcium chloride R R Fluorine, wet NR NR Chemical Name 20cC(68F) 60oC(140P) Chemical Name 20cC(68F) 60oC(140P) Formic acid R R Mercuric chloride R R Glycoli R R Methanol R NR Glycolis R R Methylene chloride R R Resistance R R Molasese R R Glycolis R R Molasese R R Glycolis acid R R Nonoethanolamine ND ND Hexamethylene diamine R R Noiteal ar R R Hydrochoric acid (50%) R R Nitrite acid (25%) R R Hydrochoric aci	Beer	R	R	Fatty acids (>C6)	R	R
Benzoic acid R R Boric acid R R Boric acid R R Brines, saturated R R Brines, saturated R R Borid (K) solution R R Bury acetate R LR Calcium chloride R R Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formic acid R R R R Mercuric chloride R R R Mercuric chloride R R Gilycolis R R Mercuric chloride R R Gilycolis acid R R Mercuric chloride R R Gilycoli cacid R R Mercuric chloride R R Gilycoli cacid R R Moist air R R Gilycoli cacid R R Nonoethanolamine ND ND Hexamine R R Nitrates of Na, K and NH3 R R Hydrocholinic acid (10%) R R Nitric acid (20%) R R Hydrophonic acid (50%) R R	Benzaldehvde	B	ND	Ferric chloride	B	B
Boric acid R R Brines, saturated R R Broinde (K) solution R R Butyl acetate R LR Calcium chloride R R Calcium chloride R R Chemical Name 20oC(68F) 60oC(140F) Formic acid R R Fruit juices R R Resistance Level Chemical Name 20oC(68F) Formic acid R R Resistance Level Chemical Name 20oC(68F) Formic acid R R Resistance Level Chemical Name 20oC(68F) Glyceine R R Glycoli R R Glycoli acid R R Resistance Level Metrunol R Glycoli acid R R R R Metrunol R Glycoli acid R R Resistance Level Molasses R Glycoli acid R R R R Molasses R Hexamine R R Hydrochoric acid (50%) R R Hydrochoric acid (50%) <t< td=""><td>Benzoic acid</td><td>R</td><td>R</td><td>Ferrous sulphate</td><td>R</td><td>R</td></t<>	Benzoic acid	R	R	Ferrous sulphate	R	R
Boric acid R R Fluorinated refrigerants R NR Brines, saturated R R Fluorine, dry NR NR Browide (K) solution R R R Fluorine, dry NR NR Butyl acetate R LR Fluorine, dry NR NR NR Calcium chioride R R R Formalderyde (40%) R R Chemical Name 20cC(68F) 60oC(140F) Chemical Name 20oc(68F) 60oC(140F) Formic acid R R Mercuric chloride R R Rigitatine R R Mercury R R Glycolic acid R R Methanol R NR Glycolic acid R R Moist air R R Glycolic acid R R Molasses R R Hexamethylene diamine R R NR NR NR Hydrochloric acid (50%) R R Nitric acid (25%) R R Hydrochloric acid (50%) R R Nitric acid (50%) R R Hydrochloric acid (75%) R R Nitric acid (50%) R </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>						
Brines, saturated R R Fluorine, dry NR NR Bornide (K) solution R R LR Fluorine, wet NR NR Butyl acetate R LR Fluorine, wet NR NR NR Calcium chloride R R LR Fluorine, wet NR NR Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formit acid R R Mercury R R Fruit juices R R Mercury R R Glycolic acid R R Metrylene chloride R R Glycol, ethylene R R Methylene chloride LR NR Hexamine R R Moist air R R Glycolic acid R R Moist air R R Hydrochoric acid (50%) R R Nitrates of Na, K and NH3 R R Hydrochoric acid (10%) R R Nitrite acid (22%) R R Hydrochoric acid (10%) R R Nitrite acid (20%) R R Hydrochoric acid (10%) R R Nitrite acid (22	Boric acid	R	R	Fluorinated refrigerants	R	NR
Bromide (K) solution R R Flourine, wet NR NR Bulyl acetate R LR Flourone, wet NR R Calcium chloride R R R Formaldehyde (40%) R R Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formic acid R R Mercury R R Galatine R R Mercury R R Glycoline R R Mercury R R Glycolic acid R R Metroury R R Glycolic acid R R Moist air R R Glycolic acid R R Molasses R R Hexamethylene diamine R R Molasses R R Hydrochloric acid (10%) R R Nitric acid (25%) R R Hydrochloric acid (00nc.) R R Nitric acid (25%) R R Hydrochloric acid (75%) R R Nitric acid (20%) R R Hydrochlorites (Na 12.14%) R R Nitric acid (10%) NR NR Hyd	Brines, saturated	R	R	Fluorine, dry	NR	NR
Butyl acetate R LR Fluorosilic acid R R Calcium chloride R R Formaldehyde (40%) R R Chemical Name 20oC(68F) 60oC(140F) Chemical Name Resistance Level Resistance Level Formic acid R R Mercuric chloride R R Formic acid R R Mercury R R Gelatine R R Methanol R NR Glycolis R R Methanol R NR Glycolis R R Moist air R R Glycolis R R Moiseses R R Hexamethylene diamine R R Moisel asits R R Hydrochloric acid (10%) R R Nitritates of Na, K and NH3 R R Hydrochloric acid (20%) R R Nitritates of Na, K and NH3 R R Hydrochloric acid (20%) R R Nitrita acid (20%) R NR Hydrochloric acid (40%) R R Nitrita acid (20%) R NR Hydrochloric acid (20%) R R Nitrita acid (30%) R R	Bromide (K) solution	R	R	Flourine, wet	NR	NR
Calcium chloride R Formaldehyde (40%) R R Chemical Name 20oC(68F) 60oC(140F) Chemical Name 20oC(68F) 60oC(140F) Formic acid R R Mercuric chloride R R Formic acid R R Mercury R R Glycoli R R Mercury R R Glycoli R R Methanol R NR Glycoli R R Milk products R R Glycoli acid R R Molassee R R Hexamine R R Nonoethanolamine ND ND Hydrochloric acid (10%) R R Nitric acid (25%) R R Hydrofuluoric acid (40%) R R Nitric acid (90%) NR NR Hydrofuluoric acid (40%) R R Nitric acid (90%) NR NR Hydrofuluoric acid (40%) R R Nitric acid (90%)	Butyl acetate	B	LB	Fluorosilic acid	B	R
Caucian culcular It It<	Calcium chloride	R	R	Formaldebyde (40%)	R	R
Chemical NameResistance Level 20oC(68F)60oC(140F)Resistance Level 20oC(68F)60oC(140F)Formic acidRRMercuric chlorideRRFruit juicesRRMercuric chlorideRRGlatineRRMercuric chlorideLRNRGlycorineRRMethanolRRGlycol, ethyleneRRMilk productsRRGlycolic acidRRMoist airRRHexamethylene diamineRRMoist airRRHexamethylene diamineRRNoorebhanolamineNDNDHexamethylene diamineRRNapthaNRNRHydroschoric acid (10%)RRNitric acid (25%)RRHydroschoric acid (10%)RRNitric acid (25%)RRHydrospanic acid (10%)RRNitric acid (25%)RNRHydrogen peroxide (30 - 90%)RRNitric acid (10%)NRNRHydrogen peroxide (30 - 90%)RRNINRNRHydrogen peroxide (30 - 90%)RRNINRNRHydrogen peroxide (30 - 90%)RRNRNRNRHydrogen peroxide (30 - 90%)RRNRNRNRHydrogen peroxide (30 - 90%)RRNRNRNRLead acetateRROils, essentialRRLead ac						
Formic acid R R Mercury R R Fornit juices R R R Mercury R R Gelatine R R R Methanol R NR Glycolic R R R Methanol R NR Glycolic acid R R R Moist air R R Glycolic acid R R Moist air R R R Hexamethylene diamine R R Molasses R R ND ND Hydrochioric acid (50%) R R R Naptha NR NR Hydrochioric acid (10%) R R Nitritaecid (<25%)	Chemical Name	Resistan	ce Level	Res	sistance Leve	el 60=C(140E)
Formic acidRRMercuryRRFruit juicesRRRMercuryRRGelatineRRRMethanolRNRGlycolsRRRMethanolRNRGlycolsRRRMilk productsRRGlycol, ethyleneRRRMoist airRRGlycol, ethyleneRRRMoiset airRRGlycol, ethyleneRRRMoiset airRRHexamethylene diamineRRMoiset airRRHexamethylene diamineRRNapthaNRNRHydrochioric acid (50%)RRNapthaNRNRHydrochoric acid (10%)RRNitric acid (25%)RRHydrochoric acid (10%)RRNitric acid (25%)RRHydrofluoric acid (40%)RRNitric acid (25%)RRHydrofluoric acid (75%)RRNNRNRHydrofluoric acid (30 - 90%)RNRNitrobenzeneNRNRHydrochorites (Na 12-14%)RROils, essentialRNRHypochlorites (Na 12-14%)RROils, essentialRNRHypochlorites (Na 12-14%)RRNRNRLead perchlorateNDNDOils, mineralRRLead perchlorateNDND<			6U0U3U4UE1			BUOL:140E/
Fruit juicesRRMercuryRRGelatineRRRMethanolRNRGlycorineRRRMethylene chlorideLRNRGlycolsRRRMilk productsRRGlycolic acidRRRMoist airRRHexamethylene diamineRRMonoethanolamineNDNDHexamineRRMonoethanolamineNDNDHydrozhioric acid (50%)RRRNapthaNRRHydrochloric acid (10%)RRRNitrates of Na, K and NH3RRHydrochloric acid (conc.)RRNitric acid (25%)RRRHydrofluoric acid (conc.)RRNitric acid (50%)RRRHydrofluoric acid (conc.)RRNitric acid (50%)RNRHydrofluoric acid (conc.)RRNitric acid (50%)NRNRHydrofluoric acid (conc.)RRNitric acid (50%)NRNRHydrofluoric acid (25%)RRRNRNRHydrogen peroxide (30*9%)RRRNRNRHydrofluorics (Na 12-14%)RRNRNRNRHydroflorites (Na 12-14%)RRRNRNRLaed acetateNDNDOils, dieselRRRLead acetateRRROils, vegetabl		2000(001)	600C(140F)		2000(00F)	606C(140F)
Gelatine R R Methanol R NR Glycorine R R R Methylene chloride LR NR Glycols R R R Milk products R R Glycolic acid R R Moist air R R R Glycolic acid R R Moist air R R R Hexamethylene diamine R R Monoethanolamine ND ND Hexamethylene diamine R R Monoethanolamine ND ND Hydrobromic acid (50%) R R Naptha NR NR Hydrochoric acid (10%) R R Nitrates of Na, K and NH3 R R Hydrocplanic acid (10%) R R Nitric acid (50%) R NR Hydrocpluoric acid (75%) R R Nitric acid (90%) NR NR Hydrogen peroxide (30%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30%) R R NR NR NR <td>Formic acid</td> <td>R</td> <td>808C(140F)</td> <td>Mercuric chloride</td> <td>R</td> <td>R</td>	Formic acid	R	808C(140F)	Mercuric chloride	R	R
GlycerineRRRGlycolsRRRGlycolsRRRGlycolc acidRRRHexamethylene diamineRRHexamethylene diamineRRHexamethylene diamineRRHexamethylene diamineRRHexamethylene diamineRRHexamineRRHydrobromic acid (50%)RRRRNitckel saltsRHydrochloric acid (10%)RRRNitric acid (25%)RRRNitric acid (25%)RRRHydrochloric acid (10%)RRRNitric acid (50%)RRHydrofluoric acid (10%)RRRHydrofluoric acid (10%)RRRHydrofluoric acid (10%)RRRHydrofluoric acid (10%)RRRHydrogen peroxide (30%)RRNitric acid (fuming)NRNRHydrogen peroxide (30%)RRRHydrogen sulphideRRRHypochlorites (Na 12-14%)RRRLead cetateRROils, essentialRRLead cetateRRParaffin waxRRLead acidRRRPhenolRR <td>Formic acid Fruit juices</td> <td>R</td> <td>R R</td> <td>Mercuric chloride Mercury</td> <td>R</td> <td>R</td>	Formic acid Fruit juices	R	R R	Mercuric chloride Mercury	R	R
Glycoline II II III III IIII IIII IIIII IIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	Formic acid Fruit juices Gelatine	R R R	R R R R	Mercuric chloride Mercury Methanol	R R R	R R NR
Glycols H H H H H H Glycol, ethylene R R Moist air R R Glycolic acid R R Molasses R R Hexamethylene diamine R R Monoethanolamine ND ND Hexamethylene diamine R R Naptha NR NR Hydrobromic acid (50%) R R R Nitrates of Na, K and NH3 R R Hydrochloric acid (10%) R R Nitric acid (50%) R NR NItric acid (50%) R NR Hydrochloric acid (10%) R R Nitric acid (50%) R NR Hydropolic acid (00%) R R Nitric acid (50%) R NR Hydrogen peroxide (30 - 90%) R R Nitric acid (fuming) NR NR Hydrogen sulphide R R Oils, diesel R NR Hydrogen sulphide R R Oils, essential R NR Hydrogen sulphide R R Oils, inineral<	Formic acid Fruit juices Gelatine	R R R R	R R R R	Mercuric chloride Mercury Methanol	R R R	R R NR
Glycol, ethylene R R Moist air R R Glycolic acid R R Molasses R R Hexamethylene diamine R R Monoethanolamine ND ND Hexamine R R Naptha NR NR Hydrochloric acid (50%) R R Nitrates of Na, K and NH3 R R Hydrochloric acid (10%) R R Nitric acid (<25%)	Formic acid Fruit juices Gelatine Glycerine Glycols	R R R R R	R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products	R R R LR R	R R NR NR R
Glýcolic acid R R Molasses R R Hexamethylene diamine R R Monoethanolamine ND ND Hexamine R R Naptha NR NR Hydrazine R R Naptha NR NR Hydrobromic acid (50%) R R Nickel salts R R Hydrochloric acid (10%) R R Nitrice of Na, K and NH3 R R Hydrochloric acid (conc.) R R Nitrice acid (50%) R NR Hydrofluoric acid (40%) R R Nitrice acid (50%) R NR Hydrofluoric acid (75%) R R R Nitrice acid (90%) NR NR Hydrogen peroxide (30%) R R Nitrice acid (90%) NR NR Hydrogen sulphide R R Oils, diesel R NR Hypochlorites (Na 12-14%) R R Oils, sesential R NR Leatic acid (90%) R R Oils, vegetable and animal R R	Formic acid Fruit juices Gelatine Glycerine Glycols	R R R R R R	R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products	R R R LR R	R R NR NR R
Hysamethylene diamineRRMonoethanolamineNDNDHexamethylene diamineRRRNapthaNRNRHydrazineRRRNapthaNRNRHydrobromic acid (50%)RRRNitrates of Na, K and NH3RRHydrochloric acid (10%)RRRNitric acid (25%)RRRHydrochloric acid (conc.)RRRNitric acid (50%)RRRHydrochloric acid (adw)RRRNitric acid (50%)RNRHydrogen peroxide (30%)RRRNitric acid (fuming)NRNRHydrogen peroxide (30%)RRRNitric oblewiceNRNRHydrogen sulphideRRROils, dieselRNRHypochlorites (Na 12-14%)RROils, lubricating+ aromatic additivesRRLead acetateNDNDOols, wegetable and animalRNRLead acetateRROils, vegetable and animalRRLead perchlorateNDNDOzoneRRLime (CaO)RRRPerchloric acidRRMaleic acidRRRPerchloric acidRRManganate, potassium (K)RRPercoloric acidRRManganate, potassium (K)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol. ethylene	R R R R R R	R R R R R R R R	Mercuric chloride Mercury Methanol Milk products Moist air	R R R LR R R	R R NR NR R R
Hexamine R R R Napitha NR NR Hydrobromic acid (50%) R R R Napitha NR NR Hydrochloric acid (10%) R R Nitrates of Na, K and NH3 R R Hydrochloric acid (conc.) R R Nitric acid (25%) R R Hydrocyanic acid (adv) R R Nitric acid (50%) R NR Hydrocyanic acid (40%) R R Nitric acid (50%) R NR Hydrofluoric acid (40%) R R Nitric acid (50%) NR NR Hydrofluoric acid (75%) R R R Nitric acid (10%) NR NR Hydrogen peroxide (30%) R R Nitric acid (10%) NR NR Hydrogen sulphide R R Oils, diesel R NR Hypochlorites R R Oils, diesel R NR Hypochlorites (Na 12-14%) R R Oils, ubricating + aromatic additives R R Lead acetate ND ND Oils, wege	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid	R R R R R R R	BUOC(140F)	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses	R R R LR R R R	R R NR NR R R R R
Hydrazine N N N N N N Hydrobromic acid (50%) R R N Nitrates R R ND Hydrobromic acid (10%) R R Nitrates of Na, K and NH3 R R Hydrochloric acid (10%) R R Nitric acid (25%) R R Hydrochloric acid (200.) R R Nitric acid (50%) R NR Hydrofluoric acid (40%) R R Nitric acid (50%) R NR Hydrofluoric acid (75%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30°- 90%) R NR Nitrobenzene NR NR Hydrogen sulphide R R Oils, diesel R NR Hypochlorites (Na 12-14%) R R Oils, mineral R R Iso-butyl-acetate ND ND Oils, mineral R R Lead acetate R R Oils, vegetable and animal R NR Lead perchlorate ND ND Ozone R	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine	R R R R R R R R R R	R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Moncethanolamine	R R LR R R R ND	R R R R R R R ND
Hydrozhire N N Naputatele N ND Hydrozhloric acid (50%) R R Nickel salts R R Hydrozhloric acid (10%) R R Nitrates of Na, K and NH3 R R Hydrozhloric acid (conc.) R R Nitric acid (250%) R R Hydrozyanic acid R R Nitric acid (50%) R NR Hydrofluoric acid (40%) R R Nitric acid (50%) R NR Hydrogen peroxide (30%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30°-90%) R NR Nitric benzene NR NR Hydrogen sulphide R R R Oils, diesel R NR Hypochlorites R R R Oils, essential R NR Hypochlorites (Na 12-14%) R R Oils, mineral R R Lead acetate ND ND Oils, mineral R R Lead acetate R R Oils, vegetable and animal R <	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hoxemice	R R R R R R R R R R R	R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine	R R LR R R R R ND	R R NR NR R R R ND
Hydrobromic acid (50%)RRRNickel saltsRRHydrochloric acid (10%)RRRNitrates of Na, K and NH3RRHydrochloric acid (conc.)RRRNitric acid (-25%)RRRHydrocyanic acidRRRNitric acid (50%)RNRNRHydrofluoric acid (40%)RRRNitric acid (90%)NRNRHydrogen peroxide (30%)RRRNitric ocid (fuming)NRNRHydrogen peroxide (30-90%)RNRNRNitrobenzeneNRNRHydrogen sulphideRROils, dieselRNRHypochloritesRRROils, dieselRNRHypochlorites (Na 12-14%)RROils, nineralRRIso-butyl-acetateNDNDOils, wegetable and animalRNRLead acetateRROils, vegetable and animalRRLead perchlorateNDNDOzoneRRLime (CaO)RRRParaffin waxRRMaleic acidRRRPercoloric acidRRMaleic acid (50%)RRRPhosphoric acid (20%)R	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hexamine	R R R R R R R R R R R R R	R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha	R R R LR R R R ND NR	R R NR R R R R ND NR
Hydrochloric acid (10%) R R Nitrates of Na, K and NH3 R R Hydrochloric acid (conc.) R R R Nitric acid (<25%)	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine	R R R R R R R R R R R R R	R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene	R R R LR R R R ND NR R	R R NR R R R R ND NR ND
Hydrochloric acid (conc.)RRNitric acid (<25%)RRHydrocyanic acidRRRNitric acid (<20%)	Formic acid Fruit Juices Gelatine Glycerine Glycoline Glycolic acid Hexamethylene Hexamine Hydrazine Hydrobromic acid (50%)	R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts	R R LR R R ND NR R R	R R NR NR R R R ND NR ND NR ND
Hydrocyanic acid (conc.) H </td <td>Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%)</td> <td>R R R R R R R R R R R R R R R R</td> <td>R R R R R R R R R R R R R R R R R R R</td> <td>Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na K and NH</td> <td>R R R LR R R ND NR R 3 R</td> <td>R R R R R R R ND NR ND R R R</td>	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%)	R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na K and NH	R R R LR R R ND NR R 3 R	R R R R R R R ND NR ND R R R
Hydrofluoric acid (40%)RRRNitric acid (90%)RNRHydrofluoric acid (40%)RRRNitric acid (90%)NRNRHydrofluoric acid (75%)RRRNitric acid (fuming)NRNRHydrogen peroxide (30%)RRRNitric acid (fuming)NRNRHydrogen peroxide (30%)RRRNitric acid (fuming)NRNRHydrogen sulphideRRROils, dieselRNRHypochloritesRRROils, essentialRNRHypochlorites (Na 12-14%)RROils, lubricating + aromatic additivesRRLactic acid (90%)RRROils, vegetable and animalRNRLead acetateRROils, vegetable and animalRRLead perchlorateNDNDOzoneRRLime (CaO)RRRPerchloric acidRRMaleic acidRRRPercolour spiritsRRMaleic acid (50%)RRRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%)	R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na, K and NHR	R R LR R R ND NR R R 3 R R	R R NR R R R R ND NR ND NR ND R R R R
Hydrofluoric acid (40%) R R Nitric acid (90%) NR NR Hydrogen peroxide (30%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30%) R R Nitric acid (fuming) NR NR Hydrogen peroxide (30%) R NR NR Nitrobenzene NR NR Hydrogen sulphide R R Oils, diesel R NR Hypochlorites R R Oils, essential R NR Hypochlorites (Na 12-14%) R R Oils, ulubricating - - Los-butyl-acetate ND ND Oils, mineral R R Lead acetate R R Oils, vegetable and animal R NR Lead perchlorate ND ND Ozone R LR Lime (CaO) R R Paraffin wax R R Maleic acidR R Petroleura spirits R R Man	Formic acid Fruit Juices Gelatine Glycerine Glycoline Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (conc.)	R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methylene chloride Milk products Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na, K and NH3 Nitric acid (<25%)	R R LR R ND NR R 3 R 3 R	R R NR R R R R ND ND NR ND R R R R R R
Hydrofluoric acid (75%)RRRNitric acid (fuming)NRNRHydrogen peroxide (30%)RRRNitrite (Na)RRHydrogen peroxide (30 - 90%)RNRNRNitrobenzeneNRNRHydrogen peroxide (30 - 90%)RNRNRNitrobenzeneNRNRHydrogen sulphideRROils, dieselRNRHypochloritesRROils, essentialRNRHypochlorites (Na 12-14%)RROils, iubricating	Formic acid Fruit Juices Gelatine Glycerine Glycols Glycols Glycols acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%)	R R R R R R R R R R R R R R R R R	BUOCCITAUP)	Mercuric chloride Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na, K and NH Nitric acid (<25%) Nitric acid (50%)	R R R R R R ND NR R 3 R R 3 R R NP	R R NR R R R R ND NR ND NR ND R R R R NR
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Hydrogen peroxide (30 - 90%) RNRNitrobenzeneNRNRHydrogen sulphideRRRHypochloritesRROils, dieselRNRHypochloritesRRROils, essentialRNRHypochlorites (Na 12-14%) RROils, lubricating + aromatic additivesRRRIso-butyl-acetateNDNDOils, mineralRRRLactic acid (90%)RRROils, vegetable and animalRNRLead acetateRRROils, vegetable and animalRRLime (CaO)RRParaffin waxRRRMaleic acidRRPerchloric acidRRRManganate, potassium (K)RRPetroleum spiritsRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrofluoric acid (40%)	R R R R R R R R R R R R R R R R R R R	BUDGE (140F) R R R R R R R R R R R R R R R R R R R	Mercury Metroury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitric acid (<25%)	R R LR R R ND NR R R R R R R R R NR	R R NR NR R R R ND NR ND NR NR NR NR NR
Hydrogen sulphideRRROils, dieselRNRHypochloritesRRROils, dieselRNRHypochlorites (Na 12-14%)RROils,Ilubricating + aromatic additivesRRIso-butyl-acetateNDNDOils, mineralRRLead acetateRROils, vegetable and animalRNRLead acetateRROils, vegetable and animalRRLead perchlorateNDNDOzoneRRLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRManganate, potassium (K)RRPetroleum spiritsRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit Juices Gelatine Glycerine Glycoline Glycolic acid Hexamethylene diamine Hexamine Hydrozhoric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrofluoric acid (40%) Hydrofluoric acid (75%) Hydrogen peroxide (30%)	R R R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercury Methanol Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na, K and NH3 Nitric acid (50%) Nitric acid (50%) Nitric acid (90%)	R R LR R ND NR R 3 R R R R NR NR NR	R R NR R R R R ND NR ND R R R R NR NR NR NR R
Hypochlorites R R Hypochlorites R R Hypochlorites (Na 12-14%) R R Oils, lubricating	Formic acid Fruit Juices Gelatine Glycerine Glycolic acid Hexamethylene diamine Hexamethylene diamine Hexamethylene diamine Hydrobioric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrofluoric acid (75%) Hydrogen peroxide (30~ 90%)	R R R R R R R R R R R R R R R R R R R	BUDGL(140F) R R R R R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitric acid (<25%)	R R LR R ND NR R 3 R 3 R NR NR NR NR	R R NR NR R R R ND ND NR ND NR NR NR NR NR NR NR NR NR NR NR
Hypochlorites (Na 12-14%)RRHypochlorites (Na 12-14%)RRSoborhuarNIHypochlorites (Na 12-14%)RRNDSoborhuarOils, lubricating + aromatic additivesIso-butyl-acetateNDNDNDOils, mineralRLactic acid (90%)RRRLead acetateRRROtageRLead acetateRRRLead perchlorateNDNDOzoneLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRManganate, potassium (K)RRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycorine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (conc.) Hydrochloric acid (conc.) Hydrocyanic acid Hydrofluoric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30~90	R R R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercuric chloride Mercury Methanol Methanol Methylene chloride Milk products Moise air Monoethanolamine Naptha Napthalene Nickel salts Nitrates of Na, K and NHS Nitric acid (50%) Nitric acid (50%) Nitric acid (50%) Nitric acid (fuming) Nitrite (Na) Nitrite (Na) Nitrite acid	R R R R R R ND NR R 3 R R R NR NR R NR R R NR R R R NR R R R	R R NR R R R R ND NR ND NR NR R R NR NR NR NR
Oils, Iubricating Iso-butyl-acetate ND ND Lactic acid (90%) R R Lead acetate R R Lime (CaO) R R Perchloric acid R R Maleic acidR R Perchloric acid R Manganate, potassium (K) R R Petroleum spirits R Phosphoric acid (50%) R R Phosphoric acid (20%) R	Formic acid Fruit Juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrozhoric acid (50%) Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (0%) Hydrogen cacid (40%) Hydrogen peroxide (30°-90 Hydrogen sulphide Hydrogen sulphide	R R R R R R R R R R R R R R R R R R R	R R R R R R R R R R R R R R R R R R R	Mercury Mercury Methanol Methanol Methanol Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitric acid (25%) Nitric acid (50%) Nitric acid (90%) Nitric acid (fuming) Nitric (Na) Nitrobenzene Oils, diesel Oils, diesel Oils, diesel	R R LR R LR R R ND NR R 3 R R R R NR R NR R R NR R R R R R	R R NR NR R R ND NR ND R R R NR NR NR NR NR NR NR NR NR
+ aromatic additivesRRIso-butyl-acetateNDNDOils, mineralRRLactic acid (90%)RROils, vegetable and animalRNRLead acetateRROils, vegetable and animalRNRLead perchlorateNDNDOzoneRRLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRMaganate, potassium (K)RRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit Juices Gelatine Glycerine Glycolic acid Hexamethylene diamine Hexamine Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrofluoric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen sulphide Hypochlorites (Na 12-14%)	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Mercury Mercury Methanol Methanol Methanol Moist air Molasses Monoethanolamine Naptha Naptha Nitrates of Na, K and NH3 Nitric acid (<25%)	R R R LR R R ND NR R 3 R R R R NR NR NR R R R R R R R R	R R NR R R R ND NR ND R R R R R NR NR NR NR NR NR NR NR
Iso-butyl-acetateNDNDOils, mineralRRLactic acid (90%)RRROils, vegetable and animalRNRLead acetateRRROxalic acidRRLead perchlorateNDNDOzoneRLRLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRManganate, potassium (K)RRPetroleum spiritsRPhosphoric acid (50%)RRPhosphoric acid (20%)R	Formic acid Fruit Juices Gelatine Glycerine Glycolic acid Hexamethylene diamine Hexamine Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen sulphide Hypochlorites Hypochlorites (Na 12-14%)	R R R R R R R R R R R R R R R R R R R	8000C(140P) R R R R R R R R R R R R R R R R R R R	Othermical Name A Mercury Methanol Methanol Methanol Methanol Methanol Moist air Molasses Monoethanolamine Naptha Naptha Naptha Nitrates of Na, K and NH3 Nitric acid (<25%)	R R R R R R ND NR R 3 R R R R NR NR R R NR R R R R R R	R R NR NR R R ND NR ND NR NR NR NR NR NR NR NR NR
Lactic acid (90%)RRROils, vegetable and animalRNRLead acetateRRROxalic acidRRLead perchlorateNDNDOzoneRLRLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRManganate, potassium (K)RRPetroleum spiritsRMeat juicesRRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycorine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (20%) Hydrogen peroxide (30%) Hydrogen peroxide (30%)	R R R R R R R R R R R R R R R R R R R	600C(140F) R R R R R R R R R R R R R	Crientical Name 2 Mercury Methanol Methanol Methanol Methanol Methanol Methanol Methanol Monoethanolamine Maptha Naptha Napthalene Nickel salts Nitric acid (<25%)	R R R R R R ND NR R 3 R 3 R R 3 R R 3 R R 8 R 8 R 8 R 8	R R NR R R R R ND NR ND NR NR NR NR NR NR NR NR NR NR NR NR NR
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Lead perchlorateNDNDOzoneRLRLime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRManganate, potassium (K)RRPerchlour spiritsRRMeat juicesRRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit Juices Gelatine Glycerine Glycolic acid Hexamethylene diamine Hexamine Hydrobloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen sulphide Hypochlorites Hypochlorites Hypochlorites Hypochlorites (Na 12-14%)	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Criterinical Name A Mercury Methanol Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Naptha Naptha Naptha Nitrates of Na, K and NHS Nitrates of Na, K and NHS Nitric acid (<25%)	R R R R R R R R ND NR R S R R R NR R R R R R R R R R R R R	R R NR R R R R ND NR ND R R R NR NR NR NR NR NR NR NR NR NR NR
Lime (CaO)RRParaffin waxRRMaleic acidRRPerchloric acidRRManganate, potassium (K)RRPetroleum spiritsRRMeat juicesRRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycorine Glycols Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (20%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen sulphide Hypochlorites Hypochlorites (Na 12-14%)	R R R R R R R R R R R R R R R R R R R	BOOCUTAUP)	Criterinical Name A Mercury Methanol Methanol Methanol Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Naptha Napthalene Nickel salts Nitrates of Na, K and NH3 Nitric acid (50%) Nitric acid (50%) Nitric acid (90%) Nitric acid (fuming) Nitrobenzene Oils, diesel Oils, lubricating + aromatic additives Oils, nimeral Oils, vegetable and anima Oxalic acid	R R R R R R R ND NR R 3 R NR R NR R R R R R R R R R R R	R R R R R R R R ND NR ND R R R NR NR NR NR NR NR NR NR NR NR NR
Initial constraintInitial constraintPatalititi waxInitial constraintHMaleic acid RRPerchloric acidRRManganate, potassium (K)RRPerchloric acidRRMeat juicesRRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycorine Glycols Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (0%) Hydrogen peroxide (30%) Hydrogen peroxide (3	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Criterinical Name A Mercury Methanol Methanol Methanol Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Naptha Napthalene Nickel salts Nitrates of Na, K and NH3 Nitric acid (50%) Nitric acid (50%) Nitric acid (90%) Nitric acid (fuming) Nitrobenzene Oils, diesel Oils, lubricating + aromatic additives Oils, nimeral Oils, vegetable and anima Oxalic acid Ozone Ozone	R R R R R R R ND NR R 3 R NR R NR R R NR R R R R R R R R	R R R R R R R R ND NR ND R R R NR NR NR NR NR NR R R R
Manganate, potassium (K) R Perchloric acid R R Manganate, potassium (K) R R Petroleum spirits R R Meat juices R R Phenol R R Phosphoric acid (50%) R R Phosphoric acid (20%) R R	Formic acid Fruit juices Gelatine Glycorine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (conc.) Hydrochloric acid (conc.) Hydrocyanic acid Hydrofluoric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30°- 90 Hydrogen peroxide (30°- 90 Hydrogen sulphide Hypochlorites Hypochlorites (Na 12-14%) Iso-butyl-acetate Lactic acid (90%) Lead acetate Lead perchlorate	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Crientical Name Mercury Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Naptha Naptha Nitric acid (<25%)	R R LR R R ND NR R R NR R R NR R R R R R R R	R R R NR R R R R ND NR ND NR NR NR NR NR NR NR NR NR NR NR R R NR N
Manganate, potassium (K)RRPetroleum spiritsRRMeat juicesRRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (25%) Hydrochloric acid (40%) Hydrogen peroxide (30°-90 Hydrogen peroxide (30°-90 Hydrogen peroxide (30°-90 Hydrogen sulphide Hypochlorites Hypochlorites Hypochlorites (Na 12-14%)	R R R R R R R R R R R R R R R R R R R	B00C(140F) R R R R R R R R R R R R R	Othermical Name Mercury Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Napthalene Nickel salts Nitric acid (50%) Nitric acid (50%) Nitric acid (150%) Nit	R R R R R R R ND NR R R R R R R R R R R	R R NR R R R ND NR ND R R R NR NR NR NR NR NR R R NR NR R R R R R R R R R R R R R R R R R R R
Meat juicesRRPhenolRRPhosphoric acid (50%)RRPhosphoric acid (20%)RR	Formic acid Fruit Juices Gelatine Glycerine Glycolic acid Hexamethylene diamine Hexamine Hydrobloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen sulphide Hypochlorites Hypo	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Criterinical Name A Mercury Methanol Methanol Methanol Methanol Methanol Moist air Molasses Monoethanolamine Naptha Naptha Naptha Naptha Naptha Nitric acid (<25%)	R R R R R R R R ND NR R R R R R R R R R	R R NR NR R R ND NR ND NR NR NR NR NR NR NR NR NR NR NR NR NR
Phosphoric acid (50%) R R Phosphoric acid (20%) R R	Formic acid Fruit juices Gelatine Glycorine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (20%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen peroxide (30%) Hydrogen peroxide (30°%) Hydrogen peroxide (30°%) Hydrogen peroxide (30°%) Hydrogen peroxide (30°%) Hydrogen peroxide (30°%) Hydrogen peroxide (30°%) Hydrogen sulphide Hypochlorites Hypochlorites (Na 12-14%) Iso-butyl-acetate Lactic acid (90%) Lead acetate Liead perchlorate Lime (CaO) Maleic acidR Manganate, potassium (K)	R R R R R R R R R R R R R R R R R R R	BOOCTAUP)	Crientical Name 2 Mercury Methylene chloride Milk products Moist air Molasses Monoethanolamine Naptha Naptha Napthalene Nickel salts Nitric acid (<25%)	R R R LR R R ND NR R R NR R R NR R R R R R R R	R R R R R R R R R R R R R R R R R R R
	Formic acid Fruit juices Gelatine Glycerine Glycols Glycol, ethylene Glycolic acid Hexamethylene diamine Hexamine Hydrazine Hydrobromic acid (50%) Hydrochloric acid (50%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (10%) Hydrochloric acid (40%) Hydrogen peroxide (30~90 Hydrogen sulphide Hypochlorites Hypochlorites Hypochlorites Hypochlorites Lead perchlorate Lime (CaO) Maleic acidR Manganate, potassium (K) Meat juices	R R R R R R R R R R R R R R R R R R R	B00C(140F) R R R R R R R R R R R R R	Criterinical Name A Mercury Methanol Methanol Methanol Methanol Methanol Milk products Moist air Molasses Monoethanolamine Naptha Naptha Napthalene Nickel salts Nitric acid (50%) Nitric acid (50%) Nitric acid (150%) Nitric acid (150%) Oils, uegetable and animal Oxalic acid Ozone Paraffin wax Petroleum spirits Phenol	R R R R R R R ND NR R R R R R R R R R R	R R NR R R ND NR ND NR NR NR NR NR NR NR NR NR NR NR NR R R NR N

1181 Appendix 3- Growth Experiment Pictures

1182 Appendix 3.1 – Day 0: all 15 tanks with light setup and slight coloration in tanks 1-3.



1184 Appendix 3.2 - Day 2: all 15 tanks with coloration in all tanks, but slightly less in tanks 9-15



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- 1188 Appendix 3.3 Day 4: All 15 tanks visible growth based on coloration, almost similar
- 1189 coloration in all 15 tanks.



1191 Appendix 3.4 – Day 5: All 15 tanks with great coloration in tanks 4-15, while tanks 1-3 have

1192 more bottom waste and less coloration.



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- 1194 Appendix 3.5 Day 7 (End of experiment): All 15 tanks with coloration and bottom waste,
- 1195 but with reduced coloration in tanks 1-3.



- 1197
- 1198 Pictures Taken by Hans Henriksen Marki before sampling throughout the experiment.