Effects of Salt Interactions in Oil Sands Process Waters on *Ceriodaphnia dubia* using an Isobolographic Approach

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ABSTRACT

Canada's oil industry is booming in large part due to the oil sands mining of Alberta's Athabasca River area. The hot water extraction process being used to separate the bitumen from the sand produces large amounts of liquid tailings with elevated levels of naphthenic acids and salts. Naphthenic acids and total salinity are known to cause adverse effects on aquatic biota, but the study of individual ion interactions in oil sands process waters (OSPW) may be a significant factor in OSPW toxicity that has been largely unexplored. Major ions contributing to total salinity in OSPW were studied using an isobolographic study design to determine the nature of the toxic interactions between NaCl and Na_2SO_4 on the cladoceran *Ceriodaphnia dubia*. The toxic interaction determined on the LC_{50} isobole graph between NaCl and Na_2SO_4 suggested additivity. There was a slight deviation towards less than additive interactions at ratio levels of 6:10, 1:1, and 10:6 of NaCl:Na_2SO_4. The IC_{50} isobole was within 95% confidence limits of additivity. With further work (studying other major salt ion interactions) the data presented here will help determine the nature of toxic interactions between the different salinity ions present in OSPW and may contribute to industry's reclamation plans of OSPW tailings.

INTRODUCTION

Alberta's Athabasca Oil Sands are the largest single oil deposit in the world and a growing part of the Canadian economy (Rogers *et. al*., 2002). Oil collected from the Athabasca region accounts for more than 25% of Canada's annual oil production (Leung *et. al.*, 2003). Naturally occurring bitumen, which must be processed and refined, makes up these lucrative oil sands of North Eastern Alberta. The resulting products of oil sands mining are a profitable synthetic crude oil along with oil sands process water (OSPW), tailings, and other remains.

Most often, the Modified Clarke Hot Water Extraction process is used to separate out the profitable oil from the sand. In the process of extraction, oil sands industry produces approximately 4 m^3 of OSPW for every m^3 of oil sands processed (Peters *et. al.*, 2007). Operating under a zero-discharge policy (Clemente & Fedorak, 2005), the oil sands industry sequesters OSPWs in settling basins allowing fines to settle out, densification to occur, and much of the water to be recycled back into the extraction process (Leung *et. al.*, 2003).

In an effort to implement reclamation strategies, OSPW components and their associated toxicity have been studied and naphthenic acids were found to be the major factor in aquatic toxicity (MacKinnon and Boerger, 1986). OSPWs are known to contain elevated levels of naphthenic acids ranging from 40 to 120 mg total naphthenic acids/L (Holowenko *et. al.*, 2002). Multiple studies have determined the acute lethality of fresh tailings water on aquatic organisms (Leung

et. al., 2003). Upon treating tailings water to remove naphthenic acids, OSPW toxicity levels significantly decrease (MacKinnon and Boerger, 1986).

In addition, OSPWs contain elevated levels of salts and dissolved organics resulting primarily from the leaching of ore, the addition of process chemicals, and the recycling of the waters (Leung *et. al.*, 2003). Significant toxic effects due both to salts, at levels as low as 700 µS/cm, and naphthenic acids, at 6-20 mg total naphthenic acids/L (Leung *et.al.*, 2001), have been observed in microcosm and mesocosm studies on phytoplankton and zooplankton (Leung *et. al.* 2003). Salinity's effects on community structure in OSPWs have not been definitively characterized (Leung *et. al.*, 2001).

Saltinity has been acknowledged as a significant contributor to OSPW toxicity but the majority of research fails to study the individual ion compositions of OSPWs. Regularly, the salinity of OSPW is quantified by a measure of total conductivity. In studies focused solely on the salinity tolerance of aquatic organisms (i.e. outside of the context of oil sands processing), individual ion compositions of saline waters show an effect on the toxicity levels (Dwyer *et. al.*, 1992). When studying toxicity due to conductivity, Mount *et. al.* (1997) pointed to studies such as Dickerson *et. al.* (1996) with *Ceriodaphnia dubia* LC_{50} values around 3700 µS/cm and contrasted them with Jop and Askew's work (1994) finding *C. dubia* tolerance as low as 1800 µS/cm. Multiple studies are pointing to ionic concentrations, not solely total conductivity, as a factor of toxicity.

This study separated the major individual ionic components of OSPW and looked at the possibility of toxic interactions of the salt ions on a well-known laboratory-cultured aquatic invertebrate, *Ceriodaphnia dubia*. Using an iosobolographic approach (Christensen *et. al.*, 2001), effects of NaCl and Na_2SO_4 dilutions were observed on *C. dubia* and graphed to determine the nature of toxic interactions between the salts. A better understanding of individual salt ion toxicity (particularly some of the major ions contributing to OSPW salinity – sodium, sulfate, and chloride) would aid in characterizing OSPW toxicity.

As part of land use agreements with the Alberta government, the oil sands industry will incorporate OSPW filled ponds into future reclamation strategies. In order for the industry to create sustainable and successful reclamation, a greater understanding of the factors of OSPW toxicity needs to be considered. This research aims to analyze OSPW toxicity by reporting individual major ion toxicities and ion interaction toxicities in order to improve reclamation of oil sands tailings ponds.

MATERIALS & METHODS

1) Isobologram Study Design

An isobolographic technique (Christensen *et. al.*, 2001) was used to create the study design and analyze the data resulting from *C. dubia* bioassays. A general representation of an Isobologram is presented in Figure 1. Multiple studies have examined the merits of using isobole methods in biological assays to determine the interaction of two compounds (Christensen *et. al.*, 2001; Merino-Garcia *et. al.*, 2003; Gessner, 1995).

An isobole is a curve of constant response graphed versus two compound concentrations (Christensen *et. al.*, 2001). The endpoint of one xenobiotic alone is graphed against the same endpoint of the second xenobiotic on x and y axes. Connecting these two points by a straight line results in a theoretical line of additivity. If the compounds work in a manner where the concentration of one could be substituted for the other and produce the same toxic effect, the compounds are said to be additive.

The interaction of the two compounds together can also act in ways outside of the simple line of additivity. If an isobole line forms below the line of additivity, the compounds are said to act in a greater than additive (or synergistic) manner. A mixture of synergistic compounds would have a lower LC_{50} value (i.e. cause a higher lethality) than the LC_{50} values of either one of the two xenobiotics alone at comparable concentrations.

The opposite is true for isoboles that fall above the line of additivity. Isoboles above the line of additivity (and below the line of no addition – a line perpendicular from the axis at the point of both compounds' LC_{50} points) are considered to have an effect that is less than additive. A less than additive line suggests that the combination of the compounds acts in a way that depreciates the individual toxicities of either substance.

Points falling outside of the lines of no addition (sometimes referred to as lines of independent action) are considered to have an antagonistic interaction. In

this case, one compound may be acting to prevent the adverse effects of the opposing compound.

The first bioassays conducted were to determine the LC_{50} values of the individual salts. LC_{50} values were determined using a logistic regression model for both NaCl and Na_2SO_4 and plotted on the x and y axes of the isobologram, respectively. Six ratios of salts were then chosen to be tested. Salt ratio bioassays were conducted at 1:0, 10:3, 10:6, 1:1, 6:10, 3:10, and 0:1 of NaCl to Na_2SO_4. Each isobole's maximum dilution concentration was chosen at a point twice that of the expected LC_{50} concentration of additivity to allow for the possible graphing of less than additive effects (i.e. an LC_{50} point falling above the line of additivity).

2) *Ceriodaphnia dubia* bioassays

Organism culturing, handling, and toxicity tests were conducted according to Biological Test Methods: Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia* (Environment Canada, 2007).

Ceriodaphnia dubia were cultured in medium hard water (80-100 mg/L as $CaCO_3$) on a 16:8, light to dark photoperiod. Each water change (on days 0, 4, and 6 of a 7-day life cycle) included neonate disposal of 1st and 2nd broods (in order to be certain of the age of the organisms) and replenishment with food and nutrients. Nutrients added were Vitamin B12 and Selenium, both at final concentrations of 2 µg/L. Cultures were fed 2 mL of yeast, Cerophyll™, and trout chow (YCT) and 10-15 mL of *Pseudokirchneriella subcapitata* algae per 2 L culture jar, daily. Organisms were transferred via pipette with minimal handling.

The bioassays performed were 3-brood, chronic toxicity, static renewal tests during a 7 day period. Endpoints studied were lethality and reproduction. Each test had 6 concentration dilutions including one control (in medium hard water) with 10 replicates per treatment and one *C. dubia* per replicate, totaling 60 organisms in one bioassay. Neonates chosen for bioassays from culture water were less than 24 hours old and from the third brood of one week old adults.

Serial dilutions were performed to obtain concentrations of 100%, 50%, 25%, 12.5%, 6.25% and a control. Salts were weighed and diluted with control water to create a 100% stock solution. Each 250 mL dilution concentration then received 125 µL of Selenium and Vitamin B12 for a final concentration of 2 µg/L.

Dilutions were then poured into six sets of ten 30 mL beakers with approximately 25 mL of solution each. Each 30 mL beaker then received 250 µL of algae (*Pseudokirchneriella subcapitata*) and 25 µL of YCT. One neonate organism was transferred via pipette into each 30 mL beaker from the culture water.

Water was changed every 24 hours, neonates were counted and discarded, and nutrients and food were replenished. Water chemistry was measured in control, 100%, and 25% solutions at the beginning, middle, and end of each 7-day bioassay. Measurement of temperature, dissolved oxygen, pH, conductivity, hardness, and alkalinity were conducted in three replicates of each concentration tested. Temperature was kept at 25 ±1°C, dissolved oxygen at 90-100% saturation, and pH at 6.0-8.5. Conductivity varied in accordance with salt concentrations, but

was stable day to day. Hardness and alkalinity measurements were also within Environment Canada's range of acceptability for 7-day chronic *C. dubia* bioassays.

All bioassays were within Environment Canada's limits of validity for reproduction and mortality. Mean mortality of controls never exceeded 20%, at least 60% of controls produced 3 broods in 8 days, and an average of at least 15 live young were produced per surviving control adult.

3) Analyses

LC_{50} values were determined using the Trimmed Spearman-Karber (TSK, US EPA) regression model analysis, and IC_{50} values were determined using a linear interpolation method for sublethal toxicity: the Inhibition Concentration (ICp, US EPA) approach. 95% confidence limits were estimated with the same programs. Once single salt lethality and inhibition concentrations were determined, endpoints were graphed on isobole axes to determine an isobolographic theoretical line of additivity. Salt ratios were then graphed on the isobologram to visually determine whether or not salt interactions were less than, greater than, or exactly additive.

RESULTS & DISCUSSION

Lethality and inhibition concentrations are presented in Table 1. NaCl and Na_2SO_4 LC_{50} values were first determined (1.9 & 2.1 g/L, respectively) and graphed on the axes of the Isobologram. Subsequent salt ratio volumes were determined based on original single salt LC_{50} values.

The shape of the LC_{50} isobole (Figure 2) suggests a line that is additive with slight deviations toward less than additive effects. The LC_{50} isobole is comprised within 95% confidence limits of a line of additivity. All points except one fall with confidence limits within the 95% confidence limit of a line of additivity. The 10:6 ratio of NaCl:Na_2SO_4 falls completely outside of the line of additivity and its 95% confidence limits. This 10:6 point may be an outlier.

The 10 NaCl: 6 Na_2SO_4 ratio may suggest evidence of interactions between the salts at a level other than additivity. At this salt proportion, sodium chloride and sodium sulfate show effects of no additivity or antagonism on *Ceriodaphnia dubia*. An interaction of no additivity is likely for two compounds that act by two completely different mechanisms. This probable result of no additivity seems unlikely for salts that could be toxic by similar mechanisms, suggesting the ratio point 10:6 could be an outlier. This point could also reflect an interaction between Cl^- and SO_4^{2-}, or a combined interference with Na^+ uptake. More research is necessary to answer this question.

The IC_{50} isobole (Figure 3) is a line completely within the 95% confidence limits of a hypothetical line of additivity. The IC_{50} isobole suggests the two salts cause similar effects at similar doses on *C. dubia*, irrelevant of the salt ion. Being that the slope of the sigmoidal curve of toxicity of the salts on *C. dubia* was so steep, inhibition concentrations, and thus the line of additivity, have very wide confidence limits. The IC_{50} isobole's line of additivity has confidence limits that more easily include the range of effects produced by the varying salt ratios. The points making

up the IC_{50} isobole trend toward more than additive effects at the 3:10 and 10:3 ratios of NaCl:Na_2SO_4.

The markedly additive effects of NaCl and Na_2SO_4 could suggest that the observed toxicity differences of OSPWs may be due to other major ions. The low toxicity, high sulfate tailings pond waters of the Syncrude Canada Ltd. site suggest protective salt interactions between sulfate and another compound. The high sulfate waters have a high total conductivity with relatively low effects on *Ceriodaphnia dubia*. These results suggest that Na^+ or Cl^- are more toxic than SO_4^{2-}. Current research of D. Turcotte *et. al.* is considering the interactions between salt ions (not total ionic content) as the cause of OSPW toxicity in the tested Syncrude waters. The isoboles studied here suggest sodium chloride is likely not the salt interacting with sulfate to act protectively is OSPW (NaCl and Na_2SO_4 act additively). The additive interactions of NaCl and Na_2SO_4 may infer that community differences of microcosm studies of OSPWs (Leung *et. al.*, 2001), if influenced by salinity, may be resulting from other ion interactions or effects of salinity on other (non-*C. dubia*) species of the communities.

Salinity as a confounding factor of community changes cannot be ruled out without further studies of salt interactions. Further studies, perhaps determining NaOH and Na_2SO_4 isoboles, or $MgCl_2$ and NaCl isoboles (and eventually all of the isoboles of major ions in the OSPW) would be beneficial in understanding salt interactions of process waters. Based on the characterization of salt interactions in *C. dubia*, salt interactions in larger aquatic communities could be hypothesized and

thus help to explain the seemingly confounding factor of salinity in ecological studies of OSPW. With further work, a better knowledge of the toxicity of OSPW salinity may be used as a tool to enhance the reclamation of tailings ponds in the Athabasca oil sands.

CONCLUSION

NaCl and Na_2SO_4 salt interactions trended towards additive effects on *Ceriodaphnia dubia*. With experimentally determined LC_{50} values of 1.9 and 2.1 g/L for NaCl and Na_2SO_4 respectively, an isobolographic method aided in exposing the major salt interactions of OSPW. Application of this knowledge to oil sands reclamation will aid in the characterization of toxic compounds in tailings ponds that will be re-integrated into sustainable ecosystems. With further studies of the interactions of other major salt ions of OSPW and a greater knowledge of salt ion toxicities in other aquatic organisms, reclamation plans of tailings ponds of the vast Canadian oil sands industry may be more successful.

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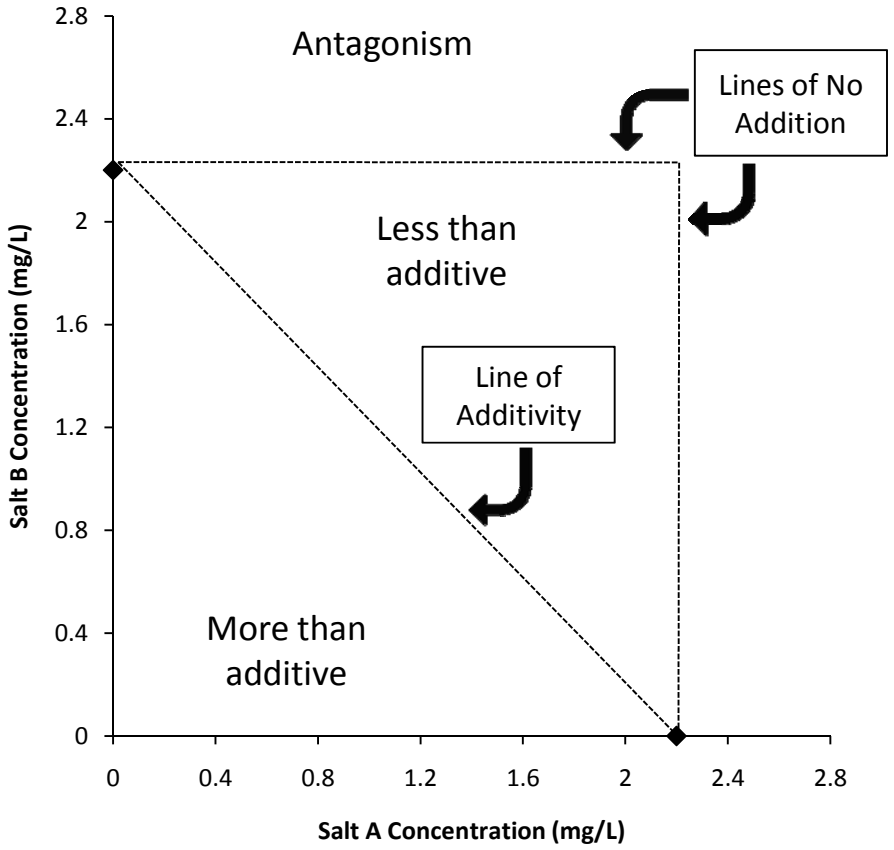

Figure 1 - Theoretical Isobologram: Compounds that act additively fall on the line of additivity. Endpoints below the line of additivity cause greater than additive effects, points above the line of additivity cause less than additive effects. Endpoints of substances that do not interact fall on the lines of no addition. Points falling outside the lines of no addition act antagonistically (Adapted from Christensen *et. al.*, 2001).

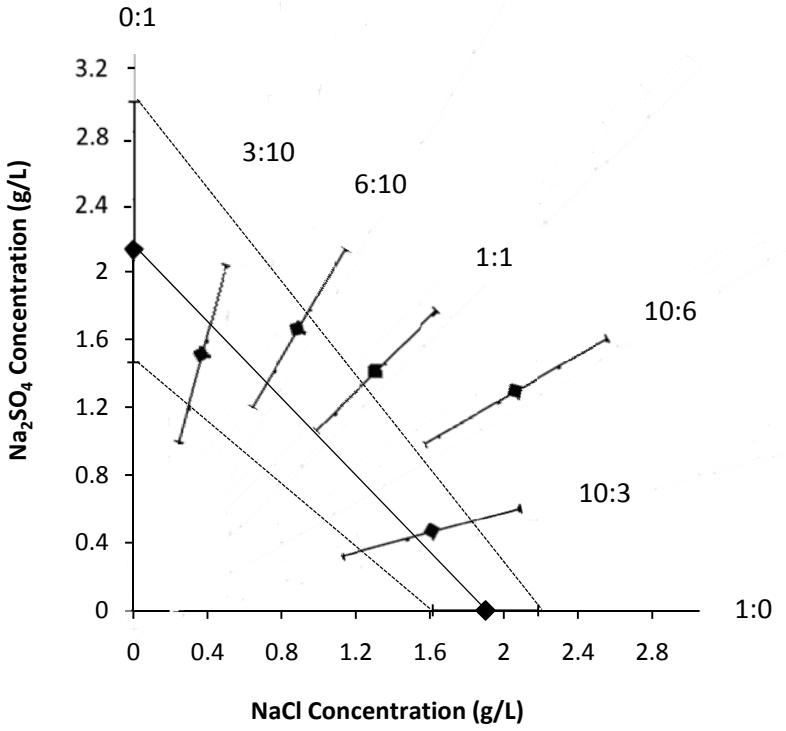

Figure 2. LC_{50} Isobole – NaCl LC_{50} (1.9 g/L) and Na_2SO_4 LC_{50} (2.1 g/L) are graphed along the X and Y axes respectively. A theoretical line of additivity (with 95% confidence limits) is drawn between the NaCl and Na_2SO_4 LC_{50} points. Ratio points of 3:10 and 10:3 (NaCl:Na_2SO_4) lie within the confidence limits of a line of additivity. Points 6:10 and 1:1 (NaCl:Na_2SO_4) lie just above the line of additivity. Point 10:6 (NaCl:Na_2SO_4) lies above the line of additivity.

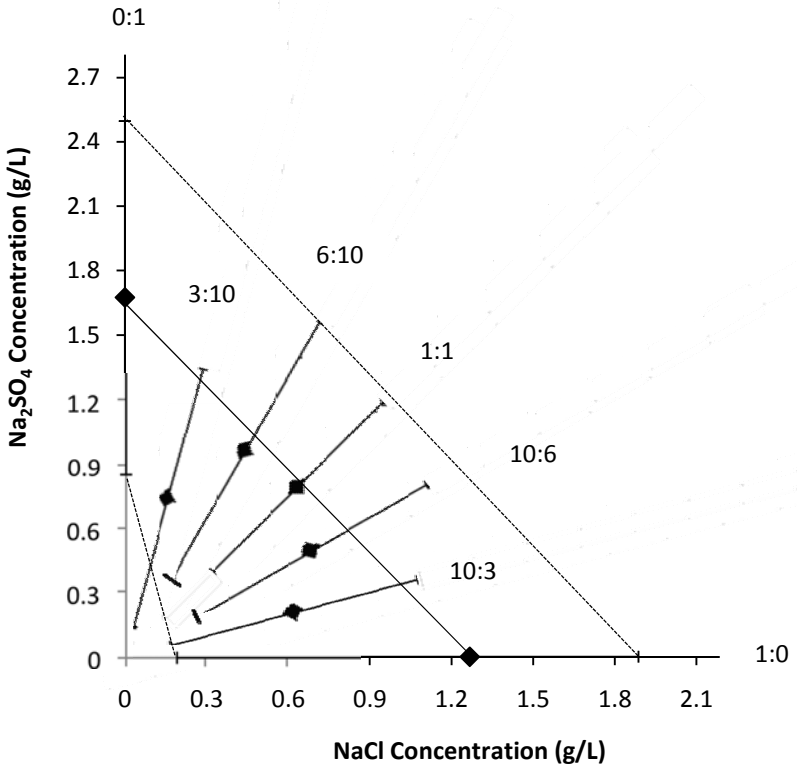

Figure 3. IC_{50} Isobole – NaCl IC_{50} (1.27 g/L) and Na_2SO_4 IC_{50} (1.68 g/L) are graphed along the X and Y axes respectively. A theoretical line of additivity (with 95% confidence limits) is drawn between the NaCl and Na_2SO_4 IC_{50} points. All ratios lie within 95% confidence limits of the line of additivity. A markedly more-than-additive trend is observable.

Table 1: LC_{50} and IC_{50} Values (With Lower and Upper Confidence Limits – LCL & UCL) of NaCl:Na_2SO_4 Mixtures in 7-day *C.dubia* Bioassays.

		LC_{50} Composition	
LC_{50}	**Mixture Tested**	**NaCl g/L (95% LCL - 95% UCL)**	**Na_2SO_4 g/L (95% LCL - 95% UCL)**
	1:0 NaCl:Na_2SO_4	1.9 (1.6 - 2.2)	0
	0:1 NaCl:Na_2SO_4	0	2.1 (1.5 - 3.0)
	1:1 NaCl:Na_2SO_4	0.9 (0.7 - 1.2)	1.0 (0.8 - 1.3)
	6:10 NaCl:Na_2SO_4	0.7 (0.5 - 0.9)	1.2 (0.9 - 1.6)
	3:10 NaCl:Na_2SO_4	0.7 (0.5 - 0.9)	0.9 (0.6 - 1.2)
	10:6 NaCl:Na_2SO_4	0.6 (0.5 - 0.8)	2.0 (1.6 - 2.5)
	10:3 NaCl:Na_2SO_4	0.2 (0.2 - 0.3)	1.6 (1.2 - 2.1)
		IC_{50} Composition	
IC_{50}	**Mixture Tested**	**NaCl g/L (95% LCL - 95% UCL)**	**Na_2SO_4 g/L (95% LCL - 95% UCL)**
	1:0 NaCl:Na_2SO_4	1.3 (0.2 - 1.8)	0
	0:1 NaCl:Na_2SO_4	0	1.7 (0.3 - 2.3)
	1:1 NaCl:Na_2SO_4	0.7 (0.1 - 0.9)	0.7 (0.1 - 1.2)
	6:10 NaCl:Na_2SO_4	0.5 (0.1 - 0.7)	0.9 (0.2 - 1.2)
	3:10 NaCl:Na_2SO_4	0.4 (0.1 - 0.7)	0.6 (0.1 - 1.0)
	10:6 NaCl:Na_2SO_4	0.3 (0.1 - 0.4)	1.0 (0.2 - 1.4)
	10:3 NaCl:Na_2SO_4	0.1 (0.0 - 0.2)	1.0 (0.2 - 1.5)

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