**Research Paper**

# Teratogenic effect of Sodium Metabisulphite in the Embryogenesis of *Gallus gallus domesticus*

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**Abstract**

The pinnacle of advances in food processing offered tremendous advantage preserving various foods with the aid of food preservatives. One of the most common preservatives use is the Sodium Metabisulphite. In this study, Sodium Metabisulphite was injected in chicken eggs to evaluate the embryonic effects of different concentrations of Sodium Metabisulphite. After 5 days incubation period, the injected eggs were examined. The results showed that malformations and deformities are evident in chicken embryo and there are even suppressions of growth and development especially in the 4% concentration. Thus, the study has shown that Sodium Metabisulphite probably trigger Teratogenic responses on different stages of development in chick embryo. Moreover, the researcher highly recommended the biochemical and molecular testing to confirm the teratogenic effect of Sodium Metabisulphite.

**Keywords:** Teratogen, Preservatives, Sodium Metabisulphite, Embryogenesis

## Introduction

Food industry is one of the major contributors in the economy. To continuously be profitable, every industry invest heavily on research and development to produce safe and high quality products. The discovery of preservatives contributed tremendously in the quality control of the food. The most common preservatives use is Sodium Metabisulphite. Sulphites are naturally formed in the body and are also found in food. It is used as preservatives to protect food from enzymatic and non-enzymatic browning and is extensively used in wine making specifically to maintain the color and prolong its shelf life. It has been classified by the European Union as one of the food preservatives. It can normally be produced by the human body through degradation of amino acids such as methionine and cysteine-sulphur. The

exogenous source for sulphites can be found in foods, drinks and pharmaceutical products (El Kadi et. al. 2014).

Moreover, Sodium metabisulphite is a yellowish- colored crystalline powder. It is an organic salt that are often used too as disinfectant and an ingredient in hair care products.Sodium Metabisulphite has received its generally recognized as safe rating (GRAS) and is FDA approved following doses safe for human consumption. It is also considered as a moderate hazard ingredient with concerns on cancer, developmental and reproductive toxicity, immunotoxicity, irritation and organ system toxicity, but with lesser concerns on neurotoxicity based on the Cosmetics Database. Furthermore, the Association of Occupational and Environmental Clinics considered Sodium Metabisulphite as a human immune and respiratory toxicant while other organizations classify it as an irritant; thus, it is expected to be toxic or harmful(Wright et. al.1989).

However, according to the final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite, undiluted Sodium Metabisulfite was not found to be an irritant to rabbits following occlusive exposures, but 50% concentrations was irritating to guinea pigs following repeated exposure. This is a concentration that would not be found in cosmetics, hair, or personal care products. It was also found to be negative in mutagenicity studies. The study concluded that that Sodium Metabisulfite is a safe ingredient in cosmetics and personal care products. Despite of the result, it is worth noting that the Material Safety Data Sheet for Sodium Metabisulfite labelled as Hazardous in case of skin contact (irritant), of ingestion, of inhalation. Slightly hazardous in case of skin contact (permeator), of eye contact (irritant) and that it may be toxic to upper respiratory tract, skin, and eyes(Nair and Elmore, 2003).

In contrast, the effects of sodium bisulphite on mitosis were investigated in allium showed that there is a significantly reduced Mitotic Index at all concentrations and treatment period, 7.5mg/Lt, 15mg/Lt, and 30 mg/Lt, showing that sodium bisulphite has cytotoxic, aneugenic and genotoxic effects on *Allium cepa.* The researchers recommended that it is necessary to be careful in using it as food preservative and antimicrobial substance. There are still few literatures proving that Sodium Metabisulphite can really have negative adverse effects on the organism, specifically on its development and/or reproductive parameters, thus, can affect normal mechanism of growth and development (Kayraldiz et. al. 2001).

The study on *Gallus gallus domesticus* as model organism for human development using embryo test is demonstrated to be reliable and to afford quantifiable end points for evaluation. Individual compounds, mixtures of compounds and against and antagonist can easily be Administered and tested. The chicken embryo possesses its own basic enzyme catalyzed drug - transformation capacity and moreover, it can be used for screening specific human metabolites. As a carrier of a complete set of developing morphogenetic system, the chick embryo in ovum manifests an advantage over those *in vitro* systems that employ isolated embryos or embryonic tissues that have only limited survival(Kotwani, 1998).

In the Philippines, the effect of exposure to Sodium Metabisulphite is poorly studied.Thus, this study provide evidence and investigated the possible developmental embryonic toxicity and teratogenic effects of Sodium Metabisulphite in relation to the malformations, deformities and mortality in chicken embryo as model organism of human development with the different test concentrations approved by Food and Drug administration (FDA) for human consumption.

## Materials and Methods

### Sample Collection

The eggs from *Gallus gallus domesticus* were collected in different poultries in Macarat, Abuno Iligan City, Philippines. The gathered eggs were five days old and weigh an average of 45 grams.

### Incubation

The eggs were put in the incubator manufactured by Abellar Manufacturing Incorporated at Izon Poultry and Supply and were incubated under standard conditions with Temperature of 37.0 degree Celsius and Relative Humidity of 55% until 18 days. The eggs were candled on the 5th day of incubation, wherein unfertilized eggs and those with dead embryos were discarded. On day 6 of incubation, the eggs with living embryos (n=40) were randomly divided into four equal groups; control and three doses of sodium metabisulphite concentrations (1ml of 2.5 ppm, 1 ml of 10 ppm and 1 ml of 40 ppm of Sodium Metabisulphite were used as experimental group. There are 10 eggs used in each set up namely the control without the application of sodium Metabisulfite 2.5ppm, 10 ppm N, and 40 ppm $NA_2S_2O_5$.

### Injection of Sodium Metabisulphite ($NA_2S_2O_5$)

Sodium Metabisulphite has chemical formula $NA_2S_2O_5$ with Molecular Weight of 190.11 and has CAS no. 7681-57-4. The experimental group ovum was *i*njected with 1 ml of Sodium Metabisulphite. The administered dose of Sodium Metabisulphite was calculated based on the allowable content of $NA_2S_2O_5$ as preservatives in the Philippine Food Industry. Ten (10) pieces of eggs as control were not treated with the $NA_2S_2O_5$ while the eggs of the 3

experimental groups were injected with 1 ml of 2.5 ppm, 1 ml of 10 ppm and 1 ml of 40 ppm of $NA_2S_2O_5$. The control set up has not injected with Sodium Metabisulphite. The injections were given at the small end of the egg, deeply into albumin.

**Examination of Egg**

The age, malformations, and malposition of the embryo were estimated at the moment of death. The incubated eggs were candled to determine viable egg after 18 days. The incubated egg is being candled to classify the viable and nonviable eggs. The eggs were then examined of any developmental deformities. The mortality were statistically analysed by linear correlation and regression.

**Data Analysis**

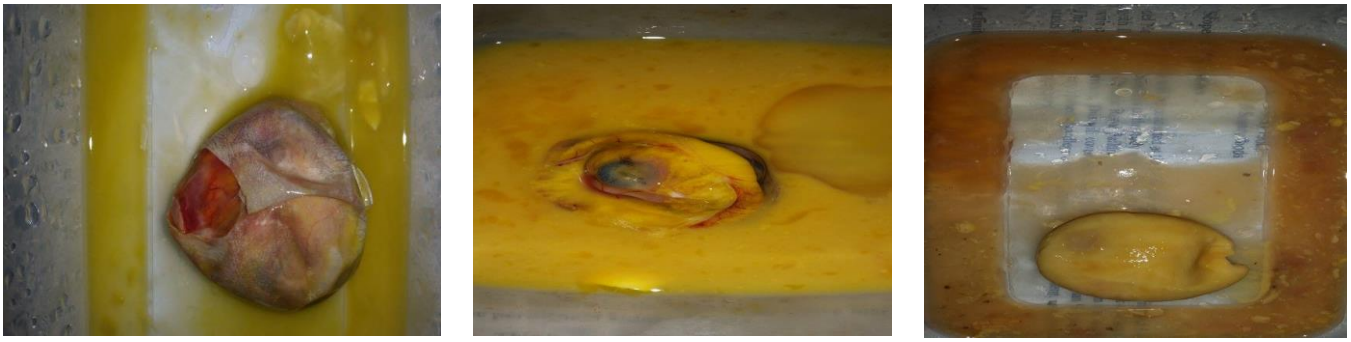
The Linear correlation and regression analysis is used to assess the obtained results. The statistical analyses were performed using Sigma-Stat (SPSS Science Software Ltd., USA).The results were presented as means ± SEM. and considered significant at $P<0.05$ and highly significant at $P<0.01$.

**Results and Discussion**

Results showed that Sodium Metabisulphite had an inhibition effect on the development of chick embryos as seen in the different test concentrations, in which, development of some embryos were hindered and malformations were visible compared to that of the control.

A. B. C.

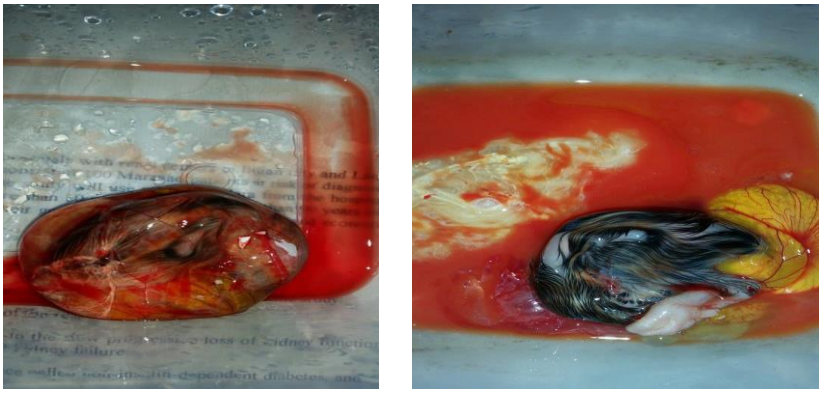
**Figure 1** is the control set up. It shows Top view of the embryo that grows rapidly assuming hatching position with the head under the right wing and beak toward the air cell. Remaining yolk sac (A-C) begins entering the body cavity. The figure shows the normal formation of the body parts of the chicken expected on its $5^{th}$ day incubation period, the appearance of the potential basic body plans such as the head, wings, visibility of the beak. There is no observed anomaly or malformations on the $5^{th}$ days of incubation period. The expressions of basic phenotypes are highly probable



A. B. C.

**Figure 2** shows the top view of embryo with 3 different developmental deformities observed in eggs injected with 2.5 ppm of $NA_2S_2O_5$. A.)Beak deformity, limb deformity, scanty feathers and wings did not develop B.)Only the eyes are developed, limb deformity, head fails to form and C.) Clumping of potential circulatory system and presumptive brain. The

figure 2 shows that Sodium Metabisulphite can cause developmental deformities in chicken embryo even at 2.5 ppm. The Beak and limb deformities, scanty feathers and failure of some vital organs were observed.

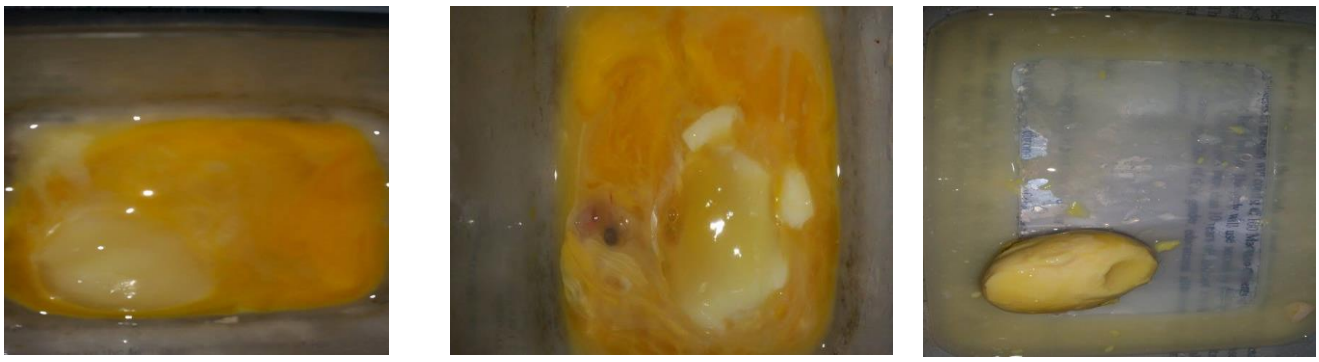


A. B.

**Figure 3** shows the top view of viable embryo injected with 10 ppm $NA_2S_2O_5$. (A)The embryo is still well covered with amniotic yolk sac and basic body plan is about to developed (B.)Embryo grows assumed a hatching position with the head under the right wing and yolk sac begins entering body cavity.The figure 3 shows that there are chicken embryos injected with 10 ppm $NA_2S_2O_5$ that shows potential formation of body plans and will likely have a normal development.

A. B. C.

**Figure 4** Shows the top view of embryo injected with 10 ppm $NA_2S_2O_5$, (A.)There is no development that took place (B.) There is a clumping of the Blastoderm; basic body plan did not develop. (C)The embryo is about to undergo differentiation but did not materialized. There is clumping of the Blastoderm and the differentiation of basic body plan was suppressed. Figure 4 shows the total inhibition of the embryonic development in eggs injected with 1.0% NaMBS, there is clumping and no cell differentiation took place



A. B. C.

**Figure 5** shows the top view of the embryo injected with 40 ppm of $NA_2S_2O_5$ (A-C) the clumping of Blastoderm, stunted development and differentiation of basic body plan is suppressed.The figure 5 which shows the embryo injected with 40 ppm of Sodium

Metabisulphite shows total inhibition of the chicken development as there was a clumping of Blastoderm, stunted development and total suppression of the body plan.

**Table 1:** The effect of different Concentration of $NA_2S_2O_5$ on the Mortality of Chicken Embryo

| Concentration(ppm) | # of Mortality | Survivor | % Mortality |
|---|---|---|---|
| Control | 1 | 9 | 10 |
| 2.5 | 8 | 2 | 80 |
| 10 | 10 | 0 | 100 |
| 40 | 10 | 0 | 100 |

The table shows that as the concentration increases, the number of mortality increases too. There are 20% of chick embryo survived and 80% of 0.25% concentration did not survive. While the effects of 1% and 4% $NA_2S_2O_5$. Concentration showed 100% mortality.

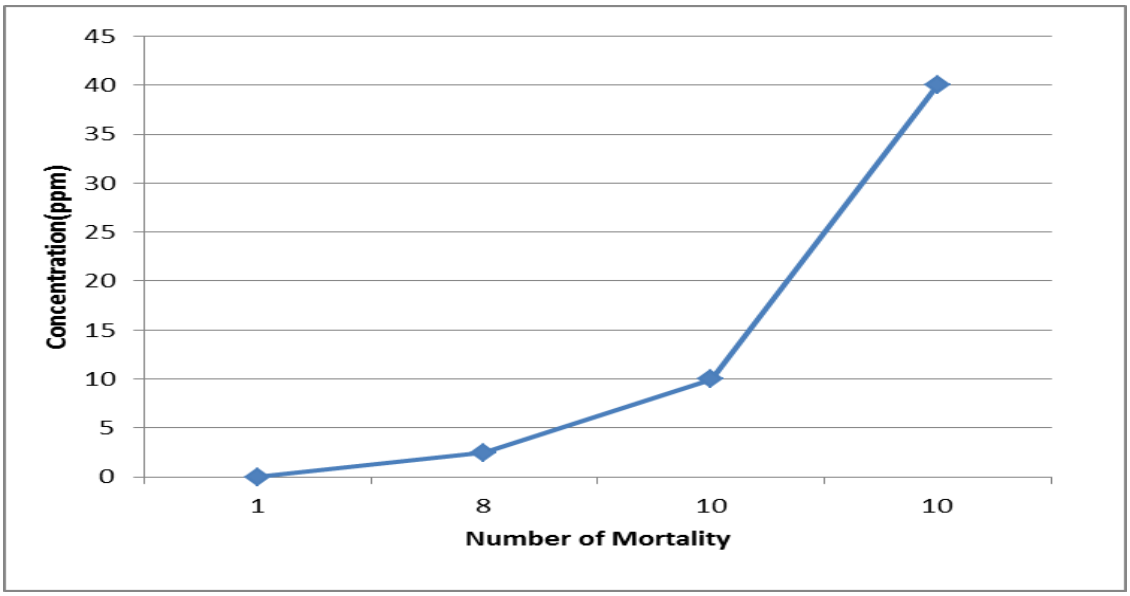
**Table 2:** The Linear correlation and regression of Different concentration of $NA_2S_2O_5$ the Mortality of Chicken Embryo

| r | $r^2$ | Slope | Y-Intercept | Std. Err. Of Estimate |
|---|---|---|---|---|
| 0.591 | 0.349 | 1.37 | 5.4516 | 4.2221 |
| t | df | P | One-tailed | 0.2047 |
| 1.035 | 2 | | Two-tailed | 0.4094 |

Note: 0.95 and 0.99 Confidence Intervals of rho

The statistical tool of Linear Correlation coefficient of 0.591 and regression coefficient 0.349 suggested the positive relationship between concentration and mortality of chick embryo. The P value which is 1.37(P=0.01) is highly significant. This goes to show that as the increased concentration of $NA_2S_2O_5$ contributes to the mortality of the test organism.

**Graph 1.**The scatter plot of the effect of Different Concentration(y) of $NA_2S_2O_5$ on the mortality(x) of Chicken Embryo




The scatter plot graph of chick embryo shows the linear relationship of NaMBS concentration and the number of mortality. This shows that there is a direct proportionality between $NA_2S_2O_5$ and chick mortality, that is, an increase in concentration causes an increase in mortality.

As shown from the different results above, there are differences in the embryotoxic effects in different test concentrations. It showed that the higher the concentration injected, the greater will be the observed morphological malformations and the more it has inhibition effects on the developing embryo. This will probably due to exposure of embryos with different concentrations in the early stage of development, thus possible deformities.

The Teratogenic effect of Sodium Metabisulfite on chick embryo is concentration dependent. As the concentration increases, the degree of deformities and malformations also increases. The Cytotoxicity and Genotoxicity of Sodium Metabisulfite using the *Allium cepa* assay were previously demonstrated[6]. The mitotic cells decreased as the concentrations and duration of treatment increased, there was a significant decrease in the Mitotic Index of the *Allium cepa* cells. Some chromosomal mutations were observed such as chromosome clumping, chromosome bridge and chromosome fragmentation. The Reduction in the mitotic activity could be due to inhibition of DNA synthesis which might be caused by the reduction ATP production, which is essential in the stages of mitosis. A decrease in Mitotic Index could be attributed to the blockage at the G2-phase of the cell cycle, preventing the cell from entering mitosis (Onyemaobi et. al.2012).

The neurotoxic effect of sulphites is due to the release of sulphur and oxygen that can damage development including the Central Nervous System. They found out that sulphite will render toxic effects that affect embryonic development (El Kadi et. al. 2014). Furthermore, Sulphite exerts toxic effects on neuronal cells grown directly or in combination with peroxynitrite. Sulphite oxidase is the key enzyme in the metabolism of sulphites in the body that causes oxidation to sulphate, thus develop severe abnormalities and early death (Reist et. al. 1998).

In addition $NA_2S_2O_5$ has been shown to induce CAs, SCEs and micronuclei and decrease MI in human lymphocytes. Bisulfite causes deamination of cytosine in both DNA and RNA(Pagano et. al.1990).The deamination of cytosine causes base-pair substitution mutations. Bisulfite has been shown to cause deamination in cytosine and adenine. Thus, studies in chicks suggested that the limb abnormalities are maybe caused by inhibition of cell division rather than cell death (Prashant and Natekar, 2007).

## Conclusion

The injection of Sodium Metabisulphite showed alterations and suppressions in the development of chicken embryo. Wherein, when the concentration is increased, mortality and severity of malformations also increases. These results will lead to considerations on the allowed amount of sulphites used as food preservatives safe for human consumption and for further determination of the potential toxic effects of this synthetic preservative, specifically for pregnant women. Furthermore, the researchers recommended among pregnant women and children to refrain from consuming foods which contents preservatives like Sodium Metabisulphite, in line with Food and Drug Association (FDA) policy of strictly regulating the sulphite content in foods.

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