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Ethylene bisdithiocarbamate pesticides Maneb and Mancozeb cause metal overload in human colon cells

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ABSTRACT

Previous studies in our laboratory have shown that ethylene bisdithiocarbamate (EBDC) fungicides Maneb and Mancozeb are equipotent gastrointestinal toxicants that produce cell loss and metal accumulation within HT-29 and Caco2 colon cells. Nabam, $MnCl_2$, $CuCl_2$ and $ZnCl_2$ exposure produced no loss of viability up to 200 μM and increases in metal levels were noted but not to the same extent as Maneb and Mancozeb. EBDC exposure caused increases in copper levels (20–200 μM). Maneb and Mancozeb treatment also caused increases in manganese and zinc concentrations (20–200 μM). Nabam plus $MnCl_2$ and Nabam and $MnCl_2$ plus $ZnCl_2$ caused decreases in viability and increases in metal levels comparable to Maneb and Mancozeb. Decreases in the ratio of reduced glutathione to glutathione disulfide were observed with Maneb and Mancozeb (20–200 μM). Maneb and Mancozeb treatment results in intracellular metal accumulation leading to the oxidative stress. The metal moiety and the organic portion of EBDCs contribute to toxicity.

1. Introduction

Dithiocarbamates (DTCs) are a class of thiocarbamate derivatives, which have been used in agriculture as pesticides, insecticides, and fungicides for more than 50 years. Today, DTCs are among the most commonly used fungicides worldwide (Acquavella et al., 2003; Drechsel and Patel, 2008; Mujawar et al., 2014). Ethylene bisdithiocarbamates (EBDCs) are a subclass of DTCs, used primarily as broad spectrum organometallic fungicides. Among these are Maneb (MB), Zineb (ZB), and Mancozeb (MZ), which are polymeric complexes of EBDC and the transitional metals manganese, zinc, or in the case of MZ, both manganese and zinc (Fig. 1). The combined amount of MB and MZ applied in the United States in 2011 was estimated at 9 million pounds (USGS.gov, 2014). As of 2010 however, the U.S. EPA canceled the registration of MB, allowing only existing stocks to be utilized (epa.gov, 2010). The European Union though remains a major producer and consumer of MB (epa.gov, 2010; efsa.europa.eu, 2014). ZB use ceased in the US in 1998, but is still applied in large quantities in South America (Astiz et al., 2009; USGS.gov, 2014). Aside from their use in the U.S., EBDCs are among the most commonly used pesticides in Denmark, Norway, Brazil, France, and Algeria, among others (Axelstad et al., 2011; Caldas et al., 2004; Medjdoub et al., 2011). Widespread use of these agents can be attributed to their good foliar protection, low environmental persistence, and reported low acute toxicity in humans (Corsini et al., 2005). However, not all of the evidence surrounding these agents supports this claim.

The generation of oxidative stress has been shown to be a major component of EBDC induced toxicity. Numerous *in vitro* and *in vivo* studies have confirmed that exposure to MB causes the generation of reactive oxygen species (ROS) and alterations in antioxidant defense systems (Barlow et al., 2005; Domico et al., 2006, 2007; Grosicka-Maciag et al., 2011; Kurzatkowski and Trombetta, 2013; Zhou et al., 2004). MZ has also been shown to demonstrate a similar pattern of toxicity. Upon MZ exposure, increases in oxidative stress markers and mitochondrial inhibition have been observed (Corsini et al., 2006; Domico et al., 2006, 2007; Medjdoub et al., 2011; Tsang and Trombetta, 2007). Further, several studies have suggested that MZ's pro-oxidant action causes the induction of apoptosis in fibroblasts and peripheral blood cells of rats and human lymphocytes (Calviello et al., 2006; Srivastava et al., 2012). ZB also has the ability to catalyze the formation of ROS and cause pro-oxidant effects. ZB treatment, in both *in vivo* and *in vitro* studies, has resulted in marked increases in oxidative stress and lower antioxidant

Abbreviations: $CuCl_2$, copper chloride; DTCs, dithiocarbamates; EBDC, ethylene bisdithiocarbamate; GI, gastrointestinal; ICP-OES, inductively coupled plasma-optical emission spectroscopy; MZ, Mancozeb; MB, Maneb; $MnCl_2$, manganese chloride; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NB, Nabam; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; $ZnCl_2$, zinc chloride; ZB, Zineb.

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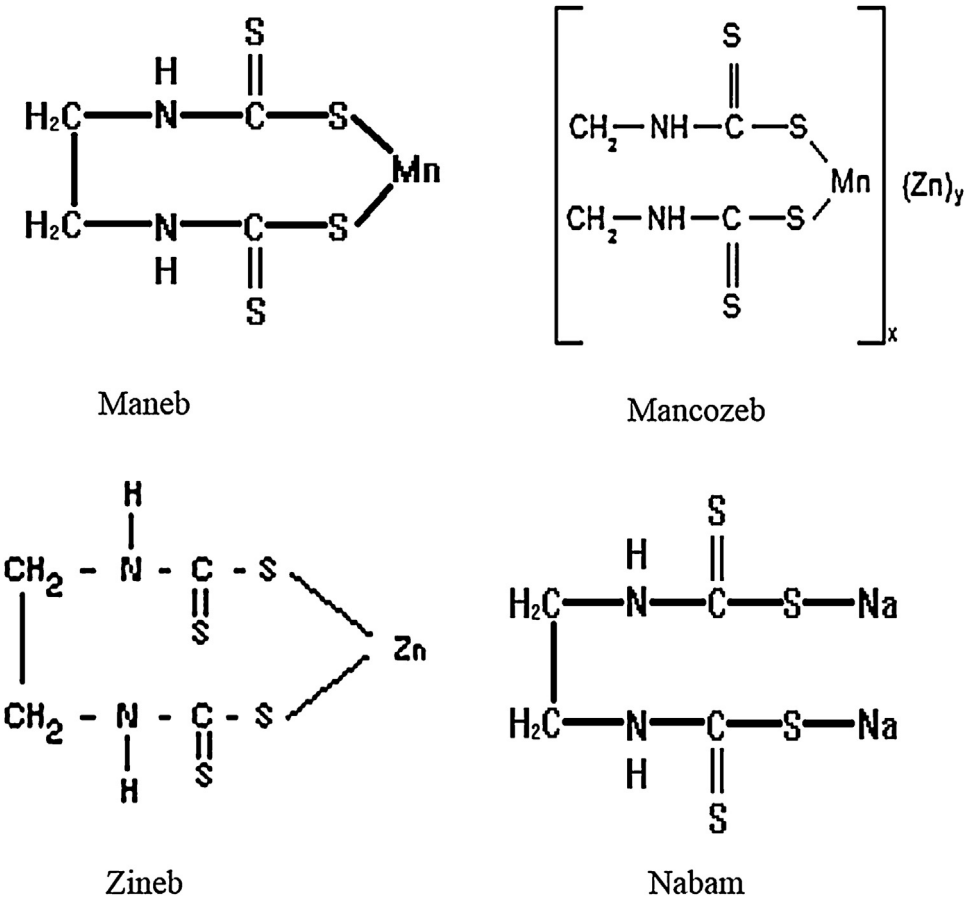

Fig. 1. Chemical structures of Maneb, Mancozeb, Zineb, and Nabam.

capabilities (Astiz et al., 2009; Jia and Misra, 2007; Soloneski et al., 2003). As a result, the potential for EBDC pesticides to induce toxicity may be related to their association with transition metals, which can generate the formation of ROS through a Fenton-like reaction (Fitsanakis et al., 2002).

The organic metal complexes in EBDCs are believed to be stable; however, degeneration to manganese and/or zinc and free EBDC may still occur (Fitsanakis et al., 2002; Grosicka-Maciag et al., 2011). It is unclear whether the breakdown of these agents can initiate toxicity, however, both the organic ligand and metal components have been shown to be potentially toxic (Domico et al., 2006, 2007; Fitsanakis et al., 2002; Nielsen et al., 2006). As thiol containing compounds, DTCs can interact with sulfhydryl groups on critical proteins, eventually inhibiting proper enzymatic function (Tilton et al., 2008; Tsang and Trombetta, 2007; van Boxtel et al., 2010). DTCs are also notorious for their propensity to chelate metals, specifically copper. The ability of DTCs to bind copper with such high affinity may lead to metal toxicity, alteration of metal containing enzymes, and/or the overproduction of ROS (Tilton et al., 2006; Viquez et al., 2008). Likewise, both manganese and zinc have been shown to cause toxicity through the formation of ROS, *via* the Fenton reaction, as a result of their redox potential (Erikson and Aschner, 2003). It has been suggested that the metal component, specifically manganese, is responsible for cytotoxic effects of EBDCs. Upon occupational exposure to MB, workers developed central nervous system disturbances similar to manganese intoxication (Ferraz et al., 1988). In addition, Vaccari et al. (1999) concluded that the manganese portion of MB and MZ and not the organic backbone, was responsible for the inhibition of glutamate transport in brain synaptic vesicles. Further, treatment with MB and MZ has been shown to cause significant increases in manganese concentrations in both *in vivo* and *in vitro* models (Hoffman and Hardej, 2012; Kurzatkowski and Trombetta, 2013; Nielsen et al., 2006; Tsang and Trombetta, 2007). These data suggest that the toxicity associated with EBDC exposure may be due, at least in part, to the metal moiety. Alternatively, Soleo et al. (1996) suggested that the organic portion of EBDCs rather than the coordinated metal ion, was responsible for the cytotoxicity induced in neuronal systems. Other studies however, have suggested that EBDC toxicity may be due to a cooperative mechanism involving both the organic backbone and the metal moiety (Domico et al., 2006, 2007; Li et al., 2013). As a result, further investigation is needed to determine the role the organic backbone and the metal component play in EBDC induced toxicity.

Previously, we investigated the potential of MB, MZ and ZB to initiate toxicity in normal and transformed gastrointestinal (GI) cells, HT-29, Caco2, and CCD-18Co. ZB exposure did not produce toxicity in colon cells within the same concentration range as MB and MZ. Further, we demonstrated that exposure to MB and MZ produced significant increases in intracellular manganese levels within colon cells. Combined, these data suggest that the manganese portion of these EBDCs may play a role in toxicity. To investigate whether the metal moiety was a critical factor in the generation of toxicity, cells were treated with $MnCl_2$ and $ZnCl_2$. If the toxicity of MB and MZ compared to ZB was related to the metal moiety, it would be expected that greater toxicity would be observed with $MnCl_2$ as compared to $ZnCl_2$. However, this was not the case, suggesting that the organic backbone is also instrumental in the cytotoxic process (Hoffman and Hardej, 2012).