

Thioacetamide and its effect on HepG2 spheroids

Thioacetamide (TAA) is a widely utilized model hepatotoxicant, yet its cellular impact in advanced three-dimensional liver-mimetic systems continues to be characterized. HepG2 spheroids—derived from hepatocellular carcinoma cells cultured under conditions that promote multicellular aggregation—offer improved physiological relevance compared with conventional 2D monolayers due to enhanced cell–cell communication, more representative metabolic profiles, and the formation of nutrient and oxygen gradients that approximate aspects of *in vivo* liver tissue. In this study context, the responses of HepG2 spheroids to TAA exposure were examined to better understand how three-dimensional architecture influences toxicant susceptibility and downstream stress signaling. The spheroids exhibited a spectrum of reactions consistent with hepatocellular injury, including metabolic perturbation, oxidative imbalance, and modulation of survival and stress pathways. These responses appeared to emerge not only from direct chemical insult but also from the layered microenvironment inherent to spheroid organization, which shapes diffusion dynamics and cellular heterogeneity. Collectively, these observations underscore the usefulness of HepG2 spheroids as an intermediate-complexity system for modeling hepatotoxicity, enabling the capture of multicellular stress patterns that are often attenuated or absent in 2D cultures. The findings support the growing interest in 3D liver models as more predictive platforms for mechanistic toxicology and preclinical safety assessment.

Thioacetamide (TAA) is widely used in toxicology research as a representative compound capable of eliciting liver-like injury responses *in vitro* and *in vivo*. In three-dimensional HepG2 spheroid cultures, TAA exposure often reveals cellular stress behaviors that are more physiologically aligned with mammalian liver tissue than those captured by traditional 2D monolayers. Spheroid architecture supports enhanced cell–cell interactions and partially restored metabolic capacity, which influences how the cells respond to hepatotoxicants. Consequently, TAA-treated spheroids frequently demonstrate complex patterns of oxidative stress, metabolic disruption, and cell survival signaling that provide a valuable window into hepatotoxic mechanisms.

Within HepG2 spheroids, TAA challenge is frequently associated with altered mitochondrial performance and shifts in energy metabolism. These disturbances can appear as reductions in overall metabolic activity, compromised structural integrity, or modulation of viability markers, depending on the exposure context. Interestingly, the spheroid format can confer a degree of resilience relative to monolayer cultures due to diffusion gradients and improved regulation of detoxification-related pathways. This makes the model particularly useful for studying dose-response trends and evaluating early indicators of cellular stress.