

## Read-across Strategy

The ability to perform scientifically valid read-across of data from one well characterized substance to another substance with little or no data requires that a minimum amount of information pertaining to the unknown substance be compared to the same information from the known substance. For example, read-across could be performed as follows:

1. Start with “known” substances, those for which obligatory toxicology information already exists
2. Develop biologically relevant baseline data for the known substances
3. Compare the same data for the new substance with the baseline data
4. Identify the “known” substance which provides the most accurate comparison and read-across to its respective toxicology

In the case of nickel compounds an assessment strategy has been devised to comply with the upcoming REACH regulations in the European Union. The assumptions used in this strategy are that:

- Exposure to the nickel ion is important in determining systemic and environmental toxicity
- Within an organism and for all endpoints the concentration of nickel ion in systemic circulation or at the intracellular site of action (*i.e.*, as in the case of carcinogenicity) will be the determining factor in whether or not toxicity will occur and a risk exists
- Bioaccessibility and transformation/dissolution data provide specific information on nickel release in relevant compartments for evaluating toxicity
- Analyses and read-across can be conducted for each toxicity endpoint (as opposed to reading-across all endpoints from one single baseline substance)

Evaluating the concentration of nickel at target sites is a two step process. First, the amount of nickel that is bioaccessible in the biological fluids associated with the route of exposure must be assessed. Subsequently, the nickel ions must be absorbed into the tissues of the organism. Finally, the absorbed dose of nickel could be related to the known toxicity of nickel compounds for which a significant amount of toxicity data exist. These “data rich” compounds will form a baseline from which to extrapolate the existing data to nickel compounds that have little or no data based upon their bioaccessibility via different routes of exposure. The baseline compounds for nickel were either 1) assessed in the EU nickel risk assessments (*e.g.*, metallic nickel, nickel sulphate, nickel chloride) or 2) have been extensively studied irrespective of the EU risk assessment (*e.g.*, nickel (sub)sulphides, nickel oxides). In all, five well characterized nickel substances have been identified (Table 1) that can serve as the basis for the comparison of nickel substances with little or no toxicological effects data. These seven compounds will form the foundation from which all read-across for the nickel REACH program will be done.

In order to accomplish the read-across, equivalent biologically relevant data must be gathered on 1) the seven baseline compounds and 2) the compounds with little or no effects data. The comparative test that will be used to perform read-across is termed “bioaccessibility testing”. Bioaccessibility testing assesses the *in vitro* release of nickel ions from nickel compounds in five (5) biologically relevant fluids. These fluids are:

1. Gastric fluid (oral route of exposure)
2. Lung lavage fluid (respiratory route of exposure)
3. Artificial sweat (dermal route of exposure)
4. Cellular interstitial fluid (related to respiratory cancer)
5. Lysosomal fluid (related to respiratory cancer)
6. Representative aquatic media (*i.e.*, dissolution kinetics for the aquatic environment)

Nickel ion release in the appropriate fluid related to the route of exposure in question for each toxicological endpoint will be assessed on the baseline substances and on the “toxicological data poor” substances. This will enable information about these unknown substances to be compared to equivalent data from the known (baseline) substances and thus the possibility of “read-across” of the effects data will be evaluated. Essentially, if the bioaccessibility data match that determined for the baseline compounds, the toxicological data for that baseline compound will be read-across to the “data poor” compound. If the bioaccessibility falls between two baseline compounds, the read-across will be to the more toxic of the boundary baseline compounds shown in Table 1.

**Table 1: Toxicological Classifications for “Data Rich” Nickel Compounds in the EU**

Endpoint	Nickel Sulfate	Nickel Chloride	Nickel Sulfide/ Nickel Sub sulfide	Nickel Monoxide	Metallic Nickel#
Acute Oral	Xn;R22	T;R25	None	None	None
Acute Inhalation	Xn;R20	T;R23	None	None	None
Dermal Irritation	Xi;R38	Xi;R38	None	None	None
Eye irritation	None	None	None	None	None
Dermal Sensitization	R43	R43*	R43	R43	R43
Respiratory Sensitization	R42	R42	None	None	None
Chronic Toxicity	T; R48/23	T; R48/23	T; R48/23	T; R48/23	T; R48/23
Reproductive Toxicity	Cat2;R61	Cat2;R61	None	None	None
Mutagenicity	Cat 3;R68	Cat 3;R68	Cat 3;R68	None	None
Carcinogenicity	Cat 1; R49	Cat 1; R49	Cat 1; R49	Cat 1; R49	Cat 3; R40
S-Phrases	53, 45, 60, 61	53, 45, 60, 61	53, 45, 60, 61	53, 45, 61	(2), 36, 37, 45
Aquatic Environment	N;R50/53	N;R50/53	N;R50/53	N;R53	Powder (<1 mm diameter): R52/53 Massive: None

Red = Changes in the 30<sup>th</sup>/31<sup>st</sup> ATP from previous classification

A summary of the strategy for acquiring data for the EU REACH regulations is presented in Figure 1. Once the bioaccessibility data for nickel compounds have demonstrated which compound a toxicity endpoint should be “read-across” from, a final step will be needed to complete the process. Specifically, limited acute toxicity testing will be conducted to verify that the assumptions behind the read-across paradigm are valid. In many cases some acute toxicity data will be available removing the need to conduct further test. In this way the read-across paradigm for nickel compounds under the EU REACH program will utilize all the elements of the 2004 OECD Chemical Category read-across methodology including verification testing.

Figure 1: Flowchart for Development of Required Toxicological Data using Read-across

