Review of welfare effects of metabolic cage housing:

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The metabolic cage and its use:

Metabolism cage housing of laboratory rodents provides researchers with unique possibilities of investigating particular biological events and their progression so it is widely used in pharmacokinetic and pharmacodynamic studies.
The metabolism cages have been designed specifically to study e.g. the eponymous metabolism (Rebuffe-Scrive et al. & Gilmore et al.), pharmacokinetics (Pollack et al.), kidney function (Gaeggeler et al.) and intestinal function. (Larauche et al.)

Metabolic cage constitutes a form of single housing on wire mesh without bedding, often without enrichment, comprising of a smaller living area with no shelter. (Kalliokoski et al.)

Metabolic cage (general specifications):

A typical metabolic cage (see photo below) is constructed with an upper chamber made of transparent, gnaw-proof polycarbonate.
A feed chamber is located outside the cage and the size of the feed chamber is designed to prevent rodents to sleep or nest inside.
The feed chamber contains of a drawer that is easy to pull out to simplify filling with minimum disturbance of the animal. This drawer is usually not designed to hold ordinary feed pellets but liquids, slurries or powders, to prevent the animal from dragging feed into the cage. The construction of the feeding chamber and drawer prevents urine from getting contaminated with feed.
The water bottle is calibrated, located outside the cage and is made of polycarbonate. Under the water bottle there is a calibrated spillage collecting tube which prevents water from entering the cage and contaminating the urine.
The spillage collecting tube is calibrated and enables the investigator to calculate the actual water intake of the animal.
The cage has grid floor and the urine flows down in the middle of a funnel under the cage to the urine collection tube which is graded in cubic centimetres. Faeces roll down on the side of the funnel into a specific faeces tube that can be removed from outside the cage to prevent disturbance of the animal. (Cvek-Hopkins).
A picture below is of aTecniplast metabolic cage (suitable for single or group housing of mice, involving a cage divider); find out more at https://www.tecniplast.it/en/product/metabolic-cages.html

The reported biological effects of metabolic cage use:

The metabolic cage exposes the animal to social isolation since the animal is placed by itself without any possibility for social contact. Social isolation is argued to be stressful for mice and rats. (Greco et al., D'Arbe et al., Nagy et al.)
Mice and rats in the wild live in social groups, with complex dominance systems, practicing passive and active social contacts. (Van der Weerd et al.)
Passive social contact is expressed when mice sleep with body contact (cage mates huddling); this ensures warmth and security (social thermoregulation, ensuring thermoneutrality in primary enclosure). Both mice and rats sleep together when held in groups and it has been shown in preference tests („ask the animal" approach) that mice prefer company over environmental enrichment i.e. supply of nesting material. When mice could choose between a cage without cage mates and an inhabited cage, there was significantly more time spent in the inhabited cage. The results showed that both young and adult mice preferred to share a common sleeping site and usually slept close together irrespective of social status. In the light phase, increasing age was correlated with a significant higher preference for social contact over nesting material. Both dominant and subordinate males preferred to sleep in near contact with other males regardless of their relationships. (Van Loo et al.)
Majority of purposly bred laboratory animals spend most of their captive lives as participants of the husbandry regime and only a fraction of their lives as participants of terminal experimental protocols regimes. All that time they are group housed.
Social isolation effects, from human point of view, are best understood and explained by the prisoners, serving a long time sentence; when comitted to a solitary confinement, according to their confessions, the harm/stress inflicted on them by social isolation is the worst possible form of punishment and the worst stressor of all.

All of the aforementioned metabolic cage structural features/factors have been associated with induction of stress or discomfort in laboratory mice and rats, as observed in e.g. Zhu et al. on enrichment, Ishida et al. on the effect of living area, Bartolomucci et al. and Cvek-Hopkins on single housing, Whitakker et al. and Banek et al. on effects of space allocation and housing density on biology (i.e. metabolism, physiology) as well as spontaneous and evoked behavior in laboratory rodents (Whittaker et al., Kalliokoski et al.) and Manser et al. on the effect of being housed on wire mesh (preference of solid bottom cage over wire-mesh cage).
There are few studies on the effect of this type of housing on the wellbeing of the test subjects. (Kurien et al., Kalliokoski et al., Cvek-Hopkins)

The effects on the wellbeing of the test subjects:

Mice and rats housed in metabolic cages can not perform some of their natural, species-specific behaviours such as making nests (mice), hide (rats) and interacting socially (both mice and rats).
Whereas it has been suggested that rats may be able to adjust to metabolism cages (Eriksson et al., Gomez-Sanchez et al., Gil et al.), mice are considered to be less malleable and more sensitive to low-level stressors. (Hennessy et al., Tabata et al., Kalliokoski et al.)
Indeed some guidelines suggest that mice should be kept in metabolism cages for the shortest possible time. (Baumans et al., Kalliokoski et al.)

The effects on reliability of the experiment:

Considering general acceptance that prolonged stress has profound consequences on body physiology, use of metabolic cageg should, on a case-by-case basis, be evaluated for its potential to interact with the reliability of the experiment, i.e. the targeted biomarkers/study endpoints.

Is the solution to expose the laboratory animals to an environment that demands an acclimatisation period of 14 days of social isolation, in order to gain unbiased results, and are the results really unbiased when the animal has been “acclimatized”? (Cvek-Hopkins)

The severity of procedures requiring housing research subjects in metabolic cages:

The current EU Directive (2010/63/EU) lists confinement in a metabolic cage for

i) up to 24 hours as a mild severity procedure,
ii) up to 5 days as a moderate severity procedure and
iii) housing for longer periods receiving a severe classification with respect to pain, suffering and/or distress.

Because of its size and construction and its potential to induce stress in research subjects, metabolic cage should only be used for sampling faeces and urine during experimental procedures. (Howard et al., eds)

It is common best practice to let animals habituate and acclimatize to changes in location and caging in order to obtain reliable results from subsequent experimentation. (Kalliokoski et al)

In mice transferred to metabolism cages, food and water intake, urinary output, and body weight do not seem to stabilize until 3–4 days after the novel housing. (Tuli et al.)

The stabilization of these parameters is sometimes interpreted as adjustment to the new environment. This thus provides a counterpoint, as reliable measures cannot be obtained until perhaps a week after housing mice in metabolism cages.

Consequently, other guidelines recommend an acclimatization period to be included in studies in metabolism cages. (Stechman et al.)

Unfortunately, a great majority of laboratory animal guidelines and handbooks do not address the conflict between the metabolism cages being considered stressful and the scientific need to let the animals acclimatize prior to experimentation. (Kalliokoski et al.)

Alternative methods for occasional urine samples:

Kurien et al. describe methods that involve the use of plastic wrap to collect pure urine from mice. They placed clear plastic wrap upon a white paper sheet located outside the animal’s home cage, on a bench top. The mouse was then transferred onto the plastic wrap and kept there until it urinated (which majority of mice do instinctively upon removal from the home cage). Using this method, urine volumes from 10 to 250 µl could be obtained as fast as in 12 seconds. (Cvek-Hopkins)

In another method for collection of small urine and faeces amounts, the rodent is placed in an empty plastic cage until it urinates. The animal is transferred back into its home cage and the samples are aspirated using a pipette. (Dahlborn et al., Augustsson et al.)

Recently, a product developed to support nonstressful urine collection from cats was proposed as a potentially useful way to collect urine from rodents.

Hydrophobic sand (for example, LabSand, Kit4Cat), a biodegradable material with a nontoxic urine-repelling coating, replaces the bedding in a normal cage during the urine collection period.

After collection is complete, the rats can be returned to their normal homecage environment, and the used hydrophobic sand is discarded as laboratory waste. (Hoffman et al.)

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